

**INVESTIGATION OF *BALANITES ROXBURGHII* AND
SOME NAPHTHOFURAN DERIVATIVES FOR
ANTIFERTILITY AND OTHER BIOLOGICAL ACTIVITIES**

THESIS

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IN

CHEMISTRY



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**DEDICATED
TO MY
PARENTS
AND FAMILY**



DECLARATION

I hereby declare that the work presented in this thesis entitled “**Investigation of *Balanites Roxburghii* and Some Naphthofuran Derivatives for Antifertility and Other Biological Activities**” is entirely original and was carried out by me in the Department of Studies in Chemistry under the supervision of Dr. V. P. Vaidya, Reader, Department of Studies in Chemistry, Kuvempu University. I further declare that the results presented in the thesis or any part thereof has not been submitted elsewhere for any other degree or diploma of similar title of any other University.

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CERTIFICATE

This is to certify that the work reported in this thesis entitled "**Investigation of *Balanites Roxburghii* and Some Naphthofuran Derivatives for Antifertility and Other Biological Activities**" submitted by Shri. Basavaraj Padmashali, to the Faculty of Science, KUVEMPU UNIVERSITY for the award of the **Doctor of Philosophy in Chemistry** is a record of the bonafide and original research work carried out by him under my guidance. The work reported in this thesis has not formed the basis for the award of any degree, diploma or any other similar title.

Date : 18th July 2003

Place : Shankaraghatta


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OVERALL CONCLUSIONS

PUBLICATIONS

PAPERS PRESENTED

INSTRUMENTATION AND METHODOLOGY

Meting Point

Melting points are in degree Celsius. These were determined in open capillary tubes and are uncorrected.

Infrared Spectra

The IR spectra were recorded in Perkin-Elmer Kbron Unicam FTIR and Perkin-Elmer FT-IR Spectrum 1000 Spectrophotometers. Wave numbers are expressed in cm^{-1} .

NMR Spectra

The ^1H NMR spectra were recorded on JEOL MODEL GSX 270 FT NMR spectrophotometer and NMR 300 MHz Supercon Machine, using CDCl_3 od DMSO-d_6 as solvent and TMS as internal standard reference. Chemical shifts are expressed as δ values [ppm].

Mass spectra

The Mass Spectra were recorded on Brucher Apex-II Mass Spectrometer.

Purity

Purity of compounds was checked by TLC.

Analysis

Calculated percentage of C, H and N are given in parenthesis.

Biological activity and Pharmacological Screening

Antifertility activity, antimicrobial, anthelmintic, anti-inflammatory, analgesic and diuretic activities were carried out at National Pharmacy College, Shimoga. Antipyretic and anticonvulsant activities were carried out at TMAE Society's SCS college of Pharmacy, Harapanahalli.



Population means the number of inhabitants in a specified territory. During the 20th century two important demographic phenomena have occurred. The first one is the unprecedented growth of human population and the second one is the movement of people from rural to urban areas. This migration of people, which started during the industrial revolution, gained significant momentum over the past 50 years. This has resulted in overcrowding of urban areas.

As per the estimation of United Nations, the world population in the middle of 1991 was 5.385 billion¹⁻³. During this period, the average rate of increase in population was 1.7%. At present the world population is growing at the rate of 176 people per minute, 10,564 people per hour, 253,542 people per day and 92,543,000 people per year. The world population has reached an amazing figure of 6 billion at the end of the last century. If the growth of population continues to increase at this rate, it is estimated that it will cross 7 billion mark by the end of 2022⁴.

Percentage wise, Asian countries contribute 58.8% to the world population, with a growth rate of 2.2%. China has largest population in the world. India is placed second with the contribution of 16% of the population.

In India, population was 344 million in 1947⁵, it was 439 million in 1961, 548 million in 1971 and 844 million in 1991⁶. It has reached the alarming figure of 1 billion in the year 2000. As per the census of 2001, carried out by the Indian government, the population of India is 1,02,70,15,247.

Uttarpradesh ranks first among the states of India, with a population of 138 million as on 1996. Amongst the cities of India, Mumbai has the largest population of 12.57 million and Kolkata comes second with 10.86 million.

Due to the advancement of medical sciences, the cruel balance between the births and deaths maintained by the dreaded diseases like smallpox, cholera, plague, malaria etc. no longer exists. People all over the world now lead healthier and longer life, when compared with the earlier situation.

The present food production is increasing in arithmetic progression, while the population is increasing in geometric progression. When compared with natural and human resources of the country, one can easily conclude that India is overpopulated. Bangladesh, Srilanka, Afghanistan and Pakistan, which are neighbouring countries of India, fall in the same category.

To control the menace of population explosion, many nations have enmarked various programmes of family planning and family welfare. Because of these steps, encouraging demographic progress has been achieved in the last 20 years. Fertility rates have declined worldwide. This has brought down the growth rate of population to some extent. Statistically, the rate of population growth, which was 2.5% per year in 1960, was brought down to 2.3% per year in 1981 and presently it is 2.0% per year. However the base is so large that this reduced rate of growth has very little impact on the population scenario. According to the forecast by experts, the world population may reach 12 or even 14 billion before it stabilizes.

Human settlement existed 1.5 million years ago in Africa as indicated by various evidences such as ^{14}C dating etc. and others. The following figures indicate the rapid rate at which population is increasing - to reach 1 billion mark of population it took a very long period and happened in the year 1800. It took 130 years, from then, to add one more billion, which occurred in the year 1930. The third billion was added in the shorter period

of time of 30 years, which happened in the year 1960. Then, only 15 years were required to add 4th billion, whereas only 12 years were required for the addition of 5th billion. This unprecedented growth was mainly attributed in the failure in making wide variety of effective, efficient and affordable methods of family planning. The rampant population growth has been viewed as the greatest single obstacle to the social and economic advancement of the majority of the people in the underdeveloped world.

In India overpopulation is due to manifold reasons. However, the major two causes are early marriages and longer span of reproductive activity. The other contributing factors are lack of respect for women and recreational facilities, tropical climate, total beliefs, ignorance, illiteracy, absence of proper welfare schemes and decline of death rate⁷.

The uncontrolled growth of population, results in the creation of various fundamental problems which include unemployment, overcrowding of urban areas, starvation and increase in crime rate. This has also resulted in communal clashes, social inequalities and conflicts.

Population growth will add many problems to the already existing ones, like limited availability of clean water, food, energy, education and job opportunities. If this continues, the process of deforestation, soil erosion and desertification⁸ will accelerate. At the end, economic and social foundation of the nation will collapse under the pressure and environmental balance will also be disturbed causing global climate changes.

The spurt in population growth has resulted in urbanization. One half of the world's population today lives in cities that occupy only 2% of the world land. Surprisingly, it consumes 75% of the resources and produce 75% of the waste material in the world. It is expected, by the year 2050, 2/3rd of the world's population will be in urban .

area. Thus large cities are faced with serious basic problems such as depletion of natural resources, mushrooming of slums, traffic congestion etc.

The rural areas continue to suffer from gross inadequacies in health services, sanitation, educational facilities, drinking water problems etc⁹.

According to the report from United Nations Family Planning Association (UNFPA), the rapid population growth is primarily attributed to the following three main reasons

1. Increase in life expectancy
2. Decrease in infant mortality
3. Development of medical science

The continuing high growth rate is also due to ignorance of married couple to use proper methods of fertility regulations. In developing countries, out of 600 million couples, nearly 500 million couples are not using suitable family planning methods. This is also due to the fact that the available methods and services of family planning are too expensive and also sophisticated to meet the demands of the underdeveloped and developing countries¹⁰.

To solve the above problems, the urgent requirement is to modernize as well as to amplify the present fertility regulation methods. Thus fertility control has become most important and urgent mainstay of all biosocial and biomedical problems faced by mankind.

Improvement and innovation in the technology of birth control are very essential in the present day situation. In the international conference on population and development held in Kairo in the year 1994, it was resolved to focus on the following major programmes

1. The eradication of illiteracy

2. Full employment opportunities for women
3. Achieving the lowest possible rates of infant mortality, and
4. Universal access to the knowledge and affordable methods of family planning to prevent unintended pregnancy.

To achieve the last aim, it is necessary to evolve more acceptable and effective means of contraception, for both male and female with nil or minimum side effects. It is also needed to update the research in human reproduction and fertility regulation, including biochemical research in order to improve safety and efficacy of the existing family planning methods.

A comprehensive discussion of the existing methods of contraception and some newly developed techniques is provided in the following pages.

1.1 Natural Family Planning methods

1.1.1 Abstinence during the fertile phase

This method emphasized on the safe period, which is also known as rhythm method involving the avoidance of sexual intercourse during ovulation period. Ovulation occurs normally on the 14th day of the estrus cycle, in a 28 days normal cycle. However, in some cases, ovulation may occur any time between the 12th and 16th day. Hence, the safe period is calculated from the 1st day of the menstrual period until 8th day of the cycle, which is generally known as pre-ovulation period. The post-ovulation period can also be regarded as the safe period, which normally occurs from 18th day to 28th day.

1.1.2 Withdrawal method (Coitus interruptus)

This is the very common method of family planning, but it is observed that it is quite inefficient in regular practice.

1.1.3 Cervical mucus method

This method is also considered as ovulation method or billing method. Cervical mucus becomes clear, slippery, profuse and smooth, at the time of ovulation. After ovulation, the mucus thickens and lessens in quality. By careful examination of the cervix by females, the sexual intercourse during this period can be avoided.

1.2 Barrier Contraceptives

1.2.1 Condom¹¹

The use of condoms is the most widely used method of contraception. The developments in technology have produced extra thin membrane sheaths, marketed in a variety of colours, with or without lubricants under different trade names. These are commonly called as “Nirodh”, which are easily available in market even in rural areas. No medication is required for this method either by male or female. Condoms have a satisfactory efficacy when used properly. The additional advantage of using condoms is that it prevents the transmission of venereal diseases and AIDS.

1.2.2 Diaphragms

These are used by females as the sacks to cover the passage of the sperms to the cervix. It has more failures than success. This is because of the fact that the sizes of diaphragm vary and desired fitness is not achieved.

1.2.3 Spermicidal agents

It has been recommended to use foams, creams and jellies containing spermicidal agents for the prevention of pregnancy. The spermicidal agents are “Surface active agents”, which attach themselves to spermatozoa and inhibit oxygen uptake and kill sperms¹². The spermicidal agents contain surfactants such as octoxynol, nanoxynol-9 and

menfegol. Recently a new agent ORF 13900 has been introduced in market, which agglutinates sperms and thus acts as spermicidal agent¹³.

1.2.4 Douching

The dutch cap, cervical cap, dumas cap, contra cap and vaginal sponge are widely used barrier devices today for the purpose of immediate postcoital douching of the vagina with spermicidal solution, to wash away the sperms.

1.3 Intrauterine Contraceptive Devices (IUD)

The antifertility action of a foreign body in the uterus was known since ancient times in India. IUD were introduced in the family planning method about 30 years back. IUD is an effective and safe method of contraception. It prevents the implantation of blastocytes in the uterus. A variety of IUDs like Lippe's loop, Dalken Shield, Soonawala Device, differing in shape have been proposed. Most of these are made up of silastic polymers with or without metallic component. However, this method suffers from major drawbacks like bleeding disorders, pain and expulsion. These side effects have been reduced in the second generation of IUD. This contains copper wire, known as Cu-IUD and the Cu-Ag IUD¹⁴. These are marketed in the trade names like Copper-T, Copper-T-200, TuCu-220c, TuCu-380 Ag, Nova-T, ML-Cu-250, ML-Cu-375.

1.4 Contraceptive Steroids (Oral Contraceptives)

Existence of a feedback control between the steroid hormones produced by ovaries and pituitary gonadotrophins which stimulates their synthesis, give rise to the property which forms the basis of utilizing progestogens and estrogens to inhibit the secretion of gonadotrophins and thereby block the ovulation.

The natural hormones, estradiol and progesterone, which are not employed due to their very short biological half-life. In place of these, synthetic steroids with estrogenic and progestogenic activity are used. In earlier days, these drugs were administered orally in the form of oral pills containing a combination of progestones with little amount of estrogens. Now a days these oral pills are available in three types.

- i). An orally active progestogen, which contains 19-nor testosterone or 17-hydroxyprogesterone derivatives. These pills suppress pituitary hormone FSH and LH and thereby prevent ovulation.
- ii). Combined oral pills containing either ethinyl estradiol or mestranol¹⁵.
- iii). Triphasic combined pills-These have been recently introduced and normal preparations contain ethinyl estradiol (EE2) and levonorgestrel (LNG).

Many side effects have been attributed to the use of oral contraceptives. Cardiovascular side effects and induction or promotion of tumors are more concerned ones. To overcome this difficulty, injectible contraceptive steroids have been evolved in recent times. The steroids commonly used are methoxy progesterone acetate or norethindrone acetate.

1.5 Interceptive Agents (Post-coital Agents)

These agents interfere with postovulatory events leading to pregnancy and are, therefore, called as “interceptives”. Ethinyl estradiol + norethisterone or levonorgestrel, ethinyl stilbesterol, mifepristone (Ru. 486) are the common examples of interceptives. These steroids have high affinity for the progesterone receptors. They act by blocking the action of progesterone on the endometrium.

Prostaglandins act by virtue of their luteolytic effect on the ovary and increased mobility effect on fallopian tube and the uterus, which prevents implantation and induce menstruation.

1.6 Immunological methods

In this method antibodies to sperm antigens are produced by introduction of enzymes like lactate dehydrogenase, hyaluronidase and acrosin. These cause immobilization and destruction of sperms. Inhibin, an anti-FSH vaccine is also under trial. Temporary sterility can be evoked by the use of β subunits of HCG (33 μ g). Zona pellucida plays an important role in implantation. If zona pellucida antibodies are developed, which either prevents sperm penetration in the ovum or shedding of zona after fertilization, it can evoke antifertility.

1.7 Sterilization (male and female)

The sterilization operation is undertaken with primary objective of preventing further pregnancy permanently. In tubectomy and vasectomy, the continuity of the duct meant for the transport of ovum and spermatozoa respectively are interrupted. The Minilap Pomeroy technique is considered a revolutionary procedure for the female sterilization. Laproscopic fulgarition is becoming more popular. It is highly effective and very quick. However, these methods have many side effects and also psychological complaints of diminution of sexual vigor and impotency. To overcome these drawbacks, new developments and improvements of the presently available methods have been devised, amongst which, the following two are the most important ones.

1.7.1 The subdermal implants norplant

In this method, a set of six celastic capsules filled with progesterone-levonorgestryl, are introduced beneath the skin under light anaesthesia. The release of the steroid at a steady rate ensures effective contraception for nearly 5 years. These can be used by young women, who require contraception for a longer time and also want to retain the option of another pregnancy.

1.7.2 Progestin release IUD

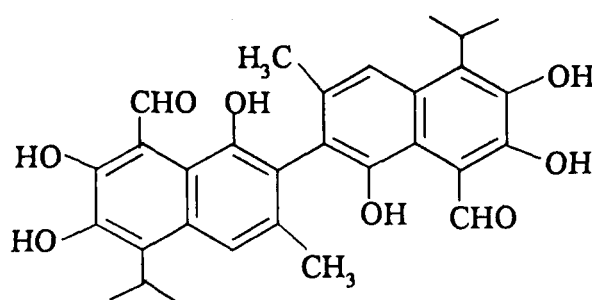
These are the new methods, which are still in trial stages.

1.7.2.1 Analogues and Antagonist of Luteinizing Hormone Release Hormone (LHRH)

Daily administration of the synthetic analogues of LHRH having prolonged biological half-life, by systematic injections or nasal spray results in blockage of ovulation in the females.

1.7.2.2 Gossypol

This compound was used by an accident in China by several thousands of males causing azospermia. This compound was extracted from the seeds of cotton. It binds with sperm proteins and causes hypokalemia in human beings. However, due to low efficacy versus toxicity ratio in conventional experiments, it is not likely to be approved for human usage.



Gossypol

1.7.2.3 Prostaglandins and Antiprogestosterone compounds for menstrual regulation and termination of pregnancy

These are contraceptive vaccines, which are still in the stage of experimental developments. In all probabilities this may emerge as the most advanced birth control vaccine.

1.8 Herbal options to control fertility

Most of the methods mentioned above are effective. However, none of them is free from one or the other drawback either in the form of side effects or complications in its use. Therefore vigorous and sincere attempts are being made for the improvement of these existing methods. Simultaneously new techniques of fertility regulations are being constantly explored. The encouraging fact is that the demand for new methods for fertility control has increased enormously in India. It is because, every newly married couple, rural or urban, educated or uneducated, want to have smaller family norms. Hence, search for harmless and inexpensive oral agents for fertility control in human beings to reduce the population size is more actually felt now than ever before.

Due to severe and long lasting side effects of modern medicines, the people are now looking back to age-old tradition of using herbal medicines for curing various diseases and also for fertility control. Fortunately India has rich heritage of use of medicinal plants for fertility control. In this context, it is appropriate to locate the large number of indigenous plants that are used as oral contraceptives by tribals and other section of people. Many such plants are even recommended in folk medicines, Ayurvedic and Yunani medicines¹⁶⁻¹⁹ very ancient times. A good number of scientific papers have

been already published related to the use of medicinal plants for antifertility. However, still many more medicinal plants are either less investigated or left uninvestigated.

Encouraged by these facts, many scientists all over the world in general and India in particular, are presently engaged in the search for a safe, acceptable both by male and female, effective, easily administrable, cheap and non-steroidal antifertility agents derived from medicinal plants, which are commonly grown in India and other parts of the world.

Plants have been screened for variety of fertility regulating activities in both females and males. The plants, which exhibit fertility control in females, may possess antiovaratory, antiimplantation, abotifacient and uterine stimulating activities²⁰. The plants, which exhibit fertility control in males, may possess androgenic, antiandrogenic, spermicidal and antispermatogeinc activities²¹.

Research on antifertility activity of Indian medicinal plants has been reviewed exhaustively by Choudhury and Haq^{22,23}, Kamboj and Dhawan^{24,25}, Sathyavathi²⁶ and Bhargava²⁷.

In this context, it is felt appropriate and necessary to gather information on such medicinal plants related to fertility control. However, detailed discussion on such plants is not within the limit of this thesis. Hence, a brief account of Indian medicinal plants, used for fertility control is given in the following pages.

1.8.1 Medicinal plants showing antiovaratory activity

Some of the medicinal plants available in India have been shown to possess antiovaratory activity. The list of such plants is given in **Table-1.1** along with the parts used with relevant references.

Table-1.1 Plants showing antioviulatory activity.

Name of the Plant	Part used	Reference
<i>Albizzia lebbeck</i>	seeds	28
<i>Aloe barbadensis</i>	leaves	29
<i>Capsella bursa-pastoris</i>	leaves and dry powdered plant	30
<i>Mentha arvensis</i>	leaves	31
<i>Polygonum hydropiper</i>	root	31
<i>Randia dumetorium</i>	fruits, seeds and pulp	32
<i>Solanum khasianum</i>	whole plant	33
<i>Taxus baccata</i>	leaves	34
<i>Vitex negundo</i>	seeds	35

Vohra and Khan²⁸ isolated some saponins from the seeds of *Albizzia lebbeck* and shown them to possess antioviulatory activity. Aqueous extracts of leaves of *Aloe barbadensis*²⁹ and dry powder of the plant *Capsella bursa-pastoris*³⁰ are also shown to exhibit considerable antioviulatory activity. Similarly, extract of leaves of *Mentha arvensis*³¹, petroleum ether extract of the root of *Polygonum hydropiper*³¹, ethanolic extracts of the fruits and seeds of *Randia dumetorium*³², ethanolic extract of the whole plant of *Solanum khasianum*³³, aqueous extract of leaves of *Taxus baccata*³⁴ and seeds of *Vitex negundo*³⁵ showed potent antioviulatory activity in albino rats.

1.8.2 Medicinal plants showing antiimplantation activity

The survey of literature revealed that there are variety of medicinal plants, which exhibit antiimplantation activity. Some of such plants are listed in **Table-1.2** and discussed in brief.

Table-1.2 Plants showing antiimplantation activity.

Name of the Plant	Part used	Reference
<i>Abroma augusta</i>	root	36
<i>Achrostichum aureum</i>	whole plant	37
<i>Allium sativum</i>	bulbs	36
<i>Artabotrys odoratissimum</i>	leaves	38
<i>Areca catechu</i>	nuts	39-40
<i>Artemisia scoparia</i>	whole plant	41
<i>Butea frondosa</i>	seeds	42
<i>Butea monosperma</i>	flowers	42
<i>Caesalpinia decapetala</i>	aerial parts	43
<i>Calatropis procera</i>	root	44
<i>Carica papaya</i>	seed oil, seeds, unripe fruits	45
<i>Cicer arietinum</i>	seed oil	42
<i>Costus speciosus</i>	rhizomes	46
<i>Cuminum cyminum</i>	seeds	47
<i>Curcuma longa</i>	rhizomes	48
<i>Cyperus rotundus</i>	rhizomes	49
<i>Dacus carota</i>	seeds	50
<i>Datura quercifolia</i>	fruits	51
<i>Desmodium gangeticum</i>	seeds	52
<i>Dieffenbachia amoena</i>	leaves	53
<i>Embelica ribes</i>	berry	54
<i>Ensete superbum</i>	seeds	55

<i>Glycyrrhiza glabra</i>	fruits	56
<i>Hibiscus rosa-sinensis</i>	flowers	57
<i>Hyptis suaveolens</i>	leaves	58
<i>Illicium anisatum</i>	seed oil	59
<i>Ischnochiton campus</i>	plant	60
<i>Michelia champaka</i>	anthers	61
<i>Nigella sativa</i>	seeds	62
<i>Ocimum sanctum</i>	leaves	63
<i>Plumbago zeylanica</i>	root, fruits	64
<i>Pueraria tuberosa</i>	tubers	65,66
<i>Revia hypercroteriformis</i>	aerial parts	67
<i>Rubus ellipticus</i>	plant excluding roots	68
<i>Salix babylonica</i>	plant excluding roots	69
<i>Salix tetrasperma</i>	stem bark	69
<i>Sarcophyton chrenbergi</i>	plant	65
<i>Sesamum indicum</i>	seeds	35
<i>Striga densiflora</i>	plant	70
<i>Striga lutea</i>	plant	71-73
<i>Thespesia populne</i>	seeds	74
<i>Trifolium alexandrium</i>	seeds	54
<i>Uraria lagopodioides</i>	whole plant	75

The root of *Abroma augusta* has been reported to possess estrogenic activity in female albino rats³⁶.

Dhar et al., investigated the antifertility activity of the ethanolic extract of the plant *Achrostichum aureum* in female albino rats. They observed that the water-soluble fraction of ethanolic extract exhibited very significant antiimplantation activity³⁷.

Investigation of the nuts of *Areca catechu* revealed that petroleum ether extract, ethanolic and aqueous extracts of nuts possess antiimplantation activity in albino rats³⁹⁻⁴⁰.

The seeds of *Butea frondosa* and the flowers of *Butea monosperma* were shown to exhibit considerable antiimplantation activity when tested on albino rats⁴².

Ethanolic extract of *Caesalpinia decapetala*, administered orally on days 1-8, postcoitum, at 500 mg/kg dose exhibited significant contraceptive activity in female hamster but devoid of any estrogenic activity⁴³.

Kamath et al., have tested the effect of ethanol extract of the root of *Calatropis procera* in albino rats, in order to explore its hormonal and antifertility activities⁴⁴. A strong antiimplantation activity and uterotropic activity was observed at the dose level of 250 mg/kg.

Keshri et al., carried out extensive research work on *Carica papaya*. Oral administration of the hexane extract of the dry seeds of this plant at the dosage of 1 gm/kg daily, to adult female rats on day 1-10 post coitum, prevented pregnancy in 70% of the animals⁴⁵. Undocumented evidences on this plant, which is used in folk medicine, indicate that this plant has considerable antiimplantation activity in human beings also.

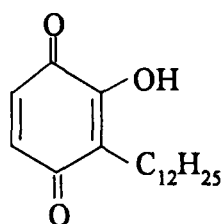
Similarly Khanna and Chowdhary carried out pharmacological investigation of seed and seed oil of *Cicer arietinum*⁴² and observed that these exhibited estrogenic activity in female albino rats.

When screened for antifertility activity, rhizomes of *Costus speciosus* were found to possess significant estrogenic activity in albino rats⁴⁶. On the other hand the rhizomes of *Cyperus rotundus* were shown to possess antiestrogenic activity⁴⁹. However, both the plants exhibited antiimplantation activity.

Various extracts of the seeds of *Dacus carota* have been extensively investigated for possible antifertility activity⁵⁰. The chromatographic fractions of petroleum ether, ethanolic and aqueous extracts of the seeds were also studied. The chloroform and methanol fractions of petroleum ether extract and ethanolic extracts showed 80-100% inhibition of implants in albino rats.

Significant interceptory and estrogenic properties were observed in the plant *Datura quercifolia*. Datura lactone isolated from this plant was found to be responsible for these properties⁵¹.

Application of Embelin isolated from the fruit of *Embelica ribes* exhibited significant antiimplantation activity⁵⁴.



Embelin

Saraf and Gomaio investigated the root of *Glycyrrhiza glabra* and reported that it exhibited considerable estrogenic activity⁵⁶.

Extensive research has been carried out on the flowers of *Hibiscus rosa-sinesis* and ethanolic and benzene extracts have been reported to possess antiimplantation activity in women⁵⁷.

The ethanolic extract of *Hyptis suaveolens* leaves completely inhibited implantation at the dose of 200 mg/kg body weight when administered orally from day 1-7 of pregnancy⁵⁸. It was observed that unsaponifiable mass isolated from the leaves and floral parts of this plant showed 20-40% implantation activity at the dose of 250 mg/kg body weight in albino rats. Further research work in this regard showed that antiimplnatation activity was due to estrogenic effect.

Antiimplantation activity to the extent of 100% was reported with the fruits of *Illicium anisatum* when administered orally at a dose of 80 mg/kg body weight in albino rats⁵⁹. The trans-anethole isolated from the oil obtained from the fruits of this plant was shown to be responsible for this activity. Significant estrogenic activity has been demonstrated by the experiments carried out on young rats.

Dhar et al., have studied the antifertility activity of *Ischochiton campus* and found that its aqueous and methanolic fractions, exhibited considerable postcoital antifertility activity⁶⁰. Investigations of the leaves of the plant revealed that ethanolic extract showed 80% and aqueous extract 60% antiimplantation activity in albino rats.

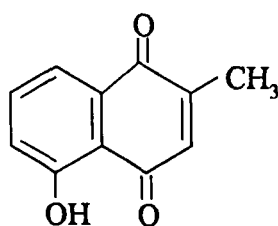
The benzene extract of *Michelia champaka* (anthers) exhibited 67% antiimplantation activity at 1 gm/kg per day dose level⁶¹.

Prevention of pregnancy in Sprague-Dawley rats was observed when n-hexane extract of the seeds of *Nigella sativa* was administered orally at the dose of 2 gm/kg body weight daily from day 1 to 10 post coitum⁶².

The leaves of well-known sacred plant *Ocimum sanctum* have been investigated for various biological and pharmacological activities⁶³. Petroleum ether extract of the

leaves of *Ocimum sanctum* has been found to possess significant antiimplantation activity in albino rats.

It was reported that fruits and root of *Plumbago zeylanica* possess antiimplantation activity when given orally at the dose of 122mg/100 gm weight to albino rats⁶⁴. Further investigations of this plant by Premakumari et al., revealed that Plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone), a crystalline compound from this plant was responsible for such an activity.



Plumbagin

Various extracts of the tubers of *Peuraria tuberosa* have been tested for their effect on early and late pregnancy in rats, mice, hamsters and guinea pigs⁶⁵. Butanol fraction of the extracts was found to be active due to its inherent hormonal properties. Shukla has described the mechanism of action and toxicological studies of this plant. Puerarin, diadzein and tuberosin were isolated from the chloroform and butanol fractions of ethanolic extract of this plant. The antiimplantation activity exhibited by this plant was attributed to the presence of these compounds⁶⁶.

Shivalingappa et al., have tested the petroleum ether, chloroform, ethanol and aqueous extracts of the aerial parts of the plant *Rivea hypocrateriformis* for antiimplantation activity and pregnancy interruption properties in albino rats⁶⁷. Among

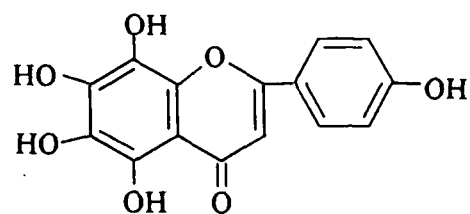
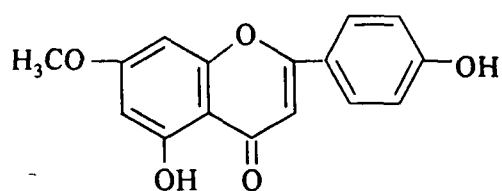
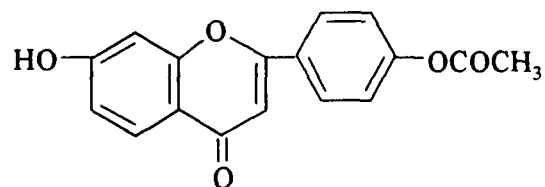
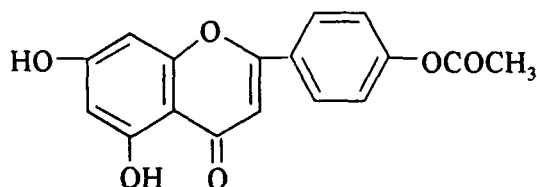
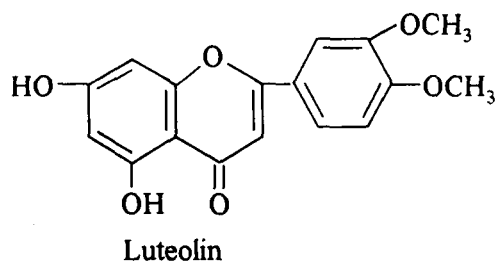
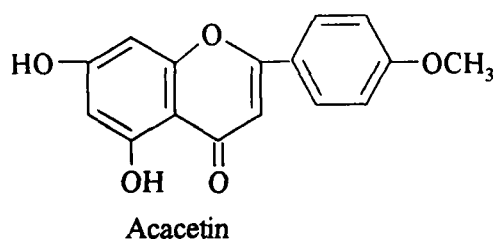
these, ethanolic extract was shown to be most effective in causing significant antiimplantation and interruption of early pregnancy.

Encouraged by various reports mentioned above, Hiremath et al., took up a major research programme of searching non-steroidal antifertility agents from medicinal plants.

The following plants have been investigated for antifertility activity.

1. *Striga densiflora*⁷⁰
2. *Striga lutea*⁷¹⁻⁷³
3. *Acalypha indica*
4. *Striga orobonchiosides*

They could isolate and identify the different flavones from these plants possessing the following structures.



The extract of the seeds of the plants *Thespesia populne*⁷⁴ and *Trifolium alexandrinum*⁵⁴ were found to possess significant antiimplantation activity in rats.

Khanna et al., reported that, the aqueous extract of the whole plant of *Uraria lagopodioides* prevented implantation in albino rats when administered orally⁷⁵.

1.8.3 Medicinal plants with abortifacient activity

The plants listed in Table-1.3 have been shown to possess abortifacient activity. A brief account of the same is described in the following paragraphs.

Table-1.3 Medicinal plants with abortifacient activity

Name of Plant	Part used	Reference
<i>Achyranthus aspera</i>	stem, bark	76
<i>Adhatoda vasica</i>	whole plant	77
<i>Ananas comosus</i>	unripe fruits, juice	78
<i>Aristolochia indica</i>	root	79
<i>Curculigo orchoiodes</i>	whole plant	80
<i>Derris brevipes</i>	whole plant	81
<i>Gardenia jasminoides</i>	flowers	82,83
<i>Marsdenia koi</i>	whole plant	84
<i>Physalis minima</i>	plant without root	85,86
<i>Pseudolarix kaempferi</i>	root, bark	87-89

Gupta et al., observed that benzene extract of *Achyranthus aspera* exhibited abortifacient effect in rabbits at a single dose of 50 mg/kg⁷⁶.

Uterotonic activity in guinea pigs was reported with vasicine, an alkaloid isolated from the plant of *Adhatoda vasica*⁷⁷. This compound also potentiated prostaglandin induced uterine contractions in rats.

The unripe fruits and juice of *Ananas comosus* were shown to exert abortifacient effect in mice, which was attributed to antiluteotropic activity⁷⁸.

Pakrashi and Shaha isolated methyl ether of aristolochic acid from the roots of *Aristolochia indica* and found that it caused 100% abortion effect at a single dose of 60 mg/kg body weight in albino rats⁷⁹.

The aerial parts of *Curculigo orchioides* were found to exhibit significant abortifacient activity due to powerful uterine stimulant property of flavone-glycoside isolated from this plant⁸⁰.

Badami et al., have shown that the ethanolic extract of *Derris brevipes* exhibited 40% antiimplantation activity when given orally at 600 mg/kg bodyweight⁸¹. However, the results of further investigation suggested that the extract possessed more abortifacient type effect than antiimplantation activity.

The ethyl acetate extract of the flowers of *Gardenia jasminoides* was found to possess significant pregnancy termination effect^{82,83}. The activity was due to triterpenoids-gardenic acid and gardenolic acid B.

Investigations of aerial parts of *Marsdenia koi* indicated that they can cause termination of early pregnancy in animals⁸⁴. Chinese workers Yuan and et al., isolated Marsdekoiside A from this plant.

Ethanolic extract of *Physalis minima* was reported to show significant abortifacient activity. Physalin B155 and Physalin D isolated from this plant were found to be responsible for this activity^{85,86}.

The root and bark of *Pseudolarix kaempferi* have been investigated extensively for their abortifacient activity⁸⁷⁻⁸⁹. This activity was due to the presence of pseudolaric acids A & B, present in this plant.

In addition to these plants listed in **Table-1.1** to **Table-1.3**, many other plants have been shown to possess considerable antifertility activity in various animals. However, detailed discussion about these plants is out of the scope of this thesis. Hence, only few plants possessing antifertility activity are listed in **Table-1.4**.

Table-1.4 Plants with antifertility activity

Name of Plant	Part used	Reference
<i>Actiniopteris radiata</i>	whole plant	90
<i>Adina cordifolia</i>	--	91
<i>Ailanthus excelsa</i>	leaves, stem bark	92
<i>Andrographis paniculata</i>	--	93
<i>Anona squamosa</i>	--	94
<i>Artabotrys odoranti</i>	leaves	95
<i>Bombax malabaricum</i>	root	96
<i>Cassia fistula</i>	seeds	97
<i>Catharanthus roseus</i>	--	98
<i>Caulerpa taxifolia</i>	whole plant	99
<i>Cedrus deodara</i>	stem	100
<i>Combretodendron africanum</i>	bark	101
<i>Coriandrum sativum</i>	seeds	102
<i>Curcuma domestica</i>	rhizomes	48
<i>Dictamnus oleifera</i>	root bark	103
<i>Dysdercus koenigii</i>	--	104
<i>Echinops echinatus</i>	--	105
<i>Ferula jaeschkeana</i>	fruits	106
<i>Gleditschia horida</i>	--	107
<i>Hagenia abyssinica</i>	flowers	39
<i>Ipomoea fistulosa</i>	--	108
<i>Juniperious communis</i>	fruits	109

<i>Lygodium flexosum</i>	whole plant	110
<i>Malvavisens conzatii</i>	flowers	111
<i>Mallotus philippensus</i>	glands, hairs from the capsules	112,113
<i>Marsdenia oreophila</i>	stem	114
<i>Momordica charantia</i>	root, leaves	115
<i>Momordica linn</i>	leaves	115
<i>Murraya paniculata</i>	root	116
<i>Nelumbo nucifera</i>	seeds	117
<i>Piper chaba</i>	fruits	118
<i>Polygonum stenophyllum</i>	root	119
<i>Podophyllum peltatum</i>	root	120
<i>Salvia fruiticosa</i>	--	121
<i>Seigesbeckia glabrascens</i>	--	122
<i>Senecia vulgaris</i>	--	123
<i>Sida carpinifolia</i>	--	124
<i>Spondias cythera</i>	bark	125
<i>Sophera japonica</i>	whole plant	126
<i>Stephania herandiifolia</i>	rhizomes	127
<i>Tabernaemontana heyneana</i>	--	128
<i>Tripterygium wilfordii</i>	--	129
<i>Wilbrandia species</i>	rhizhomes	130

1.8.4 Medicinal plants for male antifertility activity

The search for male contraceptives began in 1950. The researchers all over the world, who are working in this direction, aimed at the following aspects of male contraception.

- a) Prevention of spermatogenesis.
- b) Interfering with the storage of sperms and maturation.
- c) Prevention of the transport of sperms through vas deferens.
- d) Changing the constituents of the seminal fluid.

Most of the work carried out in this connection was devoted to prevent spermatogenesis. Surgical vasectomy, non-scalpel vasectomy and chemical occlusion of vas deferens are receiving active attention at present. Follicle Stimulating Hormone and sperm surface protein are considered as immunogens and hence has led to the development of vaccines.

An ideal male contraceptive is the one, which maintains normal level of testosterone, with simultaneous decrease in the sperm count. However, many of the contraceptives for males tend to lower the testosterone level, which suppresses the potency of the contraceptive.

Many compounds were reported to possess male antifertility activity ranging from alkylating agents to gossypol but none of them are free from toxicity. Surgical methods even though successful, have the major drawback in the form of creating weakness, loss of sexual activity etc.

Hence, researchers are looking at the use of medicinal plants for male antifertility activity. But scientists are not successful in getting the reproducible results from medicinal plants.

Several plants have been identified to possess male antifertility effect. Some of these plants are listed in Table-1.5.

Table-1.5 Medicinal plants possessing male antifertility activity

Name of the plant	Part used	Reference
<i>Abrus precatorius</i>	seeds	131,132
<i>Andrographis paniculatus</i>	--	133
<i>Austroplenckia populnea</i>		134
<i>Azadirachta indica</i>	seed oil	135
<i>Bambusa arundinacea</i>	--	136
<i>Barleria prionitis</i>	root	137
<i>Carica papaya</i>	seeds	138-142
<i>Celastrus paniculatus</i>	seeds	143
<i>Colebrookia oppositifolia</i>	leaves	144
<i>Martynia annua</i>		145
<i>Mentha arvensis</i>	leaves	146
<i>Mondia whitei</i>	--	147
<i>Mucuna urens</i>		148
<i>Piper betel</i>	--	149
<i>Quassia amara</i>		150
<i>Sarcostemma acidum</i>		151
<i>Solanum lycocarpum</i>	--	152
<i>Tripterygium hypoglaucum</i>	--	153
<i>Tripterygium wilfordii</i>	--	154-160

Rao¹³¹ and Sinha¹³² investigated male antifertility effects of ethanolic extract of seeds of *Abrus precatorius* Linn. in male albino rats. The post-testicular antifertility effects of this plant received attention by Sinha.

A well-known plant, *Andrographis paniculatus*, has been shown to exhibit significant male antifertility activity¹³³.

Mazaro et al., studied aqueous methanolic extract of *Austroplenckia populnea* for male antifertility activity. They observed that sperm concentration in cauda epididymis was decreased significantly at the dose of 500 mg/kg/day¹³⁴.

An alternative approach to vasectomy has been investigated in detail by Upadhyay et al., and they arrived at the conclusion that oil obtained from the plant *Azadirachta indica* can be effective by single intra-vas administration¹³⁵.

Gupta et al., carried out antifertility studies of the root extract of *Barleria prionitis*¹³⁷ and leaf extract of *Colebrookia oppositifolia*¹⁴⁴ in male albino rats with special reference to testicular cell population dynamics.

It has been reported that the seeds of the *Carica papaya* inhibit motility of sperms when administered orally¹³⁸⁻¹⁴². Pathak et al., and several other researchers directed their research work on the extracts of this plant for male antifertility activity. The chromatographic fraction of the chloroform extract eluted with benzene exhibited maximum activity¹⁴⁰.

The seed extracts of *Celastrus paniculatus* have been shown to possess antispermatogenic action in male albino rats with reversible changes in the liver¹⁴³.

Investigations on the effects of ethanol extract (50%) of *Martynia annua* root on reproduction in male rats revealed that, there is dose related reduction in the testicular sperm count, epididymal sperm count and motility¹⁴⁵.

Antifertility investigation and toxicological studies of petroleum ether extract of the leaves of *Mentha arvensis* in male albino mice has been reported by Sharma and Jacob¹⁴⁶.

Watcho et al., during their investigation for male antifertility agent observed that *Mondia whitei* L. has been associated with reversible antispermatogenic and antifertility activity¹⁴⁷.

Udoh et al., tested the effect of seeds of *Mucuna urens* on the gonads and sex accessory glands of male guinea pigs and found them to be potential male antifertility agents¹⁴⁸.

Similarly, the extracts of *Piper betel* L. have been shown to possess reversible antifertility effect¹⁴⁹.

Parveen et al., have tested the chloroform extract of the bark of *Quassia amara* for its impact on male reproductive system in albino rats¹⁵⁰. A marked decrease in the sperm count, motility, and viability was observed in sperm collected from the cauda epididymis of treated animals when the extract was administered daily for 15 days in the form of intramuscular injections.

It has been reported that methanol extract (70%) of the stem of *Sarcostemma acidum*, arrests spermatogenesis in male rats without noticeable side effects¹⁵¹.

The plants *Solanum lycocarpum*¹⁵² and *Typterygium hypoglaucum*¹⁵³ have been investigated for male antifertility activity.

Chinese researchers investigated antispermatogenic effect of *Trypterygium wilfordii*. They isolated multiglycosides from this plant, which are responsible for antispermatogenic activity. A series of research papers by group of these scientists have been published¹⁵⁴⁻¹⁶⁰.

1.9 Synthetic antifertility agents

Apart from plant extracts, various synthetic compounds also have been tested and proved to have antifertility activity in both female and male. Some of the compounds with promising activity are listed in the following Tables.

1.9.1 Synthetic female antifertility agents

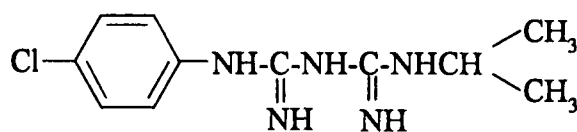
Synthetic antifertility agents interfering with different phases of reproduction have been brought to light, which can be classified under different headings as follows.

1.9.1a Synthetic agents interfering with estrous cycle

The compounds listed in Table-1.6 have been reported to disturb estrous cycle. However, the exact mechanism of their action is not yet established with certainty.

Table-1.6 Agents interfering at estrous cycle and / or reducing fertility

Agent	Species	Reference
Ascorbic acid	human female	161
Alkylendiamine derivatives	house fly	162
Chloroguanide	Rat	163
Dimethylphosphate	Rat	163
Genistein and related compounds	domestic animals	164
Mimosine	Rat	165
Nikoceptin and Galatoceptin	human female	166



Chloroguanide

1.9.1b Synthetic agents interfering with development of ova

Antimitotic agents included in the Table-1.7 are reported to interfere with the development of ova by causing the degeneration of cleaving egg.

Table-1.7 Agents interfering with development of ova

Agent	Species	Reference
6-Azauridine	human female	167
2-Deoxy-D-glucose	rabbit	168
Diethyl stilbestrol and derivatives	rodents	163
Methallibure	rat	169
Staphylococcal α -toxin	rabbit	167

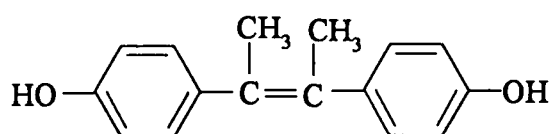
It is well established that oral administration or injection of non-steroidal estrogens after coitus, interfere with the tubal transport of ova in several rodent species. In this process, alterations in the tubal mobility, fluid volume, ciliary movement and some metabolic incompatibility between ova and tubal milieu may be involved. The exhaustive work carried out in this connection has been reviewed systematically by Pincus¹⁶³.

1.9.1c Synthetic agents having antiimplantation activity

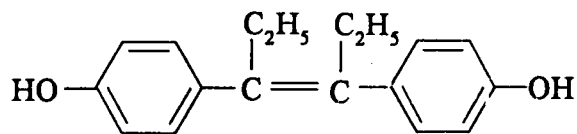
Many synthetic compounds have been reported to inhibit implantation in rats on post coital administration. Some of these compounds are listed in **Table-1.8**.

Table-1.8 Synthetic agents having antiimplantation activity

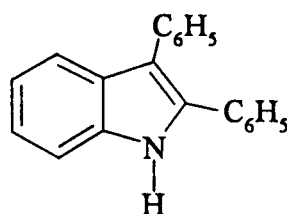
Agent	Species	Reference
Clomiphen, MRL-37	rhesus monkey, rat	170
2,3-Diphenylindenes	rodents	171
1,2-Diphenyl-3,4-dihydronaphthalenes	rodents, rhesus monkey	171
Diethyl stilbestrol, Dimethyl stilbestrol	rat	172
2,3-Diphenylindoles	rat	173
3,4-Diphenyl coumarins	rat	174
3,4-Diphenyl chromanes	rat	175
1,2,3,4-Tetrahydro-2,2-diarylnaphthalene	rat	176
2,3-Diphenylacrylophenones	rat	177
Isoflavone derivatives	rat	173
Non steroidal estrogens	rodents, rhesus monkey, human female	171



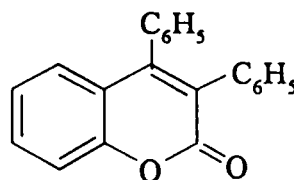
Dimethyl stilbestrol



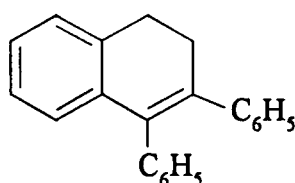
Diethyl stilbestrol



2,3-Diphenyl indole



3,4-Diphenyl coumarine



1,2-Diphenyl-3,4-dihydronaphthalene

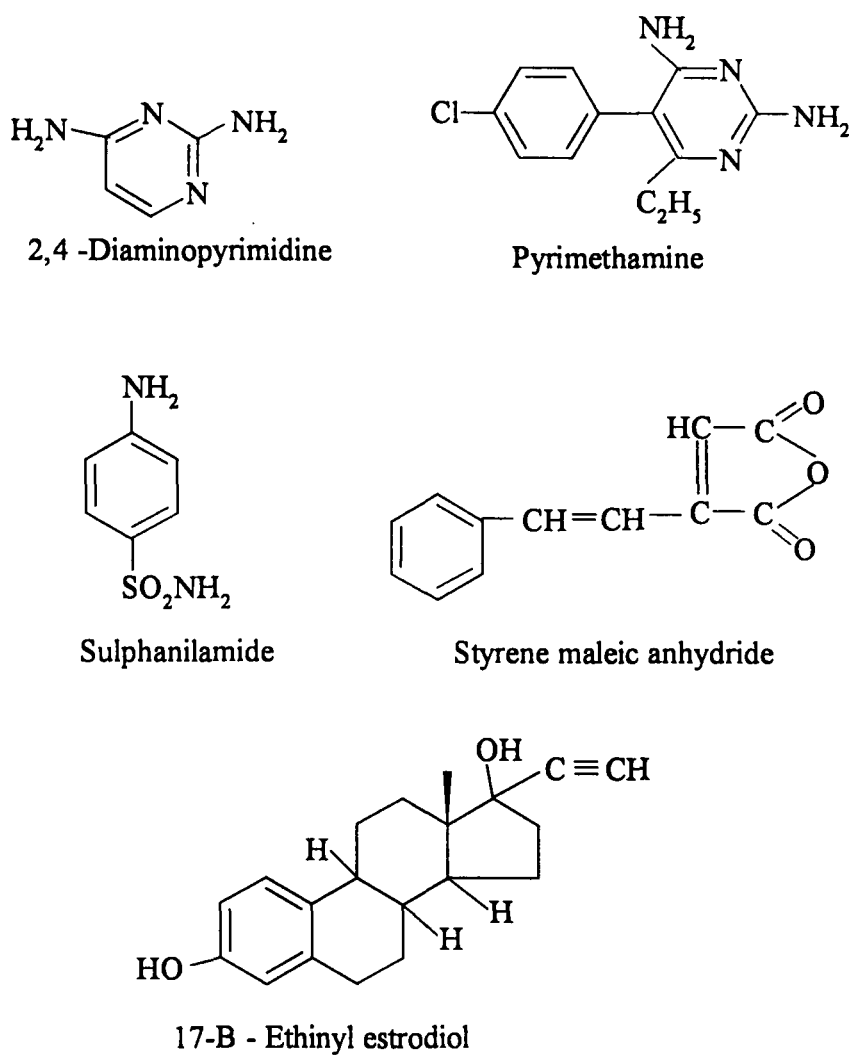
During the last 15 years, extensive research work on animal studies have been carried out. These studies have established that it is possible to prevent pregnancy by variety of non-steroidal compounds. Special attention has been focused on synthesis of potential post coital antifertility compounds with special reference to structure activity relationship studies. Increasing knowledge of the physiological process of fertilization, zygote development and transport and implantation mechanism is providing leads for more rational design for synthesis of new non-steroidal contraceptive agents. These developments would be valuable additions to the existing family planning methods and techniques for birth control.

1.9.2 Synthetic male antifertility agents

The survey of literature reveals that, till today no plant extracts have been found to be authentically useful for male antifertility activity. Hence, researchers have diverted their attention for synthetic compounds with potential male antifertility activity simultaneously with exploration of medicinal plants for such an activity. The synthetic work carried out so far has resulted in the discovery of some new antifertility agents which are listed in the **Table-1.9**.

Table-1.9 Synthetic male antifertility agents

Chemicals	Reference
α -Chlorohydrine-3-Cl-7,2-propanediol	178
6-Cl-6-Deoxyglucose	179
2,4, Diaminopyrimidines	180
Formaldehyde	181
17- β -Ethinyl estradiol and 9,11-tritiated norethindrone	182
Ornidazole	183
4-Methyl-7-hydroxycoumarin, 4-methyl-(tetrahydrobenzo furano)[6,7-b]coumarin	184
2,3,4,4a,5,9b-Hexahydroindeno(1,2-c)pyridine	185
Pyrimethamine	186
Styrene maleic anhydride	187
Sulphapyridine, N-1-Me-sulphamilamide, N-1-diethyl Sulphanilamide	188
Sulphasalazine	189



The list of compounds mentioned in Table-1.6 to Table-1.9, reveal that the compounds possessing antifertility activity both in male and female belong to different classes of compounds having diverse structures. It is also observed that structure activity relationship studies have not been established for many of the synthesized compounds. These facts encouraged several researchers to synthesize and investigate antifertility activity of new non-steroidal compounds belonging to different classes. Variety of

heterocyclic compounds containing oxygen, nitrogen and sulphur have been synthesized and screened for antifertility activity both in males and females. Some of the important class of compounds discovered so far are listed below.

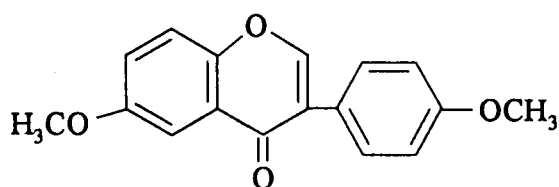
Flavones and flavonoids

Wani¹⁹⁰ et al., and Moersch¹⁹¹ et al., synthesized several flavones, isoflavones, flavonoids and coumarins and evaluated them for antifertility activity and estrogen receptor binding property.

Derivatives of benzopyran

Extensive research work has been carried out by several synthetic organic chemists in connection with the synthesis and evaluation of antifertility activity of benzopyran derivatives.

Ismail et al., have synthesized some novel 4H-benzopyran-4-one derivatives as non-steroidal antiestrogens for their uterotrophic, antiuterotrophic and antiimplantation activities in mature female albino rats¹⁹². Among the products, the 2-(4'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one exhibited highest antiestrogenic activity to the extent of 65 %.



2-(4'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one

Some derivatives of 2,3-diphenyl-4H-oxofuro[3,2-c]benzopyran have been synthesized and evaluated as possible antifertility agents¹⁹³.

Sharada et al., have synthesized 2-aryl-3-methyl-7-phenyl-5H-furo[3,2-g][1]benzopyran-5-ones for their antifertility activity. However, none of these compounds were found to exhibit antifertility activity¹⁹⁴.

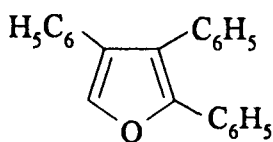
Synthesis of some 9,10-diphenyl-5-oxo-5H-benzofuro[6,5-c]benzopyran have been reported by Rao et al. Considerable anti-implantation activity was observed with some of the newly synthesized compounds¹⁹⁵.

Rao and Somayajulu have synthesized several benzopyran derivatives and noticed that many synthesized compounds possessed significant anti-implantation activity in rats^{196,197}.

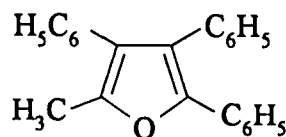
Derivatives of furans, benzofurans and naphthofurans

Several furan derivatives have been synthesized and when evaluated for in vitro for binding to the estrogen receptor and in vivo for uterotrophic and antifertility activities revealed that, 4',17-dioxo-5'H-estra-1(10),4-dieno[3,2-b]furan was capable of inhibiting (3H)E2 binding by 16%, while still eliciting high uterotrophic and post coital antiimplantation activities relative to estradiol¹⁹⁸.

Substituted furan compounds such as 2,3,4-triphenylfurans and 2,3,4-triphenyl-5-methylfurans have been synthesized and evaluated their antifertility effect on albino rats¹⁹⁹.



2,3,4-Triphenylfurans



2,3,4-Triphenyl-5-methylfurans

Kar et al., synthesized 2-phenyl-3-diethylpyrolidinoethoxy-6-methoxy benzofuran hydrochloride and investigated its biological activities. These investigations revealed that, *this compound has got antifertility activity when administered orally*²⁰⁰.

Several 2,3-diphenylbenzo and 5,6-polymethylbenzofurans, 1,2-diphenyl naphthofurans and many other related compounds have been shown to exert antifertility action²⁰¹.

Antiuterotrophic activity of some derivatives of benzofurans and naphthofurans has been reported in literature²⁰². In this connection, Saksena et al., investigated the pharmacological properties of some benzofurans with special reference to their anti-inflammatory and antifertility activities²⁰³.

Tiwari and co-workers carried out an extensive research work on synthesis of antifertility agents. In this connection, they reported the synthesis of diphenylnaphtho [1,2-b] and diphenylnaphtho[2,1-b]furans²⁰⁴.

Derivatives of pyridine

Cook et al., have synthesized analogs of (4aRS, 5SR, 9bRS)-2-ethyl-2,3,4,4a,5,9b-hexahydro-7-methyl-5-p-tolyl-1H-indeno[1,2-c]pyridyne and tested in mice for male antifertility activity. They studied this activity with special reference to the ability of the compounds to reduce testes weight and disrupt spermatogenesis. Some of the tested compounds proved to be effective antifertility agents²⁰⁵.

Derivatives of pyridazine

In continuation of the research work for the discovery of potential antiestrogens, Ismail et al., have synthesized few pyridazine derivatives and screened them for antifertility activity²⁰⁶.

Derivatives of quinolines and isoquinolines

Several derivatives of quinoline and isoquinoline have been reported to possess antifertility activity both in male and female. A new series of non steroidal female antifertility agents such as, 1-phenyl-2-phenethyl-1,2,3,4-tetrahydroisoquinolines have been shown to exhibit antifertility activity by Paul et al²⁰⁷.

Sangwan and et al., synthesized several 3-Aryl-4,5-dihydro-2-substituted-5-tosyl-2H-pyrazolo[4,3-c]quinolines and evaluated them for their CNS, CVS and antifertility activity. However, none of the compounds were found to possess considerable antifertility activity²⁰⁸.

Basic ethers of 1-(4-hydroxyphenyl)-2-phenyl-1,2,3,4-tetrahydroquinoline have been shown to exhibit antifertility activity²⁰⁹.

Kulkarni et al., took up a major research programme devoted towards the synthesis of several nitrogen heterocycles having potential antifertility activity. In this regard, they have synthesized 10,12-substituted [1,4]-benzoxazino [3,4]-quinazolin-8-ones, 2-(N,N-substituted-aminomethyl)-3-(2-pyridyl)-4(3H)-oxo-3,1-quinazolines and several derivatives of quinazolin-4-one and screened them for antifertility and anthelmintic activities. The work carried out by these scientists has resulted in several research publications²¹⁰⁻²¹².

Kelkar et al., have synthesized some 2-acetyl-3-aryl-5-tosyl-7/8-H(or methoxy)-3,3a,4,5-tetrahydropyrazolo[4,3-c]quinolines and tested them for their antifertility activity. In preliminary screening, two of the synthesized compounds have been found to prevent pregnancy at 20 mg/kg dose in female albino rats²¹³.

4-Nitroisoquinoline synthesized by Chaudhary has been shown to exhibit considerable antifertility activity²¹⁴.

Chloroquin, which is generally known as antimalarial drug, has also been shown to exhibit antifertility activity in male rats²¹⁵.

Winters et al., have tested the pregnancy terminating activity of pyrazolo[1,5-a]indoles and quinolines. The new pyrazoles were found to be more active for abortifacient activity²¹⁶.

Derivatives of benzopyranopyridazines

It is well known fact that when two or more heterocycles are connected to each other, the resulting compounds will be having enhanced pharmacological and biological activities than the individual heterocycles. Hence, Hajela et al., have synthesized some substituted benzopyrano pyridazines and pyridazines for their antifertility activity²¹⁷.

The above facts clearly reveal that compounds possessing both male and female antifertility activity could be obtained from various medicinal plants as well as by the way of synthesis in the laboratory.

The worldwide use of indigenous plant products, to regulate fertility, may indeed prove a rich source of information on a variety of possible new approaches to contraception. Research in traditional medicines and ethnopharmacology, all over the world, has received more attention from the Governments of various countries as well as the World Health Organization. It is widely acknowledged that India has tremendous wealth of medicinal plants. Hence, investigation of medicinal plants for various biological and pharmacological activities and their phytochemical studies is a very fruitful field for further research work.

Kuvempu University, which is situated in the heart of Malnad region, has enormous source of medicinal plants. More than 50% of the flora in this area is useful to mankind for the treatment of diseases starting from simple cold and cough to very complicated diseases like cancer and AIDS.

Survey of literature revealed that not much work has been carried out on these medicinal plants, as regards to their phytochemical investigation and pharmacological studies, available around Kuvempu University area. Many Ayurvedic pandits and Unani hakims of this region have been using some of the medicinal plants for fertility control both for male and female. Moreover there are plenty of unwritten evidences of usage of these medicinal plants in folklore.

These facts encouraged us to enumerate medicinal plants of this area, which are useful as antifertility agents. The same has been presented in **Chapter-2**.

Several heterocyclic compounds containing furan, benzofuran, naphthofuran, quinoline and isoquinoline moieties in them have been found to possess various pharmacological properties including antifertility activity, as discussed earlier. Many naphthofuran compounds possessing nitrogen heterocycle either in condensed form or in coupled form have been synthesized by well-established procedures in our laboratory. Many of these compounds synthesized have been found to possess wide spectrum of biological and pharmacological activities. Several research papers have been published, describing the synthesis of naphthofuran derivatives and their antimicrobial, anthelmintic, analgesic, antiinflammatory, diuretic and antifertility activities²¹⁸⁻²²⁵.

From the above discussion it is very clear that the compounds exhibiting antifertility activity possess diversified structures belonging to different classes including heterocyclic compounds. Some of the heterocyclic compounds encompass furan, benzofuran, naphthofuran and pyrimidine ring systems in their structures. This provides extensive field of research work in the direction of synthesis of many heterocyclic systems either condensed or coupled with each other. Encouraged by the wide scope of synthetic research work in this area, it was contemplated to synthesize condensed heterocyclic systems enclosing both naphthofuran and pyrimidine rings and evaluate them for various pharmacological activities and antifertility activity.

For a systematic presentation, the work now carried out is presented on the following lines.

- ◆ Enumeration of medicinal plants for antifertility activity in and around Shimoga and Chitradurga.
- ◆ Extraction and phytochemical investigation of the plant *Balanites Roxburghii*.
- ◆ Analysis of soil.
- ◆ Investigation of antifertility activity of the crude extracts in female albino rats.
- ◆ Investigation of the crude extracts for antifertility activity in male albino rats.
- ◆ Investigation of the crude extracts for other biological activities.
- ◆ Separation, isolation and identification of compounds in crude extracts.
- ◆ Antifertility activity of pure fraction (both in female and male).
- ◆ Synthesis and evaluation of various pharmacological activities of some naphtho[2,1-b]furo[3,2-d]pyrimidines derivatives.

REFERENCES

1. WHO, Global Health Situation and Projections, (1992).
2. People, 14, 25 (1987).
3. United Nations, Demographic Indication of Countries as assessed in 1980, Dept. of Int. Economic and Soc. Affairs, New York, (1982).
4. Spot light, In "Centre Calling", Ministry of Health and Family Welfare, Government of India Publication, New Delhi, 22, 3 (1987).
5. Government of India, Annual Report, 1983-84, Ministry of Health and Family Welfare, New Delhi, (1984).
6. Registrar General and Census Commission of India "Census of India 1991", Provisional Population Totals, Papers 1 & 2 of 1991.
7. K. Park, Preventive and Social Medicine, 14th ed., M/s. Banarasidas Bhanot Publication, Jabalpur, India, 282 (1995).
8. Gerturd Sigurdson, Delegation of Sweden to the International Conference on Population, (1984).
9. Editorial, In "Centre Calling", Ministry of Health and Family Welfare, Government of India Publication, New Delhi, 22, 2 (1987).
10. M.F. Fathalla, In "Research on the Regulation of Human Fertility" (ed. by E. Diczfalusy and A. Diczfalusy), 76 (1983).
11. J.D. Sherris, Barrier Methods, Population Report Series, H:6 (1982).
12. D.F. Hawkins and M.G. Elder, Human Fertility Control: Theory and Practice, Butter Worth, London (1979).
13. V. Padubidri and S.N. Daftary, 'Shaw's Text Book. of Gynaecology', 11th ed, B.I. Churchwell Livingstones, New Delhi, 226 (1995).
14. J.E. Hutching, International Family Planning Perspectives, 11 (1985).
15. V. Wynn, *Lancet*, I: 1045 (1979).
16. R.N. Chopra, I.C. Chopra, K.L. Handa and L.D. Kapur, 'Chopra's Indigenous Drugs of India', U.N. Dhur and Sons Pvt. Ltd., Calcutta, 49 (1958).

17. K.M. Nadkarni and A.K. Nadkarni, 'Indian Materia Medica', Vol. I, Popular Prakashan Pvt. Ltd., Bombay (1976).
18. G.V. Satyavati, M.K. Raina and M. Sharma, 'Medicinal Plants of India', Vol. I, Indian Council of Medical Research, New Delhi, 1976.
19. G.V. Satyavati, A.K. Gupta and N. Tandon, 'Medicinal Plants of India', Vol. II, Indian Council of Medical Research, New Delhi, 1987.
20. V.P. Kamboj and B.N. Dhawan, 'Fertility Regulating Plants', ICMR, Abstract of Young Scientists Symposium, New Delhi, 22 (1994).
21. R. Ericson, Control of Male Fertility, Harper and Row, Hagerstown, 117 (1974).
22. R.R. Chaudhury and M. Haq, *M. Bull. Medico. Ethno. Bot. Res.*, **1**, 408 (1980a).
23. R.R. Chaudhury and M. Haq, *M. Bull. Medico. Ethno. Bot. Res.*, **1**, 420 (1980b).
24. V.P. Kamboj and B.N. Dhawan, *J. Ethnopharmacol.*, **6**, 191 (1982).
25. V.P. Kamboj, *Indian J. Med. Res.*, **87**, 336 (1988).
26. G.V. Satyavati, *Ancient. Sci. Life*, **3**, 193 (1984).
27. S.K. Bhargava, *Fitoterapia*, **59(3)**, 163 (1988).
28. S.B. Vohra and M.S.Y. Khan, *Indian J. Pharm.*, **36**, 77 (1974).
29. M.L. Gupta, T.K. Gupta and K.P. Bhargava, *J. Res. Indian Med.*, **6(2)**, 112 (1971).
30. J. East, *J. Endocrinol.*, **12**, 267 (1955).
31. M. Kapoor, S.K. Garg and V.S. Mathur, *Indian J. Med. Res.*, **62**, 1225 (1974).
32. C.S. Barnes, J.R. Price and R.L. Hughes, *Loydia*, **38(2)**, 135 (1975).
33. R.P. Kohli, N. Singh and R.K. Srivastava, *J. Res. Indian Med.*, **6**, 125 (1971).
34. A.R. Chaudhury, S.K. Saksena and S.K. Garg, *J. Reprod. Fertil.*, **22**, 151 (1970).
35. S.B. Vohra, M.S.Y. Khan and S.H. Afaq, *Indian J. Pharm.*, **35(3)**, 100 (1973).
36. P.V. Tewari, H.C. Mapa and C. Chaturvedi, *J. Res. Indian Med. Yoga & Homoeop.*, **11(4)**, 7 (1976).

37. J.D. Dhar, B.S. Setty, V. Laxmi and D.S. Bhakuni, *Indian J. Med. Res.*, **96**, 150 (1992).
38. D.A. Hegde, R.L. Khosa, J.P.N. Chansouria and M. Sahai, *Indian J. Nat. Products*, **9**, 15 (1993).
39. K. Sareen, N. Mishra, D.R. Verma, M.K.P. Amma and M.L. Gujral, *Indian J. Physiol. Pharmacol.*, **5**, 125 (1961).
40. S.K. Garg and G.P. Garg, *Indian J. Med. Res.*, **59**, 302 (1970).
41. N. Chandoke, *Indian J. Exp. Biol.*, **17**, 740 (1979).
42. U. Khanna and R.R. Chowdhary, *Indian J. Med. Res.*, **56**, 1575 (1968).
43. G. Keshri, M.M. Singh, V. Laxmi, B.N. Mehrotra and D.N. Gupta, *Indian J. Med. Res.*, **87**, 377 (1988).
44. J.V. Kamath and A.C. Rana, *Fitoterapia*, **73(2)**, 111 (2002).
45. G. Keshri, M.M. Singh, V. Laxmi, D.N. Gupta and V.P. Kamboj, *Indian Drugs*, **30**, 453 (1993).
46. S. Singh, A.K. Sanyal, S.K. Bhattacharya and V.B. Pandey, *Indian J. Med. Res.*, **60**, 287 (1973).
47. S.K. Garg, *Planta Med. Res.*, **64**, 1133 (1976).
48. V. Bhatnagar, *Indian J. Physiol. Allied Sci.*, **49**, 179 (1995).
49. M. Indira, M. Sirsi, S. Radomir and S. Dev., *J. Sci. Industr. Res.*, **15C**, 202 (1956).
50. M. Gandhi, R. Lal, A. Shankaranarayana and V.S. Mathur, *Indian J. Pharmacol.*, **15**, 42 (1983).
51. N. Chandoke, *Indian J. Exp. Biol.*, **16**, 419 (1978).
52. N.R. Pillai, M. Alam and K.K. Purushothaman, *Bull. Med. Ethno. Bot. Res.*, **2**, 285 (1981).
53. R.C. De Pasquale, S. Ragusa, C. Circosta and A.M. Foresteieri, *J. Ethno. Pharmacol.*, **12**, 293 (1984).

54. K. Rathinam, G. Shanthakumari and N. Ramaiah, *J. Res. Indian Med. Yoga Homoeop.*, **11**, 84 (1976).
55. N.K. Datta, M.Y. Mhasalkar and V.R. Fernando, *Indo Soviet Symp. Chem. Nat. Prod. Including Pharma.*, 146 (1970).
56. A. Saraf and N. Gomao, *Qualit. Plant. Met. Veg.*, **20**, 271 (1971).
57. Anita Pakrashi, K. Bhattacharya, Syed N. Kabir and Alok K. Pal, *Contraception*, **34**, 523 (1986).
58. A.J. Saluja and D.D. Santani, *Indian J. Pharmacol.*, **45**, 97 (1983).
59. S.K. Dhar, *Indian J. Physiol. Pharmacol.*, **39**, 63 (1995).
60. J.D. Dhar, B.S. Setty, K. Singh, S. Jain and D.S. Bakuni, *Biological Memoirs*, **19**, 95 (1993).
61. S.K. Dhar, M.C. Nigam, K.K. Anand and V. Kaul, *Indian Drugs*, **27**, 551 (1990).
62. S.K. Batta and G. Shanthakumari, *Indian J. Med. Res.*, **59**, 777 (1971).
63. S. Kasinathan, S. Ramakrishnan and S.L. Basu, *Indian J. Exp. Biol.*, **10(1)**, 23 (1972).
64. S.K. Dhar and P.G. Rao, *Fitoterapia*, **66**, 442 (1995).
65. D.N. Gupta, V. Laxmi, B.N. Behrotra, R.S. Kapil and V.P. Kamboj, *Indian Drugs*, **27**, 372 (1990).
66. S. Shukla, *Inter J. Pharmacog.*, **53**, 324 (1995).
67. H. Shivalingappa, N.D. Satyanarayan, and M.G. Purohit, *J Ethnopharmacol.*, **74(3)**, 245 (2001).
68. B.B. Sharma, M.D. Varshnay, D.N. Gupta and A.O. Prakash, *Int. J. Crude Drug Res.*, **21**, 183 (1983).
69. S.D. Feurt and L.E. Fox. *Science*, **121**, 42 (1955).
70. S.P. Hiremath, H.K.S. Swamy, S.M. Badami, R.L. Londokar and S.B. Patil, *Int. J. Pharmacog.*, **34**, 48 (1996).
71. S.P. Hiremath, S. Hanumanth Rao, P.K. Jain, Y. Jaya and K. Sembulingam, *Indian J. Physiol. Pharmacol.*, **34**, 23 (1990).

72. S. Hanumantha Rao, Ph.D. Thesis, Gulbarga University, Gulbarga (1988).
73. S.P. Hiremath and S. Hanumanth Rao, *Contraception*, **42**, 467 (1990).
74. R.S.R. Murthy, D.K. Basu and V.V.S. Murthy, *Indian J. Pharmacol.*, **13**, 86 (1981).
75. U. Khanna, S.K. Garg, S.B. Vohra, H.B. Walia, and R.R. Choudhury, *Indian J. Med. Res.*, **57(2)**, 237 (1969).
76. O.P. Gupta, M.L. Sharma, B.J. Ray, Ghatak and C.K. Atal, *Indian J. Med. Res.*, **66**, 865 (1977).
77. R. Lal and P.L. Sharma, *Indian J. Med. Res.*, **73**, 641 (1981).
78. A. Pakrashi and B. Chakrabarthy, *Contraception*, **23**, 315 (1981).
79. A. Pakrashi and C. Shaha, *Experientia*, **34**, 1192 (1978).
80. M. Sharma, S. Shukla, G. Mishra and S.S. Mishra, *Indian J. Med. Res.*, **10**, 104 (1975).
81. S. Badami, R. Aneesh, S. Sankar, M.N. Sathishkumar, B. Suresh, and S. Rajan, *J. Ethnopharmacol.*, **84(1)**, 99 (2003).
82. R.S. Xu, Z.P. Gu, C.R. Wang and Y.S. Gao, *Chinese Pharmaceutical Bulletin*, **22**, 321 (1987a).
83. R.S. Xy, G.W. Qin, D.Y. Zhu, Z.Y. Fan, F.X. Jiang, B.X. Zhang, J.C. Wang and Y.L. Wang, *Acta. Chimica Sinica.*, **45**, 301 (1987b).
84. J.L. Yuan, Z.Z. Lu, J.P. Shi, W.P. Ding, B.N. Zhou, Y.S. Gao, C.A.J. Erdelmeirer, M.O. Hambarger, G.A. Cordell and H.H.S. Fong, Studies on the antifertility compounds from *Marsdenia koi*, Eds. Y.T. Gong, J.X. Qu and L.K. Wang, *International Symposium on Advances in Fertility Regulation Research (Abstract)*, *Shinghai, China*, 88 (1989).
85. S.S. Subramanian and P.D. Sethi, *Curr. Sci.*, **40**, 85 (1971).
86. M.B. Mulchandani, S.S. Iyer and L.P. Badheka, *Planta Med.*, **37**, 268 (1979).
87. S.N. Sanyal, *Calcutta Med. J.*, **48**, 399 (1951).
88. B.N. Zhou, B.P. Ying, G.Q. Song, Z.K. Chen, J. Han and Y.F. Fan, *Planta Med.*, **47**, 35 (1983).

89. W.C. Wang, *Reproduction and Contraception*, **9**, 34 (1989).
90. S.K. Dixit and G.K. Bhatt, *J. Res. Indian Med.*, **10**, 77 (1975).
91. M. Sabir and M.K. Razdan, *Indian J. Physiol. Pharmacol.*, **14(3)**, 10 (1970).
92. S. Dhanasekaran, B. Suresh, M. Sethuraman, S. Rajan and R. Dubey, *Indian J. Exp. Biol.*, **31**, 384 (1993).
93. M.S. Zoha, A.H. Hussain and S.A. Choudhury, *Bangladesh Med. Res. Counc. Bull.*, **15(1)**, 34 (1989).
94. A. Mishra, J.V. Dogra, J.N. Singh and O.P. Jha, *Planta Med.*, **35(3)**, 283 (1979).
95. B. Chakrabarthy, A. Choudhuri and P.R. Choudhury, *J. Indian Med. Ass.*, **51**, 227 (1968).
96. S.K. Bhargav, V.P. Dixit and P. Khanna, *Fitoterapia*, **55**, 302 (1984).
97. R. Yadav and G.C. Jain, *Adv. Contracept.*, **15(4)**, 293 (1999).
98. R. Mathur and S. Chaudan, *Acta. Eur. Fertil.*, **16(3)**, 203 (1985).
99. J.D. Dhar, V. Laxmi and B.S. Setty, *Indian Drugs*, **33**, 152 (1996).
100. M.L. Dhar, M.M. Dharm, B.N. Dhawan, B.N. Mehrotra and C. Roy. *Indian J. Exp. Biol.*, **6**, 232 (1968).
101. T. Benie, A. el Izzi, C. Tahiri, J. Duwal and M.L. Thieulant, *Contraception*, **41(4)**, 441 (1990).
102. M.S. Al-Said, K.I. Al-Khamis, M.W. Islam, N.S. Parmar, M. Tariq and A.M. Ageel, *J. Ethnopharmacol.*, **21(2)**, 165 (1987).
103. W.S. Woo, E.B. Lee, S.S. Kang, K.H. Shin and H.J. Chi, *Planta Med.*, **53(5)**, 399 (1987).
104. H. Kaur, R. Sandhu and S.S. Dhillon, *Cytobios.*, **69(277)**, 75 (1992).
105. K.S. Sharma, S. Mishra and B.K. Mehta, *Indian J. Med. Sci.*, **42(2)**, 23 (1988).
106. A.O. Prakash and S. Pathak, *Int. J. Pharmacog.*, **32**, 362 (1994).
107. S.C. Chou, S. Ramanathan, A. Matsui, J. Rogus and W.C. Cutting, *Indian J. Exp. Biol.*, **9(4)**, 503 (1971).

108. S.D. Kholkute and K.N. Udupa, *Planta Med.*, **29**, 321 (1976).
109. A.O. Prakash, B. Sisodia and R. Mathur, *Fitoterapia*, **65**, 248 (1994).
110. B.B. Gaitonde and R.T. Mahajan, *Indian J. Med. Res.*, **72**, 597 (1980).
111. V.P. Dixit, H.C. Jain, O.P. Verma and A.N. Sharma, *Indian J. Exp. Biol.*, **15**, 555 (1977).
112. M.L. Gujral, D.R. Verma, K.N. Sareen and A.K. Roy, *Indian J. Med. Res.*, **48**, 52 (1960).
113. D.R. Verma, K.N. Sareen, A.K. Roy and M.L. Gujral, *Indian J. Physiol. Pharmacol.*, **3**, 246 (1959).
114. Y.E. Zhang, J.L. Ruan and W.P. Ding, *Yao. Xue. Xue. Bao.*, **29**, 281 (1994).
115. S.K. Saksena, *Indian J. Physiol. Pharmacol.*, **15**(2), 79 (1971).
116. N.G. Wang, M.Z. Guan and H.P. Lei, *Acta Pharmaceutica, Sinica.*, **25**, 85 (1990).
117. U.K. Majumdar, M. Gupta, G. Pramanik., B.K. Mukhopadhyay and S. Sarkar, *Indian J. Exp. Biol.*, **30**, 533 (1992).
118. J.P. Tewari, K.C. Datta and S.S. Mishra, *Labdev*, **2**, 117 (1964).
119. M.N. Mats, M.I. Kiselyova, L.L. Strigina, M.M. Anisinor and Nik, *Rastitelnye Resurs.*, **25**, 583 (1989).
120. S.K. Bhatta and R.R. Chaudhury, *J. Reprod. Fertil.*, **14**, 534 (1967).
121. M.H. Al-Hamood, A. Elbetieha, A. Alkofahi and H. Bataineh, *J. Ethnopharmacol.*, **61**(1), 67 (1998).
122. X.Y. Dong, M. Chen, W. Jin, D.X. Huang, S.M. Shen and H.T. Li., *Yao Xue Xue Bao.*, **24**(11), 833 (1989).
123. Z.B. Tu, C. Konno, D.D. Soejarto, D.P. Waller, A.S. Bingel, R.J. Molyneux, J.A. Edgar, G.A. Cordell and H.H. Fong, *J. Pharm. Sci.*, **77**(5), 461 (1988).
124. S.D. Kolkute, S.R. Munshi, S.D. Naik and V.S. Jathar, *Indian J. Exp. Biol.*, **16**, 696 (1978).

125. J. Olarto, M.V. Mora, D. Yalencia, M. Guaqueta, M. Orijuella, M. Moya, C. Gonjalex and I.C. Forero, Abstract of Paper Presented at 24th Meeting of the International Congress of Research on Medicinal Plants, Munich, Section A, 6-10, September, 1976.
126. L. Ho, Y. Xu, H. Xue, W. Wang, Z. Zhou, P. Chen, M. Fan and Yu. Shengzhi, *Biyun*, **2**, 23 (1982).
127. P. Bhaduri, C.R. Ghose, A.N. Bose, B.K. Moza and U.P. Basu, *Indian J. Exp. Biol.*, **6**, 252 (1968).
128. W.E. Meyer, J.A. Coppala and L. Goldman, *J. Pharm. Sci.*, **62**(7), 1199 (1973).
129. Q. Lu, *Zhongguo, Yi Xue Ke Xue Yuan Xue Bao.*, **12**(6), 440 (1990).
130. F.R.C. Almeida, V.S.N. Rao and M.E.O. Metos, *Phytother. Res.*, **6**, 189 (1992).
131. M.V. Rao, *Acta. Eur. Fertil.*, **18**(3), 217 (1987).
132. R. Sinha, *J. Ethnopharmacol.*, **28**(2), 173 (1990).
133. M.A. Akbarsha, B. Manivannan, K.S. Hamid and B. Vijayan, *Indian J. Exp. Biol.*, **28**(5), 421 (1990).
134. R. Mazaro, L. Di Stasi, W. De Grava Kempinas, *Contraception.*, **66**(3), 205 (2002).
135. S.N. Upadhyay, S. Dhawan and G.P. Talwar, *J. Androl.*, **14**, 275 (1993).
136. G. Vanithakumari, S. Manonayagi, S. Padma and T. Malini, *J. Ethnopharmacol.*, **25**(2), 173 (1989).
137. R.S. Gupta, P. Kumar, V.P. Dixit and M.P. Dobhal, *J. Ethnopharmacol.*, **70**(2), 111 (2000).
138. N.K. Lohiya and R.B. Goyal, *Indian J. Exp. Biol.*, **30**(11), 1051 (1992).
139. N.K. Lohiya, R.B. Goyal, D. Jayaprakash, A.S. Ansari and S. Sharma, *Planta Med.*, **60**(5), 400 (1994).
140. P. Udoh and A. Kehinde, *Phytother. Res.*, **31**(1), 25 (1999).
141. N. Pathak, P.K. Mishra, B. Manikannan and N.K. Lohiya, *Phytomedicine*, **7**(4), 325 (2000).

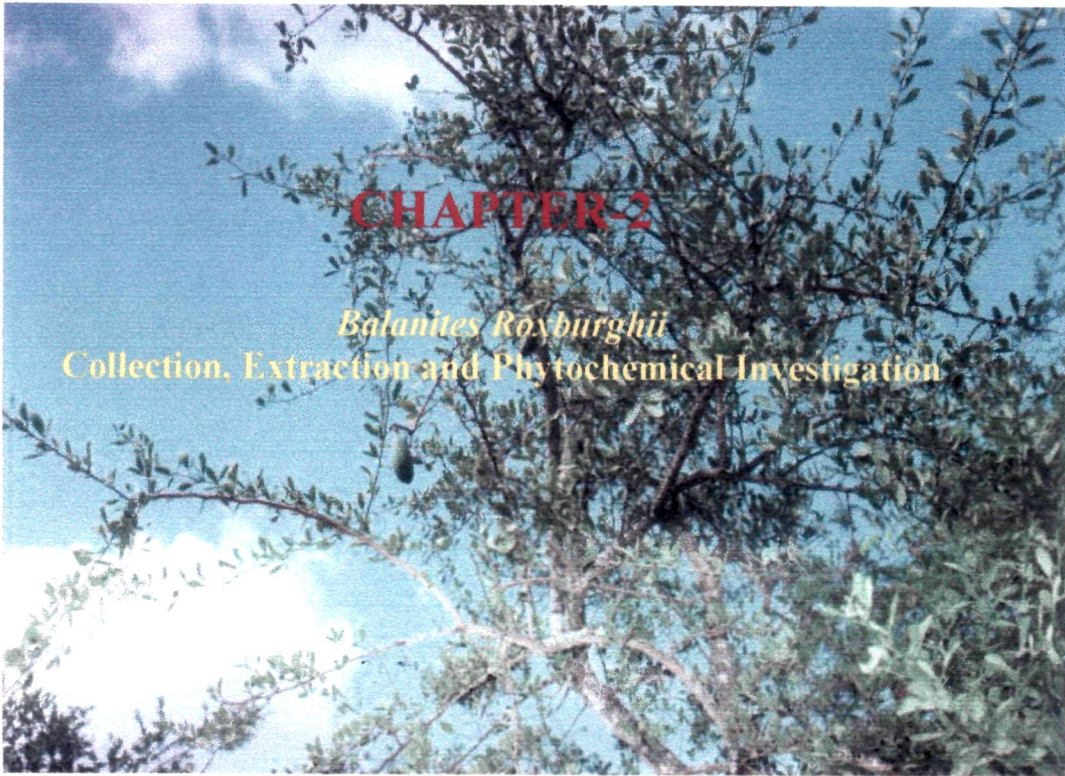
142. N.K. Lohiya, B. Manivannan, P.K. Mishra, N. Pathak, S. Sriram, S.S. Bhande, and S. Panneerdoss, *Asian J Androl.*, **4**(1), 17 (2002).
143. P.P. Bidwai, D. Wangoo and N. Bhullar, *J. Ethnopharmacol.*, **28**(3), 293 (1990).
144. R.S. Gupta, R.K. Yadav, V.P. Dixit and M.P. Dobhal, *Fitoterapia*, **72**(3), 236 (2001).
145. P.C. Mali, A.S. Ansari and M. Chaturvedi, *J Ethnopharmacol.*, **82**(2-3), 61 (2002).
146. N. Sharma and D. Jacob, *J. Ethnopharmacol.*, **75**(1), 5 (2001).
147. P. Watcho, P. Kamtchouing, S. Sokeng, P.F. Moundipa, J. Tantchou, J.L. Essame and N. Koueta, *Phytother. Res.*, **15**(1), 26 (2001).
148. P. Udoh and J. Ekpenyong, *Phytother. Res.*, **15**(2), 99 (2001).
149. M. Sarkar, P. Gangopadhyay, B. Basak, K. Chakrabarty, J. Banerje, P. Adhikary and A. Chatterjee, *Contraception*, **62**(5), 271 (2000).
150. S. Parveen, S. Das, C.P. Kundra, and B.M. Pereira, *Reprod. Toxicol.*, **17**(1), 45 (2003).
151. P.K. Verma, A. Sharma, A. Mathur, P. Sharma, R.S. Gupta, S.C. Joshi, and V.P. Dixit, *Asian J. Androl.*, **4**(1), 43 (2002).
152. R. De Cassia da Sesa, A.A. Vireque, J.E. Reis and M.O. Guerra, *J. Ethnopharmacol.*, **73**(1-2), 283 (2000).
153. S.Z. Qian, Y.Z. Hu, S.M. Wang, Y. Luo, A.S. Tang, S.Y. Shu, J.W. Zhou and T.Y. Rao, *Adv. Contracept.*, **4**(4), 307 (1998).
154. S.Z. Qian, *Contraception*, **36**(3), 335 (1987).
155. W. Ye, *Zhongguo. Yi. Xue. Ke. Xue. Yuam. Xue. Bao.*, **13**(4), 235 (1991).
156. J. Zheng, *Zhongguo. Yi. Xue. Ke. Xue. Yuam. Xue. Bao.*, **13**(6), 398 (1991).
157. S.A. Matlin, A. Belenguer, V.E. Stacey, S.Z. Qian, Y. Xu, J.W. Zhang, J.K. Sanders and S.R. Amor, *Contraception*, **47**(4), 387 (1993).
158. W. Ye, Y. Den, Y. Huang and S. Xue, *Chin. Med. Sci. J.*, **9**(2), 110 (1994).
159. Q.S. Zhen, X. Ye and Z.J. Wei, *Contraception*, **51**(2), 121 (1995).

160. J.P. Bai, and Y.L. Shi, *Contraception.*, **65**(6), 441 (2002).
161. L.I. Ivaniuta, *Pediat. Aksuh. Ginek.*, **1**, 59 (1963)
162. W.A. Skinner, M. Cory, T.E. Shellenberger and J.I. Degraw, *J. Med. Chem.*, **9**, 420 (1966).
163. G. Pincus, In "Control of Fertility", Academic press, New York (1965).
164. J.R. Price, In "Agents Affecting Fertility", Little Brown & Co., Boston, 3 (1965).
165. J.W. Hylin and I.T. Lichton, *Biochem. Pharmac.*, **14**, 1167 (1965).
166. V.T. Kaminskaia. *Zdravookhy, Beloruss.*, **9**, 70 (1963).
167. Raskova, Elis and J. Jiyaseck, *J. Clin, Pharmac. Ther.*, **7**, 162 (1966).
168. C. Lutwak-mann and M.F. Hay, *J. Reprod. Fert.*, **10**, 133 (1965).
169. M.J.K. Harper, *J. Reprod. Fert.*, **7**, 211 (1964).
170. C.W. Emmense, *J. Reprod. Fert.*, **9**, 277 (1965).
171. J.M. Morris, G. Vanwagnen, T. MacCann and D. Jacob. *Fert. Ster.*, **18**, 9 (1967).
172. C.W. Emmense, R.I. Cox and L. Martin, *J. Endocr.*, **18**, 372 (1959).
173. R. Gopalachari and R.N. Iyer, *Indian J. Chem.*, **4**, 331 (1966).
174. D. Lednicer, S.C. Lyster and G.W. Duncan, *Chem. In.*, 1032 (1966).
175. R.W.J. Carney, W.L. Bencze, J. Wojtkennsm and A.A. Renzi, *J. Med. Chem.*, **9**, 516 (1966).
176. W.L. Bencze, L.I. Barsky, R.W.J. Carney, A.A. Renzi and G. Destevens, *J. Med. Chem.*, **10**, 138 (1967).
177. R.N. Iyer, R. Gopalachari, V.P. Kamboj and A.B. Kar, *Indian J. Exp. Biol.*, **5**, 169 (1967).
178. M.S. Saini and V.R. Prasad, *Annals of Appl. Biol.*, **122**(1), 153 (1993).
179. A.R. Jones and M.S. Dobbie, *Contraception*, **44**, 649 (1991).

180. N.S. Malik, S.A. Matlin, J. Fried, R.E. Pakyz and M.T. Consentino, *J. Andrology*, **16**(2), 169 (1995).
181. A. Roy Choudhary, A.K. Gautam, K.G. Patel and H.S.Trivedi, *Indian J. Pysiol Pharmacol.*, **36**, 162 (1992).
182. H. Parnes and E.J. Shelton, *J. Label. Compd. and Radiopharma.*, **29**, 1157 (1991).
183. C.H. Yeung, G. Obserlaender and T.G. Cooper, *J. Reprod. Fert.*, **103**(2), 257 (1991).
184. V.P. Dixit, R.S. Gupta, Sulochana Sharma and B.C. Joshi, *Ann. Biol.*, **6**, 105 (1990).
185. C.E. Cook, M.C. Wani, J.M. Jump, Y.W. Lee, P.A. Fail, S.A. Anderson, Y.Q. Gu and V. Petrow, *J. Med. Chem.*, **38**(5), 753 (1995).
186. C.A. Awoniye, V. Chandrashekar, B.S. Hurst, W.K. Kim and W.D. Schlaff, *J. Andrology*, **14**(3), 174 (1993).
187. N. Sethi, R.R. Srivastava, D. Nath and R.K. Singh, *J. Med. Primatology*, **20**, 89 (1991).
188. C. Pholpramool, S. Ruchirawat, V. Verawatana Pakul, C. Paovallo and L.M. Levin, *J. Reprod. Fert.*, **92**, 169 (1991).
189. F.C.W. Wu, R.J. Aitken and A. Ferguson, *Fertility and Sterility*, **52**, 841 (1989).
190. M.C. Wani, D.H. Rector, H.D. Christensen, G.L. Kimmol and C.E. Cook, *J. Med. Chem.*, **18**(10), 982 (1975).
191. G.W. Moersch, D.F. Morrow and W.A. Neuklis, *J. Med. Chem.*, **10**(2), 154 (1967).
192. Ismail, Khadiga Ahmed, Aziem, and Tarek Abd El, *Eur. J. Med. Chem. Chim. Ther.*, **36**(3), 243 (2001).
193. B. Rajitha, Y. Geetanjali, and V.V. Somayajulu, *Indian J. Chem. Sect.B.*, **25** 872 (1986).
194. J. Sharada, Y. Kumari Ratna, and M. Kanakalingeswara Rao., *Indian J. Chem. Sect.B.*, **25**, 334 (1986).
195. B. Vittal Rao, and V.V. Somayajulu, *J. Indian Chem. Soc.*, **57**(8), 837 (1980).

196. K.V.B. Rao, and R.N. Iyer, *Indian J.Chem. Sect.B.*, **19(11)**, 992 (1980).
197. B. Vittal Rao, and V.V. Somayajulu, *Indian J. Chem. Sect.B.*, **19(3)**, 232 (1980).
198. M.E. Omar A.-Mohsen, M. Aboulwafa Omaina, M. Labouta Ibrahim, A. El-tombary Alaa and El-Mallah I. Ahmed, *Arch. Pharm.(Weinheim Ger.)*, **329(2)**, 61 (1996).
199. D.K. Dikshit, K.L. Munshi, R.S. Kapil, V.P. Kamboj and N. Anand, *Indian J. Chem.*, **12(11)**, 1144 (1974).
200. A.B. Kar, V.P. Kamboj and B.S. Setty, *Indian J. Exp. Biol.*, **5(2)**, 80 (1967).
201. H.P. Chawla, P.K. Grover, N. Anand, V.P. Kamboj and A.B. Kar, *J. Med. Chem.*, **13(1)**, 54 (1970).
202. B.S. Setty, V.P. Kamboj and A.B. Kar, *Indian J. Exp. Biol.*, **8(2)**, 139 (1970).
203. S.K. Saksena, A.V. Radhakrishnan, C.C. Kartha and S.V. Gokhale, *Indian J. Med. Res.*, **59(8)**, 1283 (1971).
204. S.S. Tiwari and S.C. Srivastava, *J. Med. Chem.*, **10(5)**, 983 (1967).
205. C. Edgar Cook, C. Wani Mansukh, M. Joseph Jump, Yue-W Lee, and A. Patricia Fail, *J. Med. Chem.*, **38(5)**, 753 (1995).
206. A. Ismail Khadiga, A. El-Tombary Alaa, M. AboulWafa Omaina, M.E. Omar A-Mohsen, H. El-Rewini Safaa, *Arch.Pharm.(Weinheim Ger.)*, **329 (10)** 433 (1996).
207. R. Paul, J.A. Coppala and E. Cohen, *J. Med. Chem.*, **15(7)**, 720 (1972).
208. K. Naresh Sangwan, M. Prabhakar Kelkar, Shri Nivas Rastogi, and N. Anand, *Indian J. Chem. Sect.B.*, **24**,639 (1985).
209. M.R. Bell, A.W. Zalay, R. Oesterlin, P. Schane and G. O. Potts, *J. Med. Chem.*, **13(4)**, 664 (1970).
210. Y.D. Kulkarni and S.H.R. Abdi, *J. Indian Chem. Soc.*, **61(8)** 721 (1984).
211. Y.D. Kulkarni and S.H.R. Abdi, *J. Indian Chem. Soc.*, **61(8)** 720 (1984).
212. Y.D. Kulkarni, S.H.R. Abdi and B. Kumar, *J. Indian Chem. Soc.*, **60**, 504 (1983).
213. M. Prabhakar Kelkar, K. Naresh Sangwan, Shri Nivas Rastogi and Nitya Anand, *Indian J. Chem. Sect.B.*, **19(4)**, 297 (1980).

214. R.R. Chaudhury, *Indian J. Med. Res.*, **56(11)**, 1720 (1968).
215. A.O. Okanlawon, C.C. Noronha and O.A. Ashiru, *West Afr. J. Med.*, **12(2)**, 118 (1993).
216. Winters Giorgio, Odasso Gianfranco, Conti Marco, Tarzia Giorgio and Galliani, Giulio, *Eur. J. Med. Chem. Chim. Ther.*, **19(3)**, 215 (1984).
217. Kanchan Hajela and R.S. Kapil, *Indian J. Chem. Sect.B.*, **29(7)**, 685 (1990).
218. H.M. Vagdevi and V.P. Vaidya, *Indian J. Heterocycl. Chem.*, **10**, 253 (2001).
219. H.M. Vagdevi, K.P. Latha, V.P. Vaidya, M.L. Vijayakumar and K.S.R. Pai, *Indian J. Pharma. Sci.*, **63(4)**, 286 (2001).
220. K.P. Latha, V.P. Vaidya, J. Keshavayya and H.M. Vagdevi, *J. Indian Council Chem.*, **17(3)**, 31 (2001).
221. K.M. Mahadevan, Basavaraj Padmashali and V.P. Vaidya, *Indian J. Heterocycl. Chem.*, **11**, 15 (2001).
222. K.M. Mahadevan, and V.P. Vaidya, *J. Indian Concil Chem.*, **18(2)**, 78 (2001).
223. K.P. Latha, V.P. Vaidya and J. Keshavayya, *Nat. Acad. Sci. Lett.*, **25**, 586 (2002).
224. H.M. Vagdevi, V.P. Vaidya and K.M. Mahadevan, *Kuvempu Uri. Sci. J.*, **2(1)**, 13 (2002).
225. K.M. Mahadevan and V.P. Vaidya, *Indian J. Pharm. Sci.*, **11**, 15 (2001).



CHAPTER-2

Balanites Roxburghii
Collection, Extraction and Phytochemical Investigation

Kuvempu University area, situated in the heart of Malnad region, comprises of three distinct regions as follows,

1. Malnad region (Chickmagalur district)
2. Semi malnad region (Shimoga district)
3. Maidan region (Chitradurga and Davanagere districts).

It has enormous wealth of flora and more than 50% of this is useful to mankind in the treatment of several diseases. All the types of plants i.e. evergreen, seasonal, grown in dry and irrigated lands, are available in this area, which are of medicinal importance.

Many government and non-government organizations have set up medicinal plant gardens in this area. In these gardens, different types of medicinal plants have been collected and maintained.

The important medicinal plant gardens are listed below.

- a) "Ashwini Aushada Vana" - At Gajanur near Shimoga.
Maintained by Department of Forest, Government of Karnataka.
- b) Medicinal Plant Garden - At Malladihalli (Chitradurga district).
Maintained by private Ayurvedic Hospital at Malladihalli.
- c) Garden of Medicinal Plants - At Agumbe (Shimoga district).
Maintained by Foundation for Revitalization of Local Health Traditions (Government of Karnataka).
- d) "Garden of Aushadi Sasyagalu" - At Koppa (Chickmagalur district).
Maintained by private ayurvedic college.

However, survey of literature revealed that, not much work has been done regarding biological, pharmacological and phytochemical investigations of these plants. Hence, there is a plenty of scope to take up research work in this direction.

Thus, study of these medicinal plants with special reference to their antifertility activity and other pharmacological activities has been initiated in our laboratory. In addition, phytochemical investigation was also undertaken. Such type of work has been done on *Momordica dioica* Roxb. and few papers have already been published¹⁻².

In continuation of this work, it was contemplated to investigate some more plants for antifertility activity. Survey was carried out in all districts coming under Kuvempu University area. Discussions with native Ayurvedic physicians and Unani hakims, who practice age-old Indian System of Medicine (ISM), revealed that the fruits of the plant *Balanites Roxburghii* have been in use as antifertility agents especially in males. Hence it was planned to take up detailed investigation of the fruits of *Balanites Roxburghii* for its antifertility, other biological activities and chemical constituents present in it. This plant is available in Maidan region and semimalnad region. Chitradurga district, which comes under Maidan region, has some protected forest area, in which this plant is available in plenty. Hence, the plant was collected from Jogimatti protected forest area near Chitradurga during fruiting season i.e. in the month of June. The location of the area from which the plant is collected is shown in **Figure-2.1**.

2.1 About the plant *Balanites Roxburghii*

The genus *Balanites* belongs to the family *Simarubaceae*, which has *Ailanthus* and *Samadera* as two other members. The plants of this genus are generally shrubs. There are three species in this genus, which are as follows.

1. *Balanites Roxburghii*
2. *Balanites aegyptica*
3. *Balanites pedicellaris*

Figure-2.1 The location of area from which the plant *Balanites Roxburghii* is collected i.e. Jogimatti protected forest area.

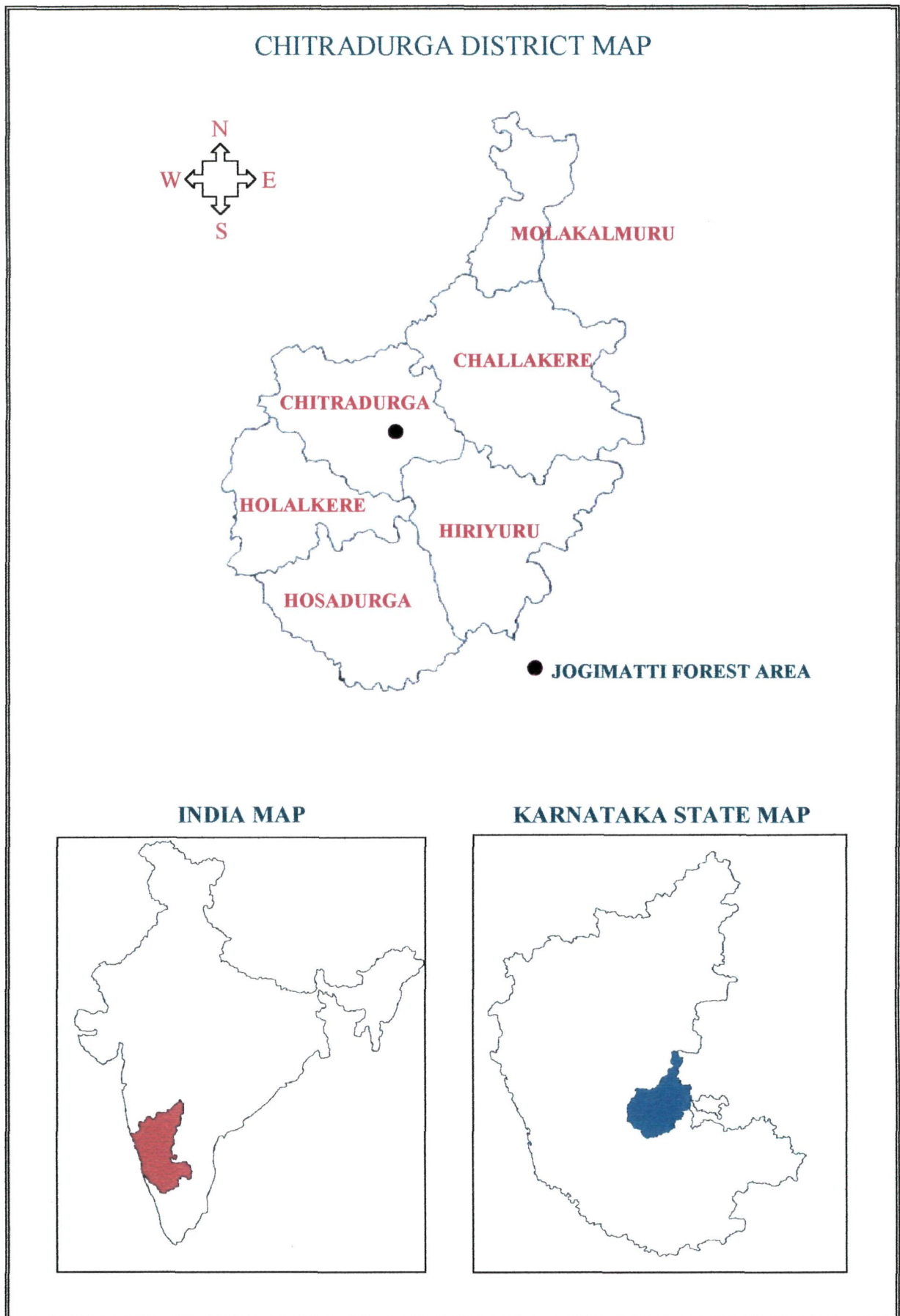


FIGURE – 2.1

Balanites Roxburghii is sometimes placed in a separate family called *Balanitaceae*³. It is popularly known as Hingan or Hingota in Hindi, Hingala in Kannada, Ringri in Telugu, Nanjunda in Tamil, and Ingudam in Sanskrit⁴.

2.1.1 Geographical distribution

Balanites Roxburghii is a small evergreen thorny tree growing in drier parts of India, Arabia, Egypt, Eritria and tropical Africa. In India, it is distributed in Bihar, Punjab, Delhi, Gujarat, Sikkim and in Deccan peninsula. It is frequently available in scrub forests of southern interior Karnataka ie. Bangalore, Belgaum, Chitradurga, Dharwad, Mysore and Tumkur during the months of March-July^{4,5,7}.

2.1.2 Morphology:

The young parts of the plant are pubescent or tomentous or glabrescent. Twigs armed with stout axillary or supra axillary spines, 1-6 cm long, which often bear leaves or flowers.

Leaves : Leaves are alternate, 2-foliate ; petioles 3-6 mm. long ; leaflets elliptic or varying from ovate or obovate pointed, 1.5 cm long ; petiolules up to 5 mm long.

Flowers : Flowers are small, greenish white, fragrant, in axillary few or many-flowered, short peduncled cymes or fascicles. Sepals 5, oblong-obovate, glabrous outside, silky villous or glabrous inside, very little longer than the sepals. Stamens 10 ; filaments subulate, glabrous ; anthers dorsifixed. Disc copular, with a 10-lobed glandular margin. Ovary ovoid, silky, 5-celled ; ovules solitary in each cell ; style short, conical.

Fruit : Fruit is an ovoid drupe, 2.5 - 6 cm long, on a short thick stalk, faintly 5-grooved, pale yellow when ripe ; pulp 5 mm thick with an offensive greasy smell ; stone hard, fibrous. It has an average weight of 10 gm.

Seed exalbuminous ; embryo with thick plano-convex, cornegated or 2-lobed cotyledons and a superior radicle.

The photograph of the plant and fruit are shown in **Figure-2.2** and **Figure-2.3**.

2.1.3 Medicinal uses

The survey of literature revealed that, plant as a whole and many parts of this plant, find applications for the treatment of various ailments.

In Sushruta, this plant is prescribed in the treatment of snakebite. The flowers are sweet smelling, are used to cure “watha and kapha”. The bark, unripe fruit and leaves are used as purgative and are considered to have anthelmintic properties. The fruits are also found to be associated with alexipharmic, analgesic and antidysenteric activities. It is also known to remove watha and cure ulcers. The fruit is also used in the treatment of many skin diseases including leucoderma. There are reports in the literature of fruits being used for rat bites also. The seeds are used in coughs, colics and also used as expectorants^{4,5}.

Dixit et al., have tested fruit pulp extract of this plant in dogs for hyperglycemia induced testicular dysfunction⁶.

The root bark of this plant is used for curing herpes zoster disease⁷.

2.1.4 Phytochemical investigations

Even though considerable work has been done, as for as phytochemical investigation on *Balanites aegyptica*, there are only few reports of such investigations on *Balanites Roxburghii* and *Balanites pedicellaris*. The phytochemical investigations carried out on *Balanites Roxburghii*, have been summarized in the following pages.

Figure-2.2 Photograph of the plant *Balanites Roxburghii*.

Figure-2.3 Photograph showing the fruits of the plant.



FIGURE – 2.2



FIGURE – 2.3

The leaves are found to contain diosgenin, the percentage of which varies depending upon the season⁸. This compound is also reported to be present in the stem bark of this plant⁹.

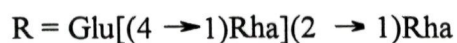
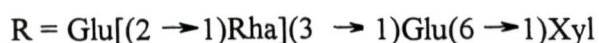
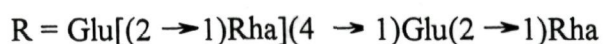
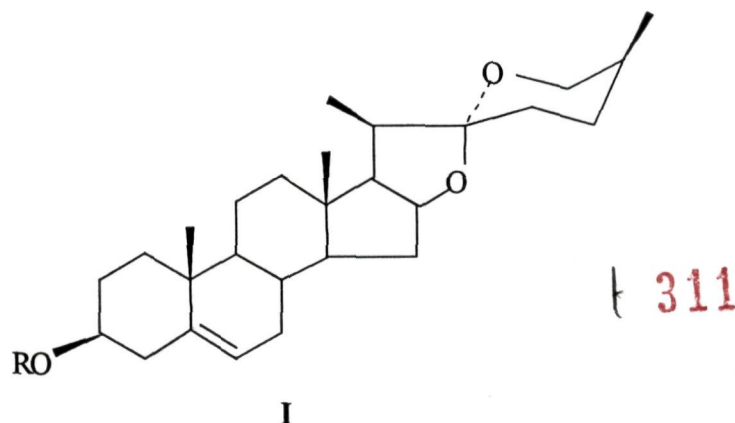
Diosgenin glucoside was isolated from fresh roots of *Balanites Roxburghii* and its structure was established on the basis of NMR and mass spectral data¹⁰.

- Amalraj carried out research on various pigments present in the leaves. The results revealed that level of chlorophyll and carotenoid pigments show similar monthly variations¹¹.

In Gujarat, it is observed that Sambar strips the bark of the trees of different families including Simurubaceae, especially in the summer season. During this season, the percentage of proteins and carbohydrates were found to be high in *Balanites Roxburghii*¹².

Amalraj and Satyanarayana carried out research on fruit development of *Balanites Roxburghii*¹³.

Some new steroidal glycosides (I) have been isolated from different parts of this plant. They have diosgenin as common aglycon whereas sugar moieties consisted of either glucose and rhamnose or glucose, arbinose, xylose and rhamnose in different proportions^{14,15}.



A new saponin with insect antifeedant activity has been isolated from the stem bark and characterized as (25R and S)-spirost-5-en-3 β -ol-3-O-[α -L-rhamnopyranosyl (1,2)]- β -D-glucopyranosyl(1,3)- β -D-glucopyranosyl(1,4)- β -D-glucopyranoside. In addition to this, two other known saponins were identified as deltonin and protodeltonin¹⁶.

Keeping in view of the above facts, it is now contemplated to subject the fruits of *Balanites Roxburghii* for a detailed investigation of its antifertility activity. It is also planned to evaluate other pharmacological activities of the fruits systematically.

It is well known that steroids have severe harmful side effects. Hence, in the present work it is envisaged to isolate non-steroidal active principles of the fruits of this plant.

2.2 Extraction of the fruits of *Balanites Roxburghii*

The fruits of *Balanites Roxburghii* were collected at Jogimatti protected forest area, near Chitradurga, in the month of June (fruiting season). The plant was authenticated by comparing with herbarium (F.D.D. 822) maintained at Department of Biotechnology, Kuvempu University, Shankaraghatta.

The collected fruits were sprayed with ethanol to prevent enzymatic reactions, and shade dried to prevent photochemical reactions. The coarsely powdered material (450 g) was extracted successively with petroleum ether (60⁰-80⁰C, 2 l), chloroform (2 l), and ethanol (2 l) in a soxhlet extractor. After each extraction, the remaining residue was shade dried and then used for next extraction. Each extraction was carried out for 16 hr (approximately 40 cycles). The remaining plant material was extracted by maceration with water at room temperature for 7 days. The petroleum ether, chloroform, and ethanol extracts were concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature. The aqueous extract was concentrated on water bath by slow evaporation at 50⁰-60⁰C. The yield, color and consistency of each extract is given in the **Table-2.1**.

Table-2.1 Yield, Color and Consistency of the extracts.

Extract	Yield	Color and Consistency
Petroleum ether	10 g	Yellow viscous liquid
Chloroform	2 g	Greenish black paste
Ethanol	28 g	Dark brown paste
Aqueous	20 g	Brown solid

All the extracts were preserved in a refrigerator.

2.3 Phytochemical investigation of the crude extracts

Each extract was subjected to phytochemical investigation to study the presence of the following constituents

1. Alkaloids
2. Steroids and Triterpenoids
3. Carbohydrates
4. Flavonoids
5. Phenolic compounds and Tannins
6. Saponin glycosides
7. Proteins and Amino acids
8. Lignans
9. Coumarins
10. Chalcones
11. Aurones
12. Anthraquinones
13. Irridoids
14. Phytosterols
15. Fixed oils and fats
16. Gums and mucilages

Standard procedures^{17,18} were followed to carry out various tests and the results are presented in **Table-2.2**.

Table-2.2 Phytochemical investigation of the crude extracts.

Test extract	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous
1. Alkaloids				
a) Mayer's test	-ve	-ve	-ve	+ve
b) Wagner's test	-ve	-ve	-ve	+ve
c) Hager's test	-ve	-ve	-ve	+ve
d) Dragendorff's test	-ve	-ve	-ve	+ve
2. Steroids and Triterpenoids				
a) Libermann sterol test	+ve	+ve	-ve	-ve
b) Salkowskis test	+ve	+ve	-ve	-ve
3. Carbohydrates				
a) Molisch's test	-ve	-ve	+ve	+ve
b) Reducing sugar test	-ve	-ve	+ve	+ve
4. Flavonoids				
Shinoda test	-ve	-ve	+ve	-ve

5. Phenolic compounds and Tannins				
a) Neutral ferric chloride test	-ve	-ve	+ve	-ve
b) Lead acetate test	-ve	-ve	+ve	-ve
c) Gelatin solution test	-ve	-ve	+ve	-ve
6. Saponin glycosides				
a) Froath test	+ve	+ve	+ve	-ve
b) Haemolytic test	+ve	+ve	+ve	-ve
7. Proteins and Amino acids				
a) Biuret test	-ve	-ve	+ve	+ve
b) Ninhydrin test	-ve	-ve	+ve	+ve
8. Lignans				
Badouin's test	-ve	-ve	-ve	-ve
9. Coumarins				
	-ve	-ve	-ve	-ve
10. Chalcones				
	-ve	-ve	-ve	-ve

11. Aurones	-ve	-ve	-ve	-ve
12. Anthraquinones				
Borntrager's test	-ve	-ve	-ve	-ve
13. Iridoids				
Trim Hill colour test	-ve	-ve	-ve	-ve
14. Phytosterols	+ve	-ve	-ve	-ve
15. Fixed oils and fats	+ve	-ve	-ve	-ve
16. Gums and mucilages	-ve	-ve	-ve	-ve

+ve = Constituent present

-ve = Constituent absent

Table-2.3 Summary of phytochemical investigations

Sl. No.	Extract	Constituent present
1	Petroleum ether extract	Steroids, triterpenoids, saponin glycosides,
2	Chloroform extract	Steroids, triterpenoids, saponin glycosides, phytosterols, fixed oils and fats.
3	Ethanolic extract	Carbohydrates, flavonoids, phenolic compounds and tannins, saponin glycosides, proteins and amino acids.
4	Aqueous extract	Carbohydrates, alkaloids, proteins and amino acids.

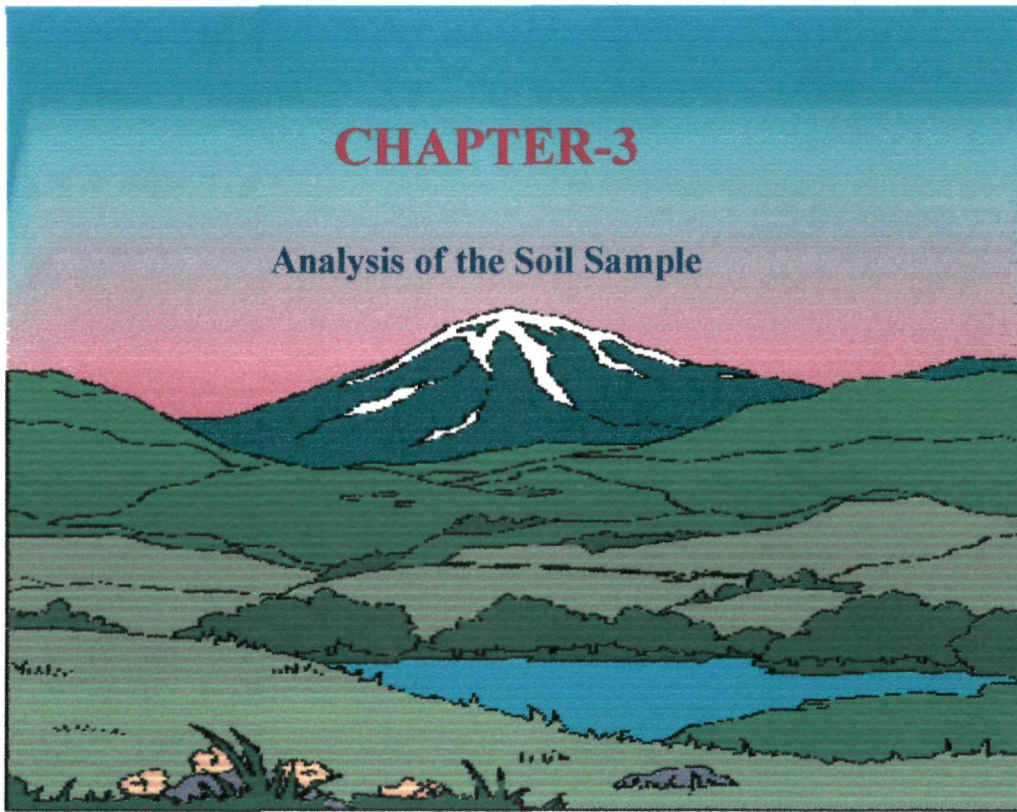
References

1. C.S. Sreedhara, K.S.R. Pai and V.P. Vaidya, *Indian J. Pharma. Sci.*, **63(6)**, 528 (2001).
2. V.P. Vaidya, C.S. Sreedhara and K.S.R. Pai, *Kuvempu University Science Journal.*, **1(1)**, 13 (2001).
3. Basak, "Fasc. Fl. India", **4**, 20 (1980).
4. George Watt, "The Economic Products of India", **1**, 363 (1972).
5. R.N. Chopra, S.L. Nayar and I.C. Chopra, "Glossary of Indian Medicinal Plants", **32**, 1992).
6. V.P. Dixit, S.K. Bhargava and R.A. Gupta, *Indian J. Exp. Biol.*, **9(10)**, 918 (1981).
7. Supriya K. Bhattacharjee, "Handbook of Medicinal Plants", **55**, (1998).
8. R.B. Patel, G.C. Bhavsar, M.R. Patel and H.K. Dhyani, *Indian Drugs.*, **24(1)**, 49 (1986).
9. B. Ganga Rao, S. Ganapathy, T. Satyanarayana and R.V. Krishna Rao, *Indian J. Nat. Prod.*, **9(2)**, 6 (1993).
10. M.S.Y. Khan, A. Anees Siddiqui and Kalim Javed, *Asian J. Chem.*, **9(2)**, 324 (1997).
11. V.A. Amalraj, "Trans. Indian Soc. Desert Technol. Univ. Cent. Desert Stud", **11(2)**, 123 (1986).
12. B.R. Pandit and O.S. Kotiwar, "Indian Forester", **126(8)**, 865 (2000).
13. V.A. Amalraj and K.A. Satyanarayan, "Indian Forester", **121(9)**, 813 (1995).
14. H.O. Rastogi and B.N. Mehrotra, "Compendium of Indian Medicinal Plants", **1**, 54 (1990).
15. H.O. Rastogi and B.N. Mehrotra, "Compendium of Indian Medicinal Plants", **2**, 92 (1991).
16. D.C. Jain, *Phytochemistry.*, **26(8)**, 2223 (1987).

17. G.E. Treace and M.C. Evans, In; "Text Book of Pharmacognosy", 12th Edn., Balliere Tindal, London, 343 (1983).
18. C.K. Kokate, "Experimental Pharmacology", 1st Edn. New Delhi (1985).

CHAPTER-3

Analysis of the Soil Sample



It is well known fact that chemical constituents of the given plant, varies depending upon factors like climatic condition and nature of the soil. The composition of soil plays vital role in the biosynthesis of various chemical components of plants. Hence, number of constituents and their relative percentage present in plant, changes from place to place and season to season. This factor also depends on the age of the plant. Many a times it is observed that there is a large variation in relative percentages of the constituents when collected from different areas.

Therefore, it was contemplated to carry out soil analysis of the soil of the area from which the plant material i.e. fruits of *Balanites Roxburghii* were collected.

The soil analysis was carried out as detailed below:

1. Sample collection
2. Testing of the soil for its constituents
 - a) Determination of pH
 - b) Determination of available carbon
 - c) Determination of available phosphorous
 - d) Determination of potassium content and
 - e) Determination of micronutrients

For these determinations standard procedures available in the literature were adopted¹⁻⁵.

3.1 Sample Collection

Soil samples were collected from different sites, from the place of collection of plant material i.e. at Jogimatti protected forest area near Chitradurga. Totally six samples were collected as described below:

Sample 1 – Collected near the plant 1 of *Balanites Roxburghii* at 1 foot depth surrounding the tuberous roots.

Sample 2 – Collected 3 mt. away from the plant.

Sample 3 – Collected 15 mt. away from the plant.

Sample 4 – Collected near the plant 2 of *Balanites Roxburghii* at 1 foot depth surrounding the tuberous roots.

Sample 5 – Collected near the plant 3 of *Balanites Roxburghii* at 1 foot depth surrounding the tuberous roots.

Sample 6 – Collected near the plant 4 of *Balanites Roxburghii* at 1 foot depth surrounding the tuberous roots.

All the samples were sun dried for 2 days, powdered and mixed in equal quantities to prepare the sample for testing. The mixed sample was heaved and divided into 4 quarters. Opposite quarters were discarded and the remaining quarters were once again mixed and heaved and divided into four quarters. This process was repeated so as to get around 500 gm of the sample for soil analysis.

3.2. Soil Testing

The collected soil sample was tested for the following parameters:

3.2.1 Determination of pH of the soil

pH of the soil is an indicative measure of chemical and biochemical properties of the soil under consideration. It includes ion exchange, retention of ions by colloids, biological activity and nutrient availability in soil. Thus, the soil pH assists in understanding the fertility status of the soil and also it is a parameter used in the classification of the soil.

The pH of the soil was determined by potentiometric method.

Potentiometric Method

The soil sample for the determination of the pH was prepared as follows,

Soil sample (10 g, 2 mm sieved) was mixed with distilled water (20 ml) and the mixture was stirred for half an hour. The two electrodes used in this experiment were glass electrode, which served as indicator electrode and calomel electrode, which served as reference electrode.

The electrodes were immersed in the suspension of the soil and emf of the cell was recorded after stabilization. The pH of the solution was calculated by using the following equation

$$\text{pH} = \frac{\text{emf of the cell (E)}}{2.303 RT/nF} \longrightarrow (1)$$

Where R = Gas constant

T = Temperature

n = Number of electrons

F = Faraday constant

E = Electrical potential of the cell

At 25⁰C, the equation (1) can be written as

$$\text{pH} = \frac{\text{emf of the cell}}{0.0591} \longrightarrow (2)$$

The emf of the cell in this case was observed to be 0.3568 V. Substituting this value in equation (2)

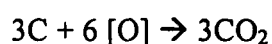
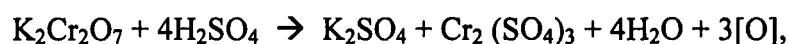
$$\text{pH} = \frac{0.3568}{0.0591} = 5.7$$

Therefore, the pH of the soil under investigation was found to be 5.7. This value indicates that the soil sample is slightly acidic in character.

3.2.2 Determination of organic carbon in soil

Nitrogen is an essential constituent of the plant, which they get from soil. Thus percentage of nitrogen in the soil, plays a significant role as far as fertility is concerned. Generally, carbon and nitrogen exists in a definite ratio of 10:1 in almost all soils. Hence, the determination of organic carbon in the soil, leads to the percentage of nitrogen in it. Generally, colorimetric method is adopted for the determination of organic carbon.

Principle : Organic matter of the soil is oxidized by potassium dichromate solution in presence of dilute sulphuric acid. Potassium dichromate, being oxidizing agent, oxidizes the organic carbon of the soil to carbon dioxide. The corresponding amount of potassium dichromate is reduced to green colored chromium sulphate. The intensity of this color thus depends on the amount of organic carbon present in the soil. Thus, by measuring the amount of chromium sulphate liberated by colorimetric method, the amount of carbon can be determined.



Colorimetric Method

The procedure for the determination of carbon content by colorimetric method involves the following steps.

Preparation of reagents :

- 1). Potassium dichromate solution (1N) was prepared by dissolving potassium dichromate (29.04 g) in distilled water (1000 ml).
- 2). Standard sucrose solution was prepared by dissolving sucrose (0.5 g) in distilled water (1000 ml).

Standard curve:

Standard curve was obtained by oxidizing the carbon content in the sucrose. Accurate volumes of standard sucrose solution i.e., 0, 1, 2, 3, 4, 5 and 6 ml were pipetted out into different conical flasks and the volume was made up to 10 ml by adding corresponding volume of potassium dichromate solution. Then, to each conical flask, concentrated sulphuric acid (20 ml) was added slowly. The resulting solution was allowed to stand for half an hour and distilled water (10 ml) was added and left overnight. The intensities of the colours developed in different conical flasks were measured next day with the help of colorimeter by using red filter at 660 nm. The graph of concentration against absorption was plotted to get the standard curve.

Soil sample (10-15 g) was ground and sieved through 100 mesh sieve. Then, 1 g of this sample was transferred to a conical flask (100 ml). To this, standard potassium dichromate solution (10 ml) and concentrated sulphuric acid (20 ml) were added slowly. After half an hour, this solution was diluted by adding distilled water (10 ml). It was left overnight and next day the intensity of the color was measured by using colorimeter.

With the help of this reading, using standard curve, the percentage of organic carbon present in the soil was calculated with the help of the following equation.

$$\begin{aligned}\text{Percentage of organic carbon} &= \text{Graph reading} \times 0.042 \\ &= 5.952 \times 0.042 \\ &= 0.25\end{aligned}$$

Hence, the percentage of carbon in soil sample was found to be 0.25% and corresponding nitrogen content was calculated as 0.025%. The results indicate that the soil is less fertile with respect to organic carbon.

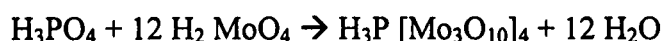
3.2.3 Determination of available phosphorous (kg/acre)

Numerous methods are available for determination of phosphorous in soil samples. However, molybdenum blue method is most sensitive and widely used method for accurate determination of available phosphorous.

Generally, available phosphorous in the soil sample is extracted in the solution form. For this purpose mainly two extractants are commonly used.

1. Olsen extractant
2. Bray's extractant

The proper extractant was selected on the basis of the nature of the soil. Olsen extractant is used for neutral and alkaline soils, whereas Bray's extractant is used for acidic soils. Bray's extractant was used, as the soil under examination was found to be acidic, to get available phosphorous in the solution form. It was estimated by converting the dissolved phosphorous into phosphomolybdate complex. This complex was reduced by using standard solution of ascorbic acid. The reduced compound has blue color, the intensity of which depends upon the concentration of phosphorous present in the solution. The intensity of the color can be measured colorimetrically.



Colorimetric method

The determination of available phosphorous by colorimetric method, involved the following steps.

Bray's extractant : It was prepared by dissolving ammonium chloride (22.2 g) in distilled water (200 ml). This solution was filtered and distilled water (18 l) containing

concentrated hydrochloric acid (40 ml) was added. The resulting solution was further diluted with distilled water to make the volume to 20 l.

Reagent A : Ammonium molybdate (12 gm) was dissolved in distilled water (200 ml). Similarly potassium antimony tartarate (0.2908 gm) was dissolved in distilled water (200 ml). These two solutions were mixed and to the mixture, sulphuric acid (5N, 1000 ml) was added and the solution was made up to 2 litres by using distilled water. The solution was stored in cold and dark place.

Reagent B : It was prepared by dissolving ascorbic acid (1.056 g) in reagent A (200 ml). This solution was prepared just before the experiment.

Stock solution of phosphorous pentoxide : Ammonium dihydrogen orthophosphate (0.479 g) was dissolved in distilled water and the volume was made up to 1000 ml with distilled water. This solution had 250 ppm concentration of P_2O_5 .

Working standard : The stock solution (20 ml) of P_2O_5 was diluted to 1000 ml by using distilled water, to reduce the concentration of P_2O_5 to 5.0 ppm.

Standard curve :

The working standard solution was accurately pipetted out 0, 2, 4, 6 and 10 ml beakers (50 ml). To this solution, Bray's extractant (5 ml) was added and the volume was made to 20 ml by adding appropriate amount of distilled water. To the resulting solution, reagent B (4 ml) was added and kept aside for 15-20 minutes. The intensity of the color was measured using colorimeter. The graph of concentration against absorption was plotted to obtain standard curve.

Soil sample (2.5 g) was taken and Bray's extractant (50 ml) was added, shaken thoroughly and filtered. The above filtrate (5 ml) was taken in a separate conical flask

and to this, sulphuric acid (5N, 0.5 ml) and distilled water (16 ml) were added. To this solution, reagent B (4 ml) was added, stirred well and allowed to stand for 15-20 minutes. The intensity of the color developed was measured colorimetrically. The concentration of available phosphorous present in the soil was determined by using standard curve as follows.

$$\text{Average P}_2\text{O}_5 \text{ kg/acre} = A \times 3.6$$

Where A is graph reading = 8.89

$$\text{Therefore, Average P}_2\text{O}_5 \text{ kgs/acre} = 8.89 \times 3.6 = 32$$

Thus, the amount of available phosphorous in the soil under examination is 32 kg/acre, which indicates that the soil is highly fertile with respect to available phosphorous.

3.2.4 Determination of potash (kg / acre)

Mineral soils generally contain potassium in larger quantities than nitrogen and phosphorous. Most of the potassium is present in the inorganic form as part of the crystalline structure of mica, feldspars and micaceous clay fraction.

The technique of flame emission spectroscopy (FES), formerly called flame photometry, is employed for the determination of potassium present in the soil in terms of available potassium oxide (in kg/acre).

Principle of FES :

In this technique, a solution containing metallic salt is aspirated into the flame. The vapours now contain atoms of the metal. Some of these gaseous metal atoms absorb energy and are raised to higher energy level to permit emission of radiation characteristic

of that metal. The intensity of the radiation can be measured by using photoelectric detectors.

The experiment is carried out in following steps.

Preparation of standard solution : The standard solution of 1000 ppm potassium oxide was prepared by dissolving potassium chloride (1.583 g) in distilled water (1000 ml). From this stock solution, 10, 20, 30 and 40 ppm K₂O solutions were prepared by diluting 10, 20, 30 and 40 ml of stock solution with neutral ammonium acetate solution (1N, 1000 ml). The neutral ammonium acetate solution helps to extract potassium present in the soil sample in solution form.

Procedure : The standard solutions having different concentrations (1.0 ml each) was aspirated into the flame and intensities of the radiation was measured by using flame emission spectrometer. The standard curve was obtained by drawing a graph of concentration (known solution) against intensity of the radiation and the similar procedure was employed for soil sample.

Soil sample (5 g) was ground and neutral ammonium acetate solution (1N, 25 ml) was added and the mixture was shaken thoroughly to get phosphorous present in the soil sample to solution form. Then, the solution was filtered and 0.5 ml of this solution was aspirated into the flame in the instrument. The intensity of radiation was recorded and compared with the standard curve. The amount of potassium present in the soil was determined by using the following equation,

$$\text{Available K}_2\text{O (Kg/acre)} = \frac{A}{10^6} \times \frac{\text{Volume of the extractant}}{\text{Weight of the sample}} \times \frac{2 \times 10^6}{2.2}$$

$$\begin{aligned}\text{Available K}_2\text{O (Kg/acre)} &= \frac{A}{10^6} \times \frac{25}{5} \times \frac{2 \times 10^6}{2.2} \\ &= A \times 4.5\end{aligned}$$

Where A = concentration of K₂O in ppm

$$\begin{aligned}\text{Therefore, Available K}_2\text{O (Kg/acre)} &= 15.56 \times 4.5 \\ &= 70\end{aligned}$$

From this value, it can be concluded that the soil sample under consideration is moderately fertile with respect to available potash.

3.2.5 Determination of micronutrients

Sixteen elements are known to be essential for plants. Out of these, seven are classified as micronutrients, since their requirement is in small quantities. They are zinc, copper, boron, molybdenum, chlorine, manganese and iron. These nutrients are present in the soil in different forms viz.,

1. Available form
2. Unavailable form

For the determination of micronutrients, Atomic Absorption Spectrophotometer (AAS) is widely used. Fe, Cu, Zn and Mn are determined using AAS by extracting them with Diethylene Triamine Penta Acetic acid (DTPA).

Principle : If a solution containing metallic salt is aspirated into a flame, a vapour containing atoms of metal may be raised to an energy level, which is sufficient to permit the emission of radiation characteristic of the metal. However, a much large number of these gaseous metal atoms will normally remain in ground state. These are capable of absorbing radiant energy of their own specific resonance wavelength, which in general is

the wavelength of radiation that the atoms would emit if excited from ground state. Hence, if the light of resonance wavelength is passed through a flame containing atoms in question, then, the part of light will be absorbed and the extent of absorption will be proportional to the number of ground state atoms present in the flame. This is the underlying principle of AAS. By using AAS, the amount of Fe, Cu, Zn and Mn present in the soil sample are determined. The procedure involves the following steps.

Preparation of reagents

Extractant : DTPA (1.97 g) and calcium chloride dihydrate (1.47 g) were dissolved in distilled water (500 ml). To this solution, triethanol amine (13.3 ml) was added and the pH of the solution was adjusted to 7.3 by using hydrochloric acid solution (5N) and the solution was diluted to 1000 ml with distilled water.

Stock solution of zinc, 1000 ppm : Zinc metal (1.0 g) was dissolved in hydrochloric acid (5N, 20 ml). Then, the solution was diluted to 1 litre with distilled water.

Stock solution of copper, 1000 ppm : Copper metal (1.0 g) was dissolved in nitric acid solution (5N, 20 ml). Then, the solution was diluted to 1 litre with distilled water.

Stock solution of manganese, 1000 ppm : Manganese metal (1.0 g) was dissolved in hydrochloric acid solution (5N, 20 ml). Then, the solution was diluted to 1 litre with distilled water.

Stock solution of iron, 1000 ppm : Iron metal (1.0 g) was dissolved in hydrochloric acid solution (5N, 20 ml). Then, the solution was diluted to 1 litre with distilled water.

Preparation of working standards

Working standards of zinc : Stock solution of zinc (10 ml) was diluted to 1 litre by using DTPA solution to obtain a solution of 10 ppm concentration. This diluted solution was

accurately pipetted out 2.5, 5, 7.5 and 10 ml and then diluted to 100 ml with DTPA solution in a standard flask. This gave 0.25, 0.50, 0.75 and 1 ppm zinc solutions.

Working standards of copper : Stock solution of copper (20 ml) was diluted to 1 litre by using DTPA solution to obtain a solution of 20 ppm concentration. This diluted solution was accurately pipetted out 2.5, 5, 7.5 and 10 ml and then diluted to 100 ml with DTPA solution in a standard flask. This gave 0.50, 1.00, 1.50 and 2 ppm copper solutions.

Working standards of manganese : Stock solution of manganese (10 ml) was diluted to 1 litre by using DTPA solution to obtain a solution of 10 ppm concentration. This diluted solution was accurately pipetted out 2.5, 5, 7.5 and 10 ml and then diluted to 100 ml with DTPA solution in a standard flask. This gave 0.25, 0.50, 0.75 and 1 ppm manganese solutions.

Working standards of iron : Stock solution of iron (10 ml) was diluted to 250 ml by using DTPA solution to obtain a solution of 10 ppm concentration. This diluted solution was accurately pipetted out 2.5, 5, 7.5 and 10 ml and then diluted to 100 ml with DTPA solution in a standard flask. This gave 1, 2, 3 and 4 ppm iron solutions.

Procedure : The working standard solution (0.5 ml) of each concentration of each metal ion) was aspirated into the flame of AAS and intensity of the radiation was recorded separately. The standard curve was obtained for each of the solution separately by plotting the graph of concentration against the intensity of radiation.

Soil sample (10 gm) was mixed with DTPA solution (20 ml). The mixture was shaken mechanically for 2 hr. The solution was filtered and the filtrate (0.5 ml) was aspirated into the flame of AAS and the intensity of radiation was recorded for zinc, copper, manganese and iron using appropriate filters. By using this intensity of radiation,

the concentration of these metals was determined with the help of standard curves. The exact amount of these micronutrients was calculated by using the following equation.

$$\text{Available micronutrients in ppm} = \frac{X \text{ ppm}}{10^6} \times \frac{\text{Volume of the extractant}}{\text{Weight of the sample}} \times 10^6$$

$$\text{Available micronutrients in ppm} = \frac{X \text{ ppm}}{10^6} \times \frac{20}{10} \times 10^6$$

$$= X \times 2$$

Thus, available zinc in the soil sample = $0.15 \times 2 = 0.30 \text{ ppm}$

Available copper in the soil sample = $0.275 \times 2 = 0.55 \text{ ppm}$

Available manganese in the soil sample = $2.25 \times 2 = 4.50 \text{ ppm}$

Available iron in the soil sample = 3.625×2

$$= 7.25 \text{ ppm}$$

From values obtained, it can be concluded that the soil sample under consideration has a deficiency of zinc and moderately fertile with respect to available copper, manganese and iron.

Table-3.1 Summary of the soil analysis

Sl. No.	Examined parameter	Unit	Report	Nature of the soil
1	pH	--	5.7	acidic
2	Organic carbon	%	0.25	highly fertile
3	Available phosphorous	Kg/acre	32	moderately fertile
4	Available potassium	Kg/acre	70	highly fertile
5	Zinc	Ppm	0.30	deficiency
6	Copper	Ppm	0.55	fertile
7	Manganese	Ppm	4.50	fertile
8	Iron	Ppm	7.25	fertile

References

1. M.L. Jackson, "Soil Chemical Analysis", Prentice Hall Publication, 128, (!973).
2. M.D. Kerwin and K. Pooch, "Exchangeable Soil Science", Soc. Anor. Proc., 125, (1951).
3. B.V. Subbaiah and G.L. Asija, *Current Science*, 259, (1956).
4. A.I. Vogel, "A Text Book of Quantitative Chemical Analysis", 1989.
5. B.R. Puri, L.R. Sharma and S. Pathania, "Principles of Physical Chemistry", 1994.



CHAPTER-4

Pharmacological Evaluation of Crude Extracts of the Plant *Balanites Roxburghii*

Section-A: Antifertility activity in female rats

Section-B: Antifertility activity in female rats

Section-C: Antimicrobial activity

Section-D: Anthelmintic activity

Section-E: Anti-inflammatory activity

Section-F: Analgesic activity

Section-G: Diuretic activity

Pharmacological evaluation of crude extracts

The plant *Balanites Roxburghii* is known to exhibit many dynamic biological and pharmacological activities¹⁻³. Particularly, the fruits of this plant are being used for male antifertility by folk physicians of Chitradurga. Hence, it was contemplated to subject the crude extracts of the fruits of this plant for investigation of the following activities.

- A. Antifertility in female
- B. Antifertility in male
- C. Antimicrobial
- D. Anthelmintic
- E. Anti-inflammatory
- F. Analgesic
- G. Diuretic

4.A Antifertility efficacy of crude extracts of the fruits of *Balanites Roxburghii* in female rats

Antifertility effect of any drug in female can be ascertained by studying the following parameters.

- 1) Post-coital antifertility activity in female rats
- 2) Estrogenic and antiestrogenic activity in young female rats.
- 3) Biochemical changes.

4.A.1 Post-coital antifertility testing in female rats

Post-coital antifertility testing was carried out in female Wistar rats as per the method described by Khanna and Chaudhury⁴. Colony bred virgin female albino rats of Wistar strain were used. All animals were maintained under controlled standard animal house conditions, fed food as prescribed by Central Food Technological Research Institute (CFTRI) and water *ad libitum*. These female rats were monitored daily for different stages of estrous cycle. The rats weighing between 150-200 g of proven fertility and with regular estrous cycle were selected for this experiment. The rats, which were in proestrous stage were separated and caged with male rats that had sired litters before, in the ratio of 2:1. Every morning the vaginal smears were examined for the presence of lumps of spermatozoa. The day when spermatozoa were detected in the vaginal smear was considered as day 1 of pregnancy. Then the pregnant rats were isolated and divided into nine groups of six animals each.

Dose fixation

Dose fixation was carried out by stair-case method⁵ on Swiss strain albino mice (80-100 g body weight). It was observed that none of the extracts were found to be lethal even at the dose of 3000 mg/kg body weight. Hence 1/10th of 3000 mg/kg i.e. 300 mg/kg body weight of the crude extracts was fixed as the dosage. As the extracts were found to be non-toxic, double of this dose i.e. 600 mg/kg body weight was also chosen to ascertain the response of the animals and also to study the dose dependency.

The extracts were administered at the dose levels of 300 mg/kg and 600 mg/kg body weight through intragastric catheter from day 1 to day 7 of the pregnancy. The

animals were laprotomised under light ether anaesthesia on day 10 of pregnancy and both the horns of the uterus were observed for number, color and size of implants.

Simultaneously, the number of corpora lutea were also noted. The abdomen was sutured and the pregnancy was allowed to go to full term. After the delivery, the litters were counted, weighed and examined for teratogenicity. The litters were allowed to grow to check post-natal growth and monitored for any congenital abnormalities. The observations made are presented in the Table-4.1. The photographs showing the uterus of the rat are represented in Figure-4.1 and Figure-4.2.

Table-4.1 Abortifacient activity of the extracts of fruits of *Balanites Roxburghii*.

Treatment	Dose mg/kg body weight	Group	No. of rats with implantation sites on day 10	Mean No. of implants on day 10	Mean No. of litters delivered	Percentage of abortifacient activity
Control	-	I	6	11.00	11.00	-
Petroleum ether extract	300	II	6	11.28	8.33	26.15
	600	III	6	10.86	7.33	32.50
Chloroform extract	300	IV	6	11.63	9.50	18.31
	600	V	6	11.92	9.16	23.15
Ethanol extract	300	VI	6	12.37	6.66	46.16
	600	VII	6	12.94	5.16	60.12
Water extract	300	VIII	6	12.60	10.16	19.36
	600	IX	6	11.62	9.04	22.20

Figure-4.1 Photograph of uterus of the rat without implantation sites.

Figure-4.2 Photograph of uterus showing the implantation sites.

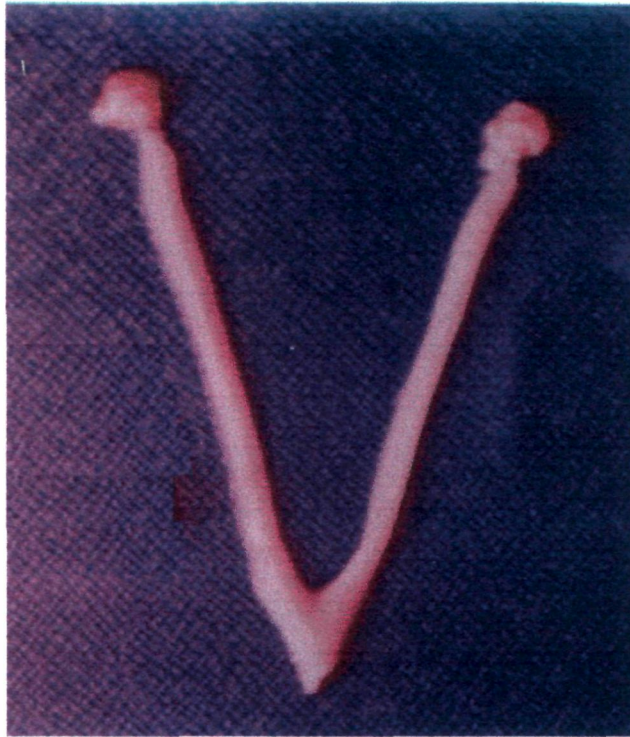


FIGURE – 4.1



FIGURE – 4.2

Petroleum ether extract and ethanol extract of the plant *Balanites Roxburghii* were found to possess abortifacient activity. The phytochemical investigations have proved the presence of steroids in petroleum ether and chloroform extracts. Hence, only the ethanolic extract was taken up for the detailed investigation of estrogenic and antiestrogenic activity in Wistar strain albino rats to investigate the possible mechanism of its antifertility action.

4.A.2 Estrogenic and antiestrogenic activity

Colony bred Wistar strain immature female albino rats, aged 21-23 days, weighing between 35-45 g were bilaterally ovariectomised under light ether anaesthesia and under sterile conditions. They were divided into 4 groups consisting of 6 rats each. The first group served as control and received vehicle only (Tween-80, 1%, 5 ml/kg). The second group received ethinyl estradiol in olive oil (1 µg/rat/day) subcutaneously. The third group received ethanol extract at the dose of 600 mg/kg body weight. The fourth group received, in addition to ethinyl estradiol (1 µg/rat/day), ethanol extract at the dose of 600 mg/kg body weight. All the above treatments were given for 7 days. Vagina and the vaginal smears were examined in all the animals in the treated groups for 7 days of treatment. On day 8, the rats were sacrificed by decapitation, the uteri were dissected out and the exact weights of the uteri were recorded. The two horns of the uterus were separated, one of the horns was used for investigation of biochemical changes and the other was utilized for histological studies. The separated horn was fixed in Bouin's fluid for 25 hr for histological studies. The diameter of the uterus, thickness of endometrium and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer. The statistical analysis was carried out by using student's 't' test. The results were expressed as mean ± SEM. The findings are

summarized in Tables-4.2 and Table-4.3. The photographs of the histological observations of the uterus of the rats are represented in Figure-4.3 to Figure-4.6.

Table-4.2 Estrogenic and antiestrogenic activity of the ethanol extract of fruits of *Balanites Roxburghii*.

Group	Treatment group (Dose/day)	Uterine weight in mg $\pm$ SEM	Vaginal cornification
I	Control	62.14 $\pm$ 4.52	nucleated and cornified cells
II	Ethinyl estradiol (1 μ g/rat)	302.72 $\pm$ 13.38*	cornified cells
III	Ethanol extract (600 mg/kg)	114.81 $\pm$ 9.17*	nucleated and cornified cells
IV	Ethinyl estradiol (1 μ g/rat) + ethanol extract (600 mg/kg)	322.16 $\pm$ 9.62*	cornified cells

*p < 0.001 vs control, student's t-test, n=6

Table-4.3 Histological changes in the uterus

Group	Treatment (Dose/day)	Diameter of the uterus (μ m) $\pm$ SEM	Thickness of the endometrium (μ m) $\pm$ SEM	Height of the endometrial epithelium (μ m) $\pm$ SEM
I	Control	356.56 $\pm$ 6.06	52.82 $\pm$ 2.18	28.14 $\pm$ 1.06
II	Ethinyl estradiol (1 μ g/rat)	861.24 $\pm$ 2.84	247.63 $\pm$ 1.92*	48.72 $\pm$ 9.12**
III	Ethanol extract (600 mg/kg)	571.40 $\pm$ 1.48**	75.92 $\pm$ 1.12**	37.63 $\pm$ 1.52*
IV	Ethinyl estradiol (1 μ g/rat) + ethanol extract (600 mg/kg)	911.10 $\pm$ 2.66*	268.32 $\pm$ 4.38*	54.44 $\pm$ 3.72*

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

Figure-4.3 Photograph of the cross section of the uterus of immature control rat showing normal myometrium and very few endometrial glands.

Figure-4.4 Photograph of the cross section of the uterus of immature rat treated with ethinyl estradiol at 1 µg/rat/day showing increase in height of myometrium, endometrium and endometrial glands.

ED	Endometrium
EG	Endometrial gland
EP	Epithelium
MY	Myometrium
L	Lumen

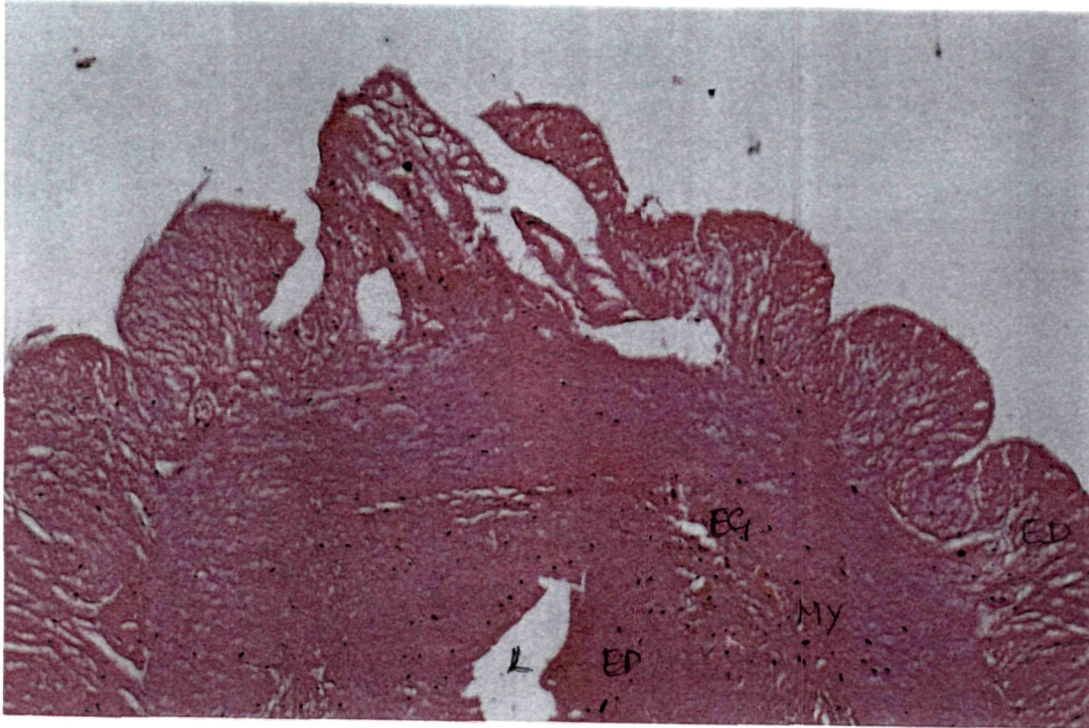


FIGURE – 4.3

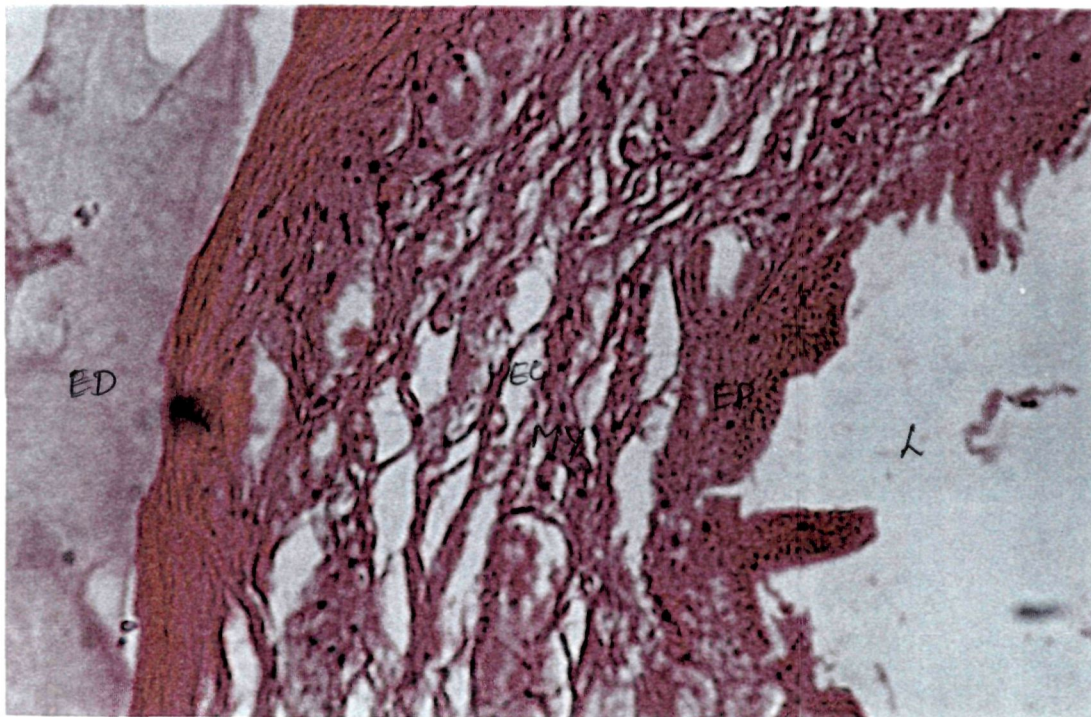


FIGURE – 4.4

Figure-4.5 Photograph of the cross section of the uterus of immature rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight showing mild estrogenic activity. Endometrial glands are visible. Height of myometrium and endometrium is increased.

Figure-4.6 Photograph of the cross section of the uterus of immature rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight along with ethinyl estradiol at 1 µg/rat/day showing endometrial glands, reduction in lumen and increase in thickness of endometrium.

ED	Endometrium
EG	Endometrial gland
EP	Epithelium
MY	Myometrium
L	Lumen

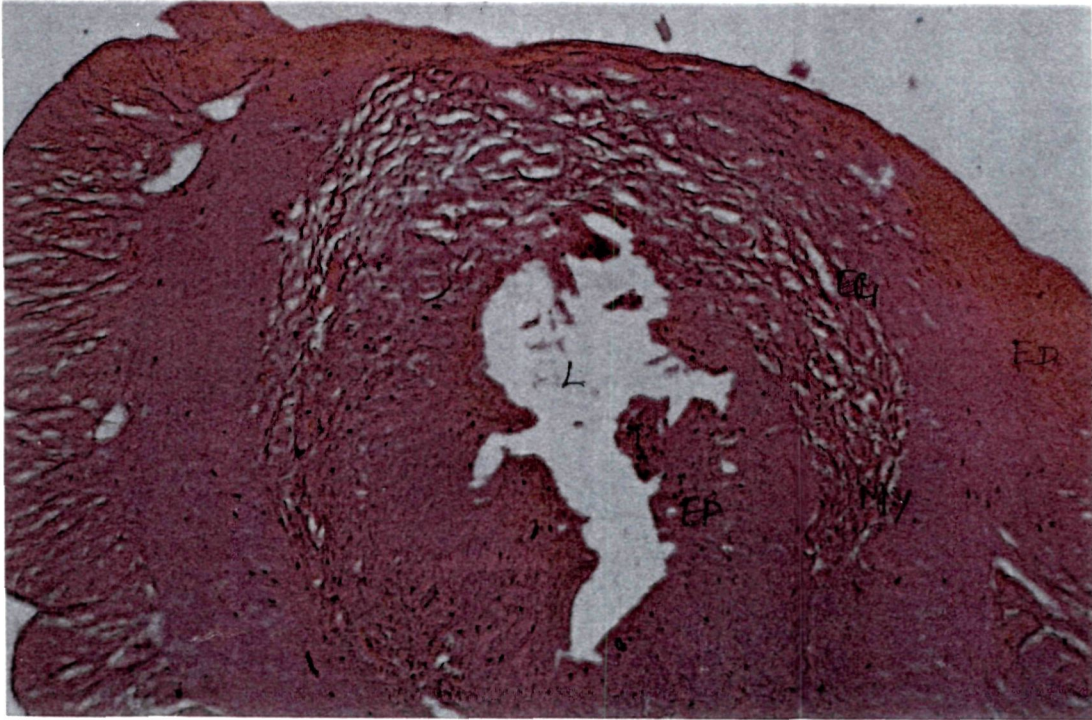


FIGURE – 4.5

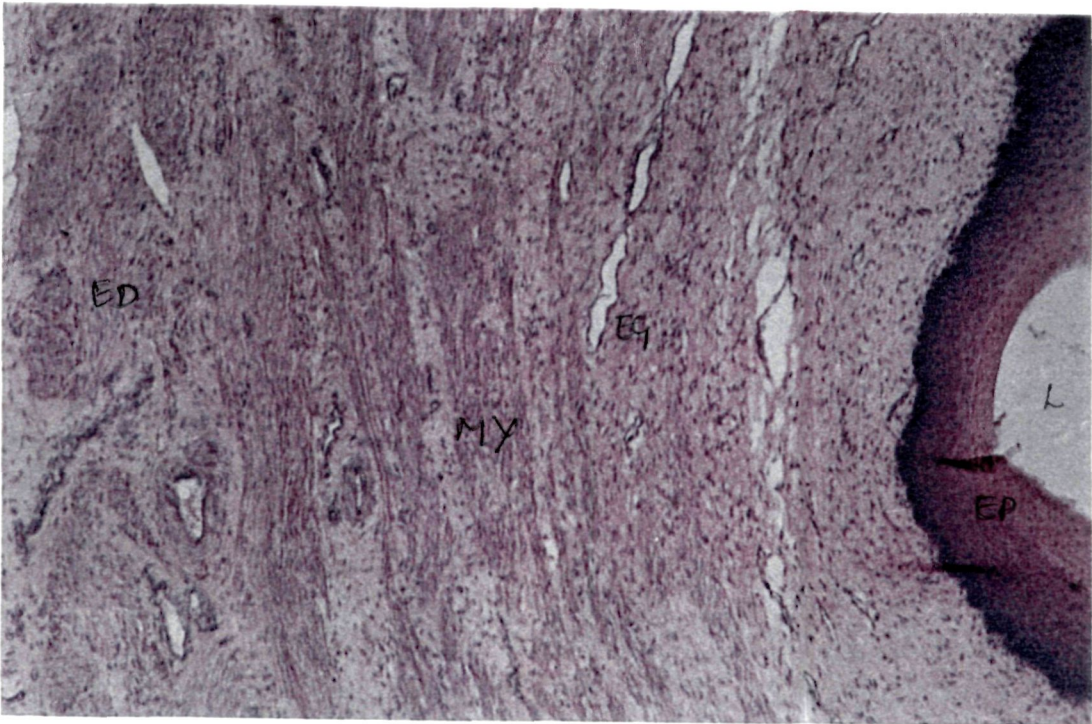


FIGURE – 4.6

4.A.3 Biochemical changes

The biochemical changes in the uterus of the treated rats were carried out to know the effect of ethanol extract on the total protein content and total glycogen content of the uterus. Total protein content and glycogen content were estimated by the method as described by Lowry⁶ et al., and Good⁷ et al., respectively.

Estimation of total protein

Proteins are the polymers of amino acids, which are linked to each other by peptide bonds. Proteins are present in all living organisms since they form the structural and functional basis of the cell.

Principle : Proteins react with Folin-Ciocalteu reagent (FCR reagent) to give a blue colored complex. The color so formed is due to the reaction of alkaline copper with protein and the reduction of phosphomolybdic-phosphotungstic components in the FCR reagent by the amino acids tyrosine and tryptophan present in the protein. The intensity of blue color is measured colorimetrically.

Procedure : The tissue (0.5 g) was ground with ice-cold water (5 ml) and diluted with distilled water (10 ml). Aliquot sample (1 ml) was taken in a separate test tube and alkaline copper reagent (5 ml) was added, mix well and incubate at room temperature for 10 minutes. FCR reagent (0.5 ml) was added to this solution, mixed well and incubated at room temperature in dark for 30 minutes. The intensity of blue color that developed was measured colorimetrically at 660 nm.

Absorbance of solutions of standard protein at different concentrations was measured and a standard curve was constructed by plotting the concentration on x-axis and absorbance on y-axis.

Amount of protein was calculated by comparing the absorbance of tissue protein solution with the graph and using the formula

$$\text{Amount of protein present present in sample (mg/100gm)} = \frac{\text{protein value from graph (mg)}}{\text{Aliquot sample used (1 ml)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of the sample (mg)}} \times 100$$

Estimation of glycogen

Glycogen is the major storage polysaccharide in the liver and muscle of the animals. It is a highly branched polymer made up of D-glucose units linked by both α -1,4 and α -1,6 glycosidic bonds. In a well-fed animal, glucose is converted to glycogen in the liver, but during starvation, the liver glycogen is broken down to glucose.

Principle: Glycogen is released from the tissue by heating with strong alkali, precipitated by ethanol and separated by centrifugation. It is then hydrolyzed by dilute hydrochloric acid, neutralized and estimated colorimetrically.

Procedure : The tissue (1.5 g) was taken into a calibrated centrifuge tube containing potassium hydroxide (5N, 2 ml). It was heated on a boiling water bath for 20 minutes with occasional shaking. The resulting solution was cooled in ice, and saturated solution of sodium sulphate (0.2 ml) was added and mix thoroughly. The glycogen present was precipitated by the addition of ethanol (5 ml), cooled in ice for 5 minutes and centrifuged.

The precipitated glycogen was dissolved in water (5 ml) by gentle warming. It was diluted with water to 10 ml. Aliquot sample (1 ml) was taken into test tube and concentrated hydrochloric acid (1 ml) was added. This solution was heated on a boiling water bath for 2 hr and neutralized with sodium hydroxide using phenol red as indicator.

The neutral solution thus obtained was diluted with water (5 ml) and heated for 8 minutes after the addition of anthrone-sulphuric acid reagent (4 ml). It was cooled rapidly and the intensity of dark green color developed was measured colorimetrically at 630 nm.

Absorbance of solutions of standard glucose at different concentrations was measured and a standard curve was constructed by plotting the concentration on x-axis and absorbance on y-axis. Amount of glycogen was calculated by comparing the absorbance of tissue glycogen solution with the graph and using the formula

$$\text{Amount of glycogen present in sample (mg/100gm)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (1 ml)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of the sample (mg)}} \times 100$$

The results are tabulated in **Table-4.4**.

Table-4.4 Biochemical changes in the uterus of the treated rats.

Treatment (Dose)	Protein content µg/mg ± SEM	Glycogen content µg/mg ± SEM
Control	8.24 ± 0.40	1.52 ± 0.20
Ethinyl estradiol (1 µg/kg)	10.92 ± 0.84	1.92 ± 0.45
Ethanol extract (600 mg/kg)	8.78 ± 0.30	1.50 ± 0.11
Ethinyl estradiol (1 µg/kg) + ethanol extract (600 mg/kg)	12.41 ± 0.15	2.06 ± 0.5

Results

All the extracts were evaluated for post-coital antifertility activity at the dose of 300 mg/kg and 600 mg/kg body weight. No toxic effects were observed either by gross visual examination or in the weight of rats. After discontinuation of the treatment, all the rats were mated. This resulted in pregnancy and delivery of normal litters, indicating that the action of the extracts of fruits of *Balanites Roxburghii* is reversible.

There was no noticeable change in the color and size of the implants when compared with rats of control group. These observations clearly indicate that none of the extracts possess antiimplantation activity. However, all the extracts exhibited abortifacient activity. Amongst these, ethanol extract showed 46.16% abortifacient activity at a dose of 300 mg/kg body weight and 60.12% at a dose of 600 mg/kg body weight. All the animals in treated group showed open vagina, while the control group had closed vagina. No vaginal bleeding was observed in any of the animals. This observation revealed that all the implants have been absorbed by the uterus.

Oral administration of the ethanol extract caused significant increase in uterine weight. The uterotrophic potency, as shown by the increase in weight of the uterus, was about 40% in the case of ethanol extract, when compared with that of ethinyl estradiol. This suggested mild estrogenic activity of the extract. This fact was well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium and height of endometrial epithelium.

It was also observed that the ethanol extract potentiated the action of ethinyl estradiol when administered together. This fact was well indicated by the significant

increase in diameter of uterus, thickness of endometrium and height of the endometrial epithelium.

Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

The results of biochemical changes also support the observations made in histological studies, that the ethanol extract of *Balanites Roxburghii* has mild estrogenic activity. Administration of ethanol extract along with ethinyl estradiol at the dose of 600 mg/kg body weight and 1 µg/rat/day respectively for seven days, resulted in increase in protein as well as glycogen content in the uterus.

Discussion

In the present study, the extracts of the fruits of the *Balanites Roxburghii* were tested at the dose of 300 mg/kg and 600 mg/kg body weight. All these extracts did not exhibit any antiimplantation activity. However, abortifacient activity was exhibited by these extracts. The ethanol extract showed 46.16% abortifacient activity at a dose of 300 mg/kg body weight and 60.12% at a dose of 600 mg/kg body weight.

It is a well known fact that for implantation and sustainance of pregnancy, exact equilibrium of secretion of estrogen and progesterone is necessary. Any imbalance in the levels of these hormones can cause antiimplantation or can induce abortion. Compounds disturbing hormonal functions may evoke infertility. In this study, the histological changes observed in the uterus of animals treated with various extracts supports an unfavorable uterine milieu. Therefore, the abortifacient activity may be due to mild estrogenic activity of the extracts causing expulsion or resorption of the implants by the uterus.

4.B Antifertility efficacy of the ethanol extract of the fruits of *Balanites Roxburghii* in males.

Many plants are known to contain chemical component/s, which can affect fertility in males. Gossypol is a very potent male antifertility agent, isolated from the cotton plant. After the discovery of this, many researchers have taken up screening of various plants and their part/s for evaluation of antifertility effect in males.

Rural folk physicians of Chitradurga use the fruits of *Balanites Roxburghii* for antifertility activity. So far, this plant has not been studied for its male antifertility activity. Therefore, the present investigation has been undertaken to examine the possible androgenic and antiandrogenic effect of the ethanol extract of fruits of *Balanites Roxburghii*.

The antifertility activity of the ethanol extract of fruits of *Balanites Roxburghii* in young male rats was ascertained by studying the following parameters.

- 1) Androgenic/antiandrogenic activity in young male rats.
- 2) Biochemical changes.

4.B.1 Androgenic/antiandrogenic activity

The androgenic/antiandrogenic activity has been carried out by following the method as described by Lohiya and Goyal⁸.

Materials and Method

Colony bred young male albino rats, (Wistar strain) 22 days old and weighing between 28-32 g, were used for the evaluation of androgenic or antiandrogenic activity. They were divided into four groups, each group comprising of six animals. The first group served as control and received the vehicle (Tween-80, 1%, 5 ml/kg). The second

group received testosterone suspension in 1% Tween-80 solution at the dose of 1 mg/kg subcutaneously. The third group received the suspension of ethanol extract in Tween-80 (0.1%, 1 ml) at 600 mg/kg body weight. The fourth group received, in addition to testosterone, a test dose of the ethanol extract at 600 mg/kg body weight. All the treatments were given for seven days. On eighth day of the experiment, all the animals were sacrificed by decapitation. The testes, epididymes, vas deferens, seminal vesicle and the ventral prostrate were dissected out, freed from surrounding tissues and weighed quickly on a sensitive balance. Gravimetric changes were recorded and shown in Table-4.5 and Table-4.6. The photographs showing the histological observations of the testes of the rats are represented in Figure-4.7 to Figure-4.10.

Table-4.5 Effect of the ethanol extract of the fruits of *Balanites Roxburghii* on the weights of male reproductive organs when fed orally to young male rats for seven days (in mg/100 g body weight $\pm$ SEM).

Group	Treatment dose (mg/kg)	Testes $\pm$ SEM	Seminal vesicle $\pm$ SEM	Ventral prostrate $\pm$ SEM	Vas deferens $\pm$ SEM	Epididymes $\pm$ SEM
I	Control	647.76 $\pm$ 16.26	49.26 $\pm$ 2.16	56.68 $\pm$ 6.21	31.52 $\pm$ 3.65	79.42 $\pm$ 6.84
II	Testosterone (1 mg/kg)	654.32 $\pm$ 14.62*	78.45 $\pm$ 0.52 ⁺	89.46 $\pm$ 4.83**	44.81 $\pm$ 4.58 ⁺	88.73 $\pm$ 8.14*
III	Ethanol extract (600 mg/kg)	586.31 $\pm$ 31.45*	36.73 $\pm$ 1.68**	42.84 $\pm$ 4.29 ⁺	26.07 $\pm$ 3.12*	61.03 $\pm$ 7.65 ⁺
IV	Ethanol extract (600 mg/kg) + testosterone (1 mg/rat)	642.05 $\pm$ 24.31***	38.31 $\pm$ 1.74*	45.97 $\pm$ 3.12**	29.76 $\pm$ 2.28**	62.82 $\pm$ 4.45*

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

*** p < 0.001 when compared with control, + p < 0.5 when compared with control

Figure-4.7 Photograph showing the cross section of testes of immature control male rat.

Figure-4.8 Photograph showing the cross section of testes of immature male rat treated with testosterone at 1 mg/rat/day.



FIGURE – 4.7

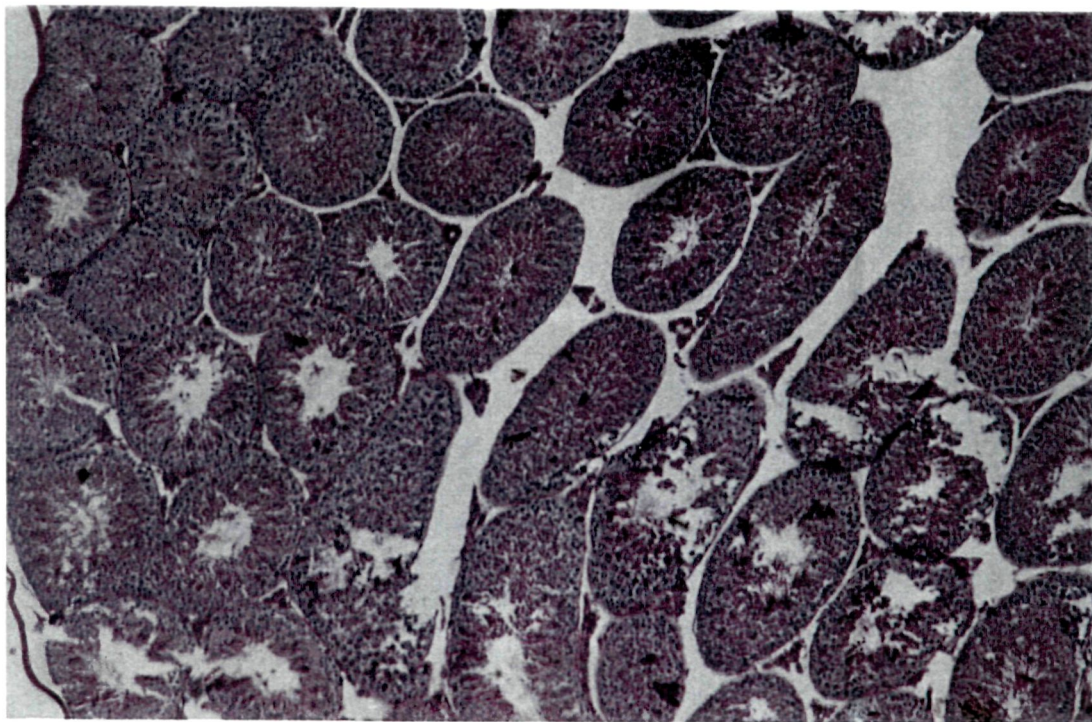


FIGURE – 4.8

Figure-4.9 Photograph showing the cross section of testes of immature male rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight.

Figure-4.10 Photograph showing the cross section of testes of immature male rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight along with testosterone at 1 mg/rat/day.

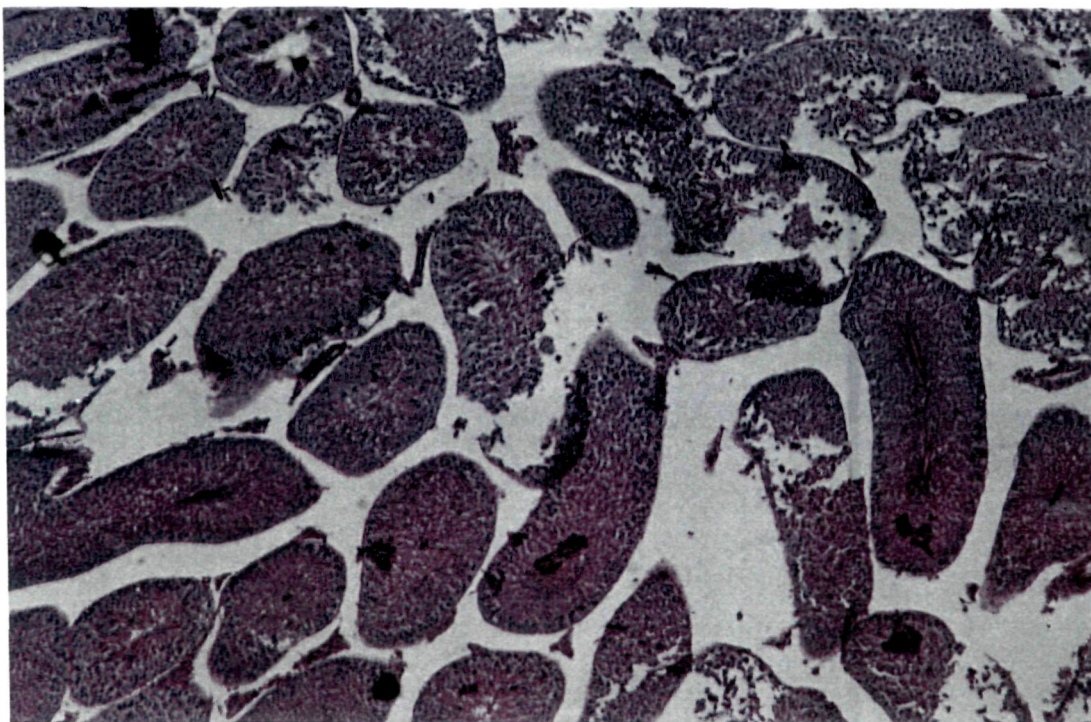


FIGURE – 4.9

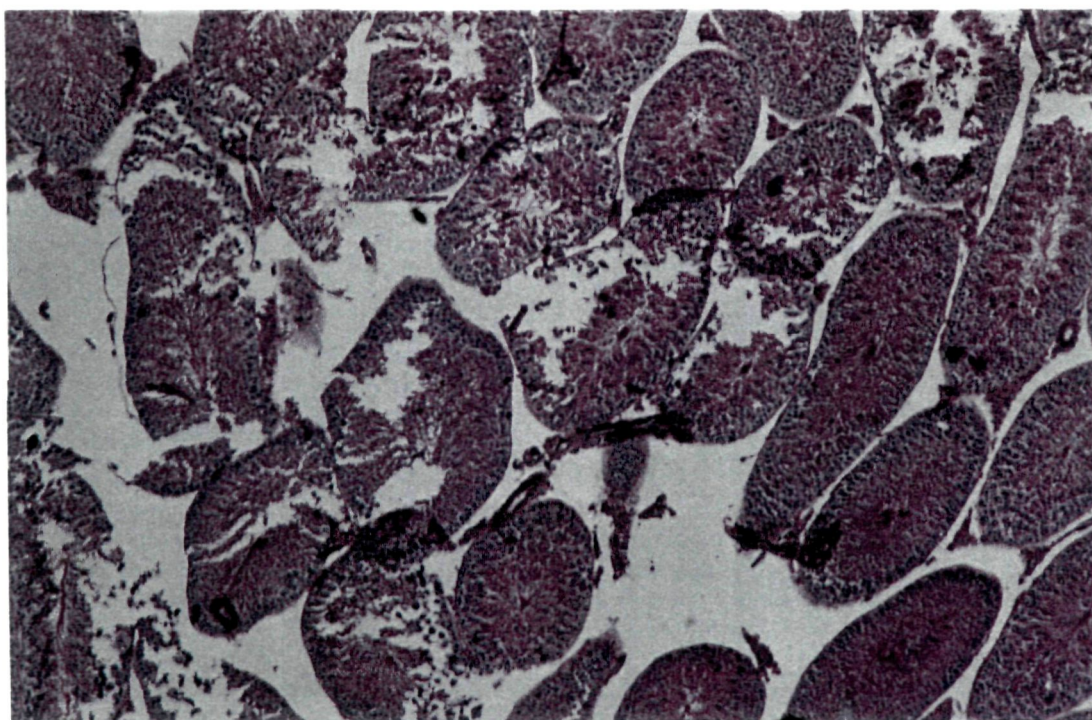


FIGURE – 4.10

Table-4.6 Changes in the testes due to oral administration of the extracts of *Balanites Roxburghii* on young male rats for seven days.

Group	Treatment (Dose)	Diameter of the testes (μm) $\pm$ SEM	Diameter of the seminiferous tubules (μm) $\pm$ SEM
I	Control	4132 $\pm$ 23.7	132.7 $\pm$ 4.1
II	Testosterone (1 mg/kg)	4145 $\pm$ 16.2*	145.8 $\pm$ 2.9**
III	Ethanol extract (600 mg/kg)	3986 $\pm$ 08.7**	124.4 $\pm$ 3.6***
IV	Testosterone (1 mg/kg) + Ethanol extract (600 mg/kg)	4016 $\pm$ 12.3*	125.8 $\pm$ 3.1**

* p < 0.1 when compared with control, ** p < 0.01 when compared with control, *** p < 0.001 when compared with control.

4.B.2 Biochemical changes

The biochemical changes of the testes were also carried out by the methods as described earlier. The results are recorded in Table-4.7.

Table-4.7 Biochemical changes in the testes of the treated rats.

Treatment	Protein content $\mu\text{g}/\text{mg} \pm$ SEM	Glycogen content $\mu\text{g}/\text{mg} \pm$ SEM
Control	9.16 $\pm$ 0.2	2.24 $\pm$ 0.4
Testosterone (1 mg/kg)	5.40 $\pm$ 0.5	1.23 $\pm$ 0.6
Ethanol extract (600 mg/kg)	6.22 $\pm$ 0.3	1.44 $\pm$ 0.1
Testosterone (1 mg/kg) + ethanol extract (600 mg/kg)	6.53 $\pm$ 0.6	1.64 $\pm$ 0.5

Results

The administration of ethanol extract of the fruits of *Balanites Roxburghii* to prepubertal male rats for seven days has significantly reduced the weights of testes, epididymes, seminal vesicle and ventral prostate when compared with the rats of control and testosterone group. A significant increase in the weights of testes and other accessory organs was observed when testosterone was administered alone, but androgenic effect of testosterone was decreased with simultaneous administration of the ethanol extract and testosterone, which was indicated by the reduction in the weights of all the organs when compared to testosterone administered animals. Considerable reduction in weights of seminal vesicle and ventral prostate were observed, thus indicating moderate antiandrogenic activity of the extract.

The diameter of the testes and seminiferous tubules decreased significantly in ethanol extract treated rats, when compared with the control rats and testosterone treated rats. The seminiferous tubules of the control rats contained healthy spermatogonia, both primary and secondary spermatocytes, and a few spermatids indicating the normal spermatogenesis. However the seminiferous tubules of ethanol extract treated rats showed less spermatocytes and a very few spermatids.

Decreased levels of glycogen and protein content proved the catabolic nature i.e., antiandrogenic effect of the ethanol extract. The inhibition of spermatogenesis may also be due to low availability of glycogen, which is a nutrient for spermatogenesis.

4.C Antimicrobial activity

It is a well known fact that the interactions between microorganisms, plants and animals are natural and constant. The ecological role of microorganisms and their importance in the bio-geochemical cycles in nature is well documented. The human food supply consists basically of plants and animals or products derived from them.

Microorganisms are ubiquitous and are so small that they can only be visualized with the aid of high-resolution microscopes.

Microbes are a heterogeneous group of several classes of living things. These were originally classified under the plant and animal kingdoms. As this proved unsatisfactory, a five-kingdom system was introduced namely,

- a. Monera (Unicellular prokaryotes)
- b. Protesta (Unicellular Eukaryotes)
- c. Fungi
- d. Plantae
- e. Animalia

Protozoa are unicellular, non-photosynthetic eukaryotic microorganisms. Various groups of protozoa exhibit differing strategies of locomotion, for example *Ameoba* can change its cell shape by extending the cell wall and thus migrates along, whereas *Paramecium* is propelled by numerous beating structures called cilia.

As group, fungi are microorganisms that obtain their nutrition from organic compounds. Their cells are usually surrounded by protective cell wall composed of chitin or other polysaccharides.

Fungi produce spores, which are specialized cells, involved in reproduction, dissemination and survival. Depending on the cell morphology fungi can be divided into four classes such as, yeast, yeast like fungi, moulds and dimorphic fungi.

– Yeasts are unicellular and other fungi called moulds are filamentous. Dimorphic fungi may grow as mycelial or yeast-form depending upon prevalent growth conditions. Fungi form multicellular filaments called hyphae. The only pathogenic yeast is *Cryptococcus neoformans*. Fungi have been recognized as causative agents of human disease earlier than bacteria. Fungal infections are extremely common and some of them are serious and even fatal.

Antimicrobial activity of the crude extracts of the fruits of *Balanites Roxburghii* was carried out in two parts namely, antibacterial and antifungal activities.

4.C.1 Antibacterial activity

Introduction

The antibacterial activity of the extracts of *Balanites roxburghii* was studied comparatively with that of standard ciprofloxacin, by cup-plate method⁹ using a gram-positive and a gram-negative organisms *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Materials and method

1. Nutrient agar
2. Sterilized petridishes, pipettes, boiling tubes and beakers
3. Sterilized test tubes
4. Sterile 6 mm borer
5. Sterile inoculation loops
6. Sterilized fine pointed forceps
7. Tuberculin syringes

Preparation of media

Nutrient agar was prepared by dissolving of nutrient agar (14 g) in distilled water (500 ml), the pH of the solution was adjusted to 7.4 and then sterilized for 15 minutes at 15 lbs pressure in autoclave.

Preparation of sub-cultures

One day prior to the experiment, the microorganisms were inoculated into sterilized nutrient broth tubes and incubated at 37°C for 24 hr. The organisms used in the present study for evaluating antibacterial activity of test compounds were obtained from the laboratory at National College of Pharmacy, Shimoga. On the day of testing, the

organisms were subcultured into sterile nutrient broth. After incubating the same for three hours, the growth thus obtained was used as inoculum for the test.

Sterilization of media and glassware

The media used in the present study, nutrient agar and nutrient broth, were sterilized in conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized in hot air oven at 160⁰C for an hour.

Preparation of test solutions of the extracts

The suspension of each extract (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.

Method of testing : Cup-Plate Method

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antibiotic.

A previously liquified medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms between 40-50⁰C and the inoculated medium was poured into petridishes to give a depth of 3 to 4 mm., ensuring that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.

The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out the set agar in each petridish (three cups were numbered for the particular compounds, solvent and a standard) using sterile pipettes, the standard and the sample (0.1 ml) of known concentration were fed into the bored cups. The dishes were left standing for 2 hr. at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24 hr at 37°C. The zone of inhibition developed, if any, was then accurately measured and recorded. Each zone of inhibition recorded was average of three measurements. Zone of inhibition for Tween-80 was done separately.

The data of antibacterial activity of standard and the extracts is given in **Table-4.8**.

4.C.2 Antifungal activity

Introduction

The antifungal activity of the extracts of *Balanites Roxburghii* was studied in comparison with that of standard antifungal drug, ciclopirox olamine, by cup-plate¹⁰ method.

Materials and method

1. Potato dextrose agar
2. Micropipette
3. Sterilized petridishes
4. Potato dextrose broth (48 hours old)
5. Sterile test tubes for preparation of solutions of the test compounds.

Sterilization of media and glassware

The media used in the present study, nutrient agar and nutrient broth, were sterilized in conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized in hot air oven at 160°C for an hour.

Preparation of test solutions of the extracts

The suspension of each extract (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.

Preparation of media

Potato dextrose agar was prepared by dissolving potato dextrose (20 g) agar in distilled water (500 ml), the pH of the solution was adjusted to 5.6 and then sterilized for 15 minutes at 121⁰C at 15 lbs pressure in an autoclave.

Preparation of sub-culture

Two-days prior to the experiment, the microorganism were inoculated into sterilized potato dextrose broth tubes and incubated at 25⁰C for 48 hr.

Method of testing : Cup-plate method

This method depends on the diffusion of an antifungal agent from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antifungal agent.

A previously liquified medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms between 40-50⁰C and the inoculated medium was poured into petridishes to give a depth of 3 to 4 mm., ensuring that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.

The dish thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out the set agar in each petridish using sterile pipettes, the standard and the sample solution (0.1 ml) of known concentrations were fed into the bored cups.

The dishes were left standing for 2 hr at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for three days at 25°C. The zone of inhibition developed, if any, was then accurately measured and recorded in the tables. Each zone of inhibition recorded was average of three measurements.

The data of antifungal activity of standard and the extracts is given in Table-4.8.

Table-4.8 Antimicrobial activities of the extracts of *Balanites Roxburghii*.

Compound	Antibacterial activity Zone of inhibition (in mm.)		Antifungal activity zone of inhibition (in mm.)	
	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. niger</i>	<i>C. albicans</i>
Control	-	-	-	-
Standard	20	22	24	26
Petroleum ether extract	08	13	10	11
Chloroform extract	11	14	12	14
Ethanol extract	07	09	08	12
Water extract	06	08	10	11

Results

The results revealed that both petroleum ether extract and chloroform extract exhibited antimicrobial activity. Considerable zone of inhibition was observed for chloroform extract against *K. pneumoniae* when compared to the standard drug. The chloroform extract has also shown good antifungal activity against *C. albicans* and *A. niger*. The antimicrobial activity of other extracts was less when compared with the standard.

4.D Anthelmintic Activity

Introduction:

Helminthiasis is a disease caused by infestation of parasitic worms living in the alimentary canal or in the other tissues of the host. This disease is one of the major prevalent diseases in the world, particularly in the tropical countries. The main reasons responsible for the widespread nature of this disease in the developing countries are the lack of adequate sanitary facilities and supply of water, coupled with poverty and illiteracy.

The helminthus infection can be acquired by,

1. Contact with infected animal
2. The ingestion of infected meat
3. The animal or human excreta via ground water
4. Means of certain mosquitoes

The worms enter the body in the form of either larvae or egg. In some cases they produce diseases immediately, whereas in other cases they take weeks or months to produce diseases. Thus to take preventive measures against these worms, one should have exact understanding of the life cycle of helminthes. The main danger of helminthiasis is that, the worms may cause injuries to the vessels and organs. They may cause loss of blood, nutritional deficiency, urticaria and other allergic conditions.

Anthelmintics are the drugs, which are used to kill or to remove the parasitic worms. Those anthelmintics, which kill the worm, are called vermucidal while those that help in expelling them by making the environment uncomfortable for living are called vermifuges.

An anthelmintic drug may act by causing narcosis or paralysis of worm either by penetrating the cuticle or by the worm ingesting. It may damage the cuticle of the worm or partially paralyze it. The specificity of anthelmintic reveals that they interfere with metabolism of the worm, each worm is having its own metabolic requirement.

The human and animal parasitic worms zoologically belong to the class *Cestodes* (Tape worm), *Schistosomes* (Trematodes, flukes), *Filaria* (Thread worm), *Nematodes* (Round worm) and *Pheritima posthuma* (Earthworms, order-Annelida, class-Oligochaeta).

Literature survey revealed that, researchers all over the world are engaged in research work to synthesize compounds, which are capable of paralyzing these worms or causing death. In animals and human beings, roundworms and tapeworms cause sufficient trouble in the form of abdominal pain or crippling of movement. Therefore scientific community has been making continuous effort to control menace of these worms.

Fayard¹¹ was the first scientist to discover piperazine, a heterocyclic compound, as the drug having anthelmintic activity. Since then many derivatives of piperazine have been synthesized in order to increase the efficacy of the drug and also to minimize side effects. In addition, many other compounds have been synthesized, but only diethyl carbazine could find place in human therapeutics.

The fruits of *Balanites Roxburghii* are reported to exhibit anthelmintic activity and henceforth, it was contemplated to investigate the various extracts of the fruits for possible anthelmintic activity. As it was difficult to procure either roundworm or tapeworm, the experiment was performed on earthworms, which have similar life cycle.

The technique adopted was that described by Giand¹² et al., with slight modification¹³⁻¹⁵.

Anthelmintic activity was evaluated by using earthworm *Pheritima posthuma* (class-Annelida and order-Oligicheata).

The worms were procured from local supplier at Shimoga at the time of carrying out the experiment. The worms were washed with water to remove adhering materials and were sorted out for uniform size and length. The worms were kept in 6% dextrose solution for acclimatization. The worms with normal motility were selected for the experiment. Petridishes of equal size were selected and in each petridish, 2 ml of each extract (50 mg/ml) in 0.1% Tween-80 suspension were placed and the volume was made up to 25 ml with an aqueous solution of dextrose (6%). In another petridish Tween-80 (25 ml, 0.1% solution) prepared in 6% dextrose solution was placed, which served as control. 1 ml of piperazine citrate (100 mg/ml) suspended in Tween-80 (0.1%, 1 ml) was placed in another petridish and the volume was made up to 25 ml using dextrose (6%) solution. The piperazine citrate solution was served as standard. In each petridish 8 worms were placed. The time taken by each worm for paralysis was noted and the paralysis of worms was tested by placing the worms in water maintained at 50°C. Non-motility at 50°C was taken as the death of the worm and correspondingly the time taken for death was recorded. The results were tabulated in Table-4.9. The Figure 4.11 shows the worms in 1% Tween-80 solution and Figure-4.12 shows the worms in the solution of chloroform extract of the fruits of *Balanites Roxburghii* in dextrose solution.

Figure-4.11 Photograph showing earthworms in 1% Tween-80 solution.

Figure-4.12 Photograph showing earthworms in the solution of chloroform extract of the fruits of *Balanites Roxburghii*.

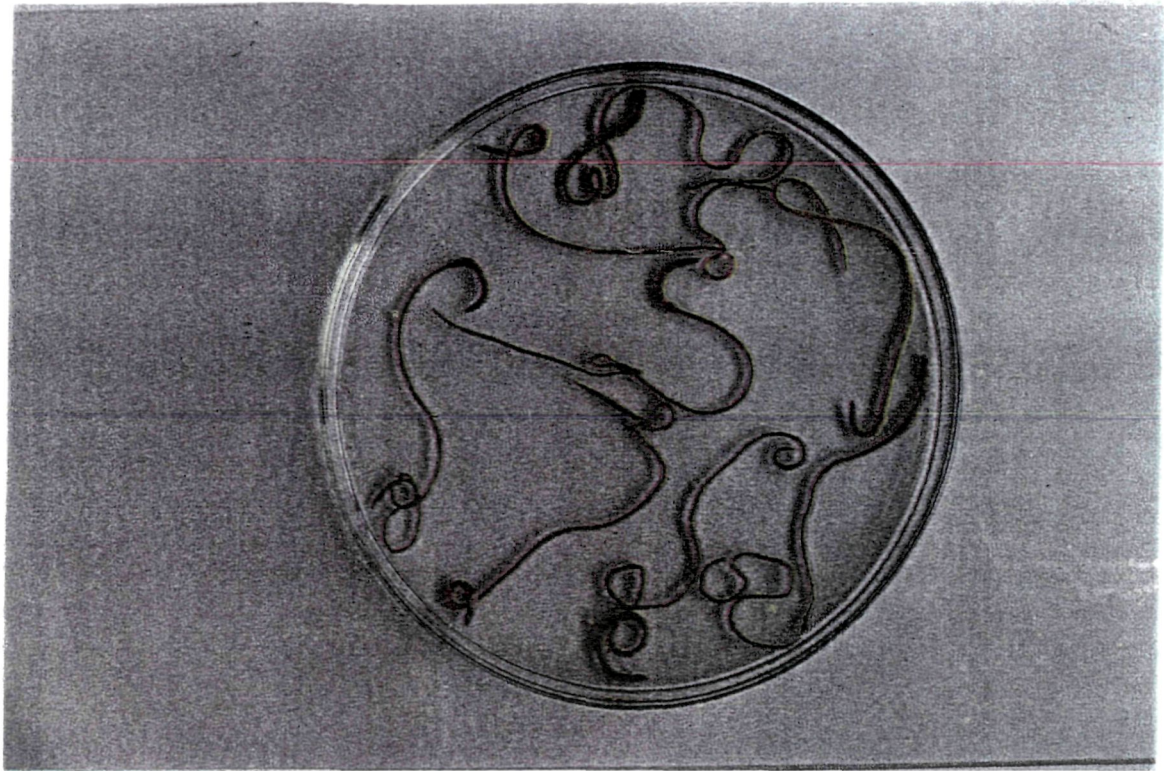


FIGURE-4.11

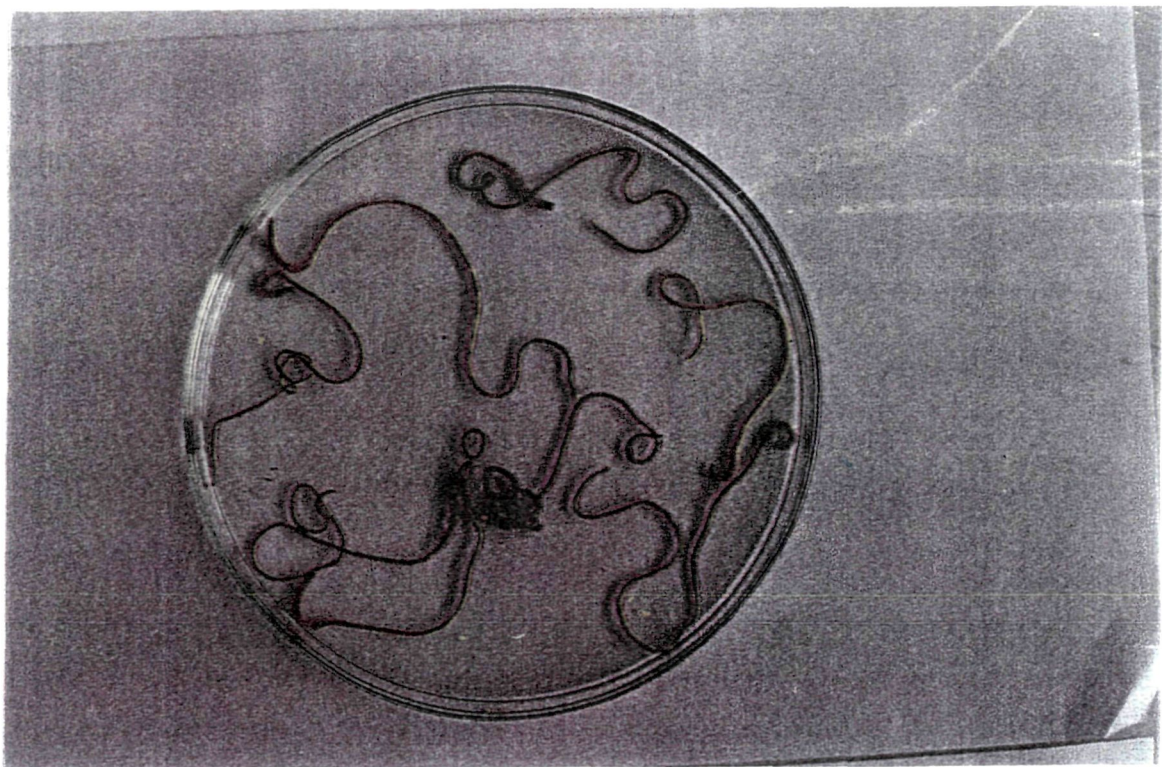


FIGURE-4.12

Table-4.9 Anthelmintic activity of the extracts of *Balanites Roxburghii*.

Compound	No. of earthworms tested	Concentration (Wt./Vol., mg)	Mean paralysis time (in min.)	Mean death time (in min.)
Control	8	-	NP	ND
Standard	8	100	120	160
Petroleum ether extract	8	100	142	200
Chloroform extract	8	100	130	190
Ethanol extract	8	100	162	ND
Water extract	8	100	NP	ND

ND = no death NP = no paralysis

Results

The chloroform extract and petroleum ether extracts of the fruits of *Balanites Roxburghii* exhibited significant anthelmintic activity against earthworm at 100 mg/ml concentration when compared with the standard piperazine citrate. However ethanol extract and water extract showed very less anthelmintic activity against earthworms.

Discussion

Earthworms have the ability to move by ciliary movement. Outer layer of the earthworm is a mucilaginous layer and composed of polysaccharides. This layer, being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layers, and this restricts its movement and can cause paralysis. This action may lead to the death of the worm. The drugs possessing anthelmintic property will paralyze the worms by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis. Since, the worm is incapable of movement, it will be expelled out from the body. The anthelmintic activity of the various extracts may be attributed to similar type of action.

4.E Anti-inflammatory Activity

Introduction

Inflammation is a normal, essential protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complete response involving the whole organism. Rheumatic diseases are inflammatory conditions that probably cause more disability than any other group of diseases and are very common among persons with age more than forty years.

In ordinary conditions the inflammatory and reparative processes progress smoothly from injury to healing and here the whole process is beneficial. Under special circumstances, eosinophils may concentrate in large number in tissue leading to restoration, as in the case of acute inflammation. In contrast, many chronic inflammatory conditions of unknown etiology, which affect organ system in the body, produce tissue destruction rather than restoration. Chronic inflammatory reactions can be divided into two groups, those with and without the formation of granuloma depending on the agent responsible for inflammation. When the irritant cannot be easily disposed by the mononuclear phagocytes, granuloma formation unusually occurs. Such inflammation may become much more complex and inflammatory response try to isolate them from the rest of the organisms by forming granuloma or pus, this is the case which is observed in pulmonary tuberculosis and in syphilis. Apart from these, there are various chronic inflammatory conditions of unknown etiology like rheumatoid arthritis, spondylitis, gout etc. Anti-inflammatory drugs are the drugs used to diminish or to reduce inflammation and pain arising from it. They modify the inflammatory response to disease. So an ideal anti-inflammatory drug should only affect the aberrant, uncontrolled inflammation and should

not interfere with the normal inflammatory system. Anti-inflammatory drugs have been evaluated by studying inflammatory responses produced in animals by injecting foreign or noxious agents. These responses mostly comprise of the development of edema.

An attempt was made to test the anti-inflammatory properties of the extracts of the fruits of *Balanites Roxburghii*.

Among several models available for evaluating anti-inflammatory activity, the rat paw edema model is the simplest and widely used method for this purpose. The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants or inflammagens. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxytryptamine etc., produce acute paw edema within a few minutes of the injection. Carrageenan induced paw edema is the most commonly used method in experimental pharmacology.

Materials and Method

Albino Rats weighing 150-200 g of Wistar strain

Plethysmograph

Beaker and measuring jar.

Standard drug (Ibuprofen).

Potassium permanganate solution (0.1%).

Tween-80 solution (0.1%).

Carrageenan (0.1%).

Rat paw edema method

This method was based on plethysmographic measurement of carrageenan-induced acute rat paw edema¹⁶⁻²⁰ produced by subplantar injection of carrageenan in hind paw of the rat. The method described by Wilhmi and Domenjoz²¹ later on modified by Sirodia and Rao²² was used for measuring the paw volume. The photographs showing plethysmograph and the measurement of rat paw volume²³⁻²⁷ are given in **Figure-4.13** to **Figure-4.16**.

This procedure of measuring the paw volume required two operators, one for dipping the paw of the rat in potassium permanganate solution and another to measure and record the paw volume simultaneously.

Before measuring the paw volume of the rat, the volume of solution in graduated tube 'F' was set to zero by operating the side tube 'G', after filling the apparatus with potassium permanganate solution without any entrapment of air bubble. The paw was dipped in limb 'A' up to the line marked on the foot of the rat by keeping the stopcock 'C' open and 'E' closed. The first operator held the foot of the rat firmly in potassium permanganate solution till the stopcock 'C' was kept closed by the second operator. The solution in the limb 'B' rises and now the stopcock 'C' was kept closed. The stopcock 'E' was slowly opened to bring back the raised solution in limb 'B' so as to bring the level of solution equal in limbs 'A' and 'B'.

Immediately the stopcock 'E' was closed. The solution rises in the graduated part 'E' of the apparatus and the increase in volume of the solution was noted. Now the side limb 'G' was opened to remove the solution in the graduated tube to bring it to zero and immediately the stopcock 'G' was closed. The stopcock 'D', meant for removing the air

Figure-4.13 Photograph showing the carrageenan being injected in the right hind paw for inducing edema.

Figure-4.14 Photograph showing the inflamed right hind paw.

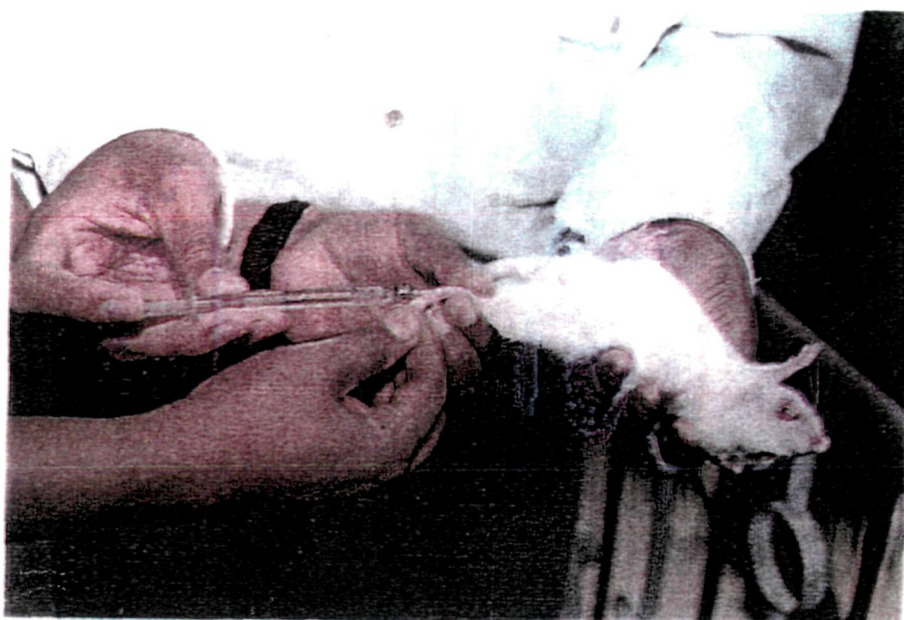


FIGURE-4.13



FIGURE-4.14

Figure-4.15 Photograph showing plethysmograph used for measuring paw volume.

Figure-4.16 Photograph showing the inflamed right hind paw of the rat being dipped in plethysmograph for measuring the paw volume.

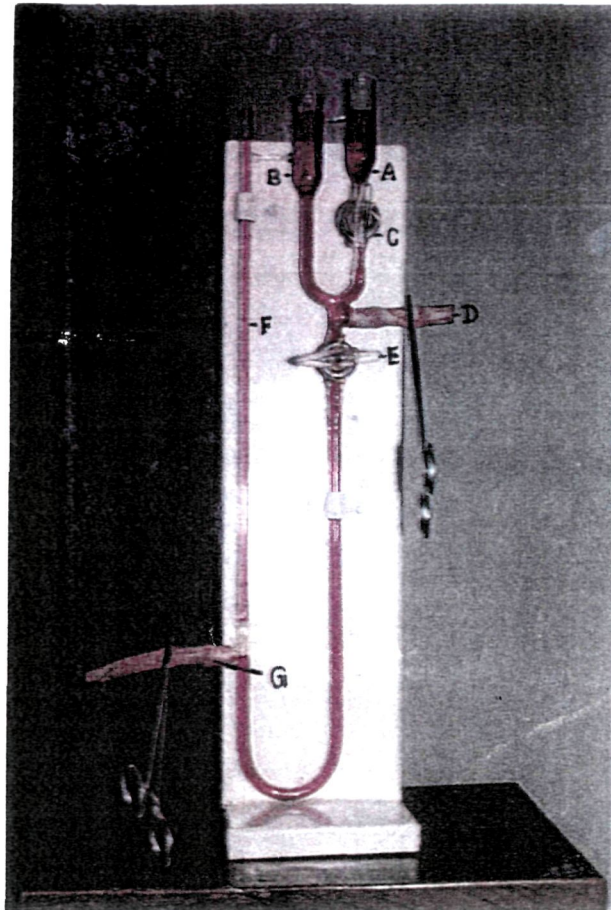


FIGURE-4.15

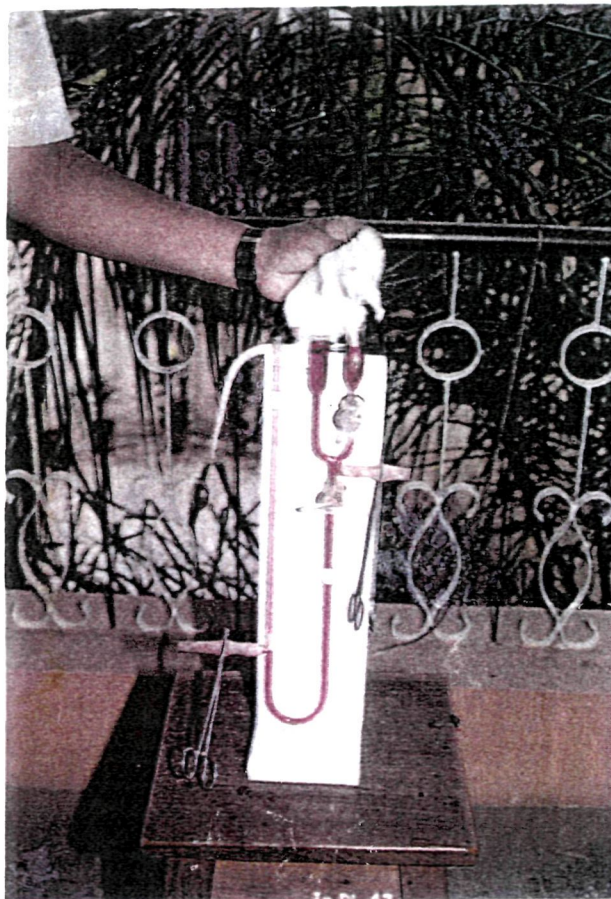


FIGURE-4.16

bubble in limbs 'A' and 'B', was kept closed through out the experiment. This procedure was repeated every time for measuring the paw volume.

For this study, albino rats (Wistar strain) of either sex weighing between 100-200 g were used and divided into 6 groups of 4 animals each. The group I served as control and received Tween-80 (0.1%, 1 ml) solution orally. The group II received Ibuprofen at a dose of 40 mg/kg body weight in Tween-80 (0.1%, 1 ml) and served as standard. The group III, IV, V and VI received orally the petroleum ether extract, chloroform extract, ethanol extract and water extract respectively at the dose of 600 mg/kg body weight in Tween-80 (0.1%, 1 ml) solution. These drugs were administered one hour before the injection of the irritant. After 1 hr all the animals were injected subcutaneously carrageenan solution (0.1%, 0.05 ml) to the right hind paw in the subplantar region, immediately the paw volume was measured ('0' hour). After 3 hr the paw volume was measured in control, in standard and in test animals.

The percentage inhibition of paw volume was calculated by using the formula,

$$1 - \frac{V_t}{V_c} \times 100$$

Where, V_t = Mean increase in the paw volume in test animal group.

V_c = Mean increase in the paw volume in control group.

Statistical analysis was carried to determine % protection,

Table-4.10 Anti-inflammatory activity of the extracts of *Balanites Roxburghii*

Group	Compound	Dose (mg/kg)	Difference in paw volume $\pm$ SEM after 3 hr	Percentage inhibition of edema after 3 hr
I	Control	-	0.600 ± 0.028	..
II	Ibuprofen	40	0.146 ± 0.046	75.7
III	Petroleum ether extract	600	0.441 ± 0.078	26.4
IV	Chloroform extract	600	0.372 ± 0.061	38.0
V	Ethanol extract	600	0.320 ± 0.420	46.7
VI	Water extract	600	0.403 ± 0.325	32.8

Index for anti-inflammatory activity.

Model	:	Acute inflammation
Method	:	Carrageenan induced edema
Animals	:	Albino rats
Number of animals per group	:	4
Route of administration	:	Oral
Standard	:	Ibuprofen
Control	:	Tween-80 (0.1%)
S.E.M.	:	Standard Error Mean.

Results

The four extracts of the fruits of *Balanites Roxburghii* were tested for anti-inflammatory activity against carrageenan-induced rat paw edema. The activity was compared with Ibuprofen. Ethanol extract and water extract showed considerable anti-inflammatory activity when compared with the standard drug.

Discussion

The results clearly indicate that ethanol extract and water extract possess anti-inflammatory activity. Carrageenan-induced rat paw edema model is commonly used for evaluating anti-inflammatory activity. The effect is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the 1 hr after administration of carrageenan, a more pronounced phase, 2nd phase, is attributed to the release of bradykinin, protease, prostaglandin and lysozyme. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. The results suggest that mechanism of action of the tested extracts requires more investigation.

4.F Analgesic activity

Introduction

In early days, the mankind depended on divine healing through laying on of hands or prayer for relief of pain. However, after sometime, man did not rely exclusive on spiritual methods for pain reduction. Portions concocted from herbal extracts were used by earlier physicians for this purpose. Alcohol and methanolic extract of opium were used by Hippocrates for the production of the insensibility to pain. It was only when the first alkaloid morphine, isolated in 1803 by Serturmer, the scientific era of analgesia was realized. Analgesia can be defined as a reduction of pain perception without the loss of consciousness. The diminution of pain may be a consequence of febrifuge effect of the drug. After the discovery of morphine as an analgesic, lot of research work has been carried out to increase the efficacy of morphine and reduce its undesired side effects. The research work mainly was on the following lines.

1. Structural modification of morphine
2. Synthesis of morphine like compounds
3. Synthesis of other heterocyclic compounds
4. Random screening of synthesized compounds
5. Subjecting different plant extracts for analgesic response on mice or rats
6. Isolation of active components from plant extracts

It is a well known fact that plant possessing anti-inflammatory property can act as analgesic also. As various extracts of the fruits of the *Balanites Roxburghii* have been shown to possess significant anti-inflammatory activity, it is envisaged now to subject various extracts for analgesic activity in albino mice.

There are mainly three methods available to evaluate analgesic activity namely,

- a. Acetic acid induced writhing method in mice
- b. Tail flick method in rats
- c. Eddy's hot plate analgesiometer method in mice

In the present investigation, analgesic activity of various extracts was evaluated by using acetic acid induced writhing method in mice.

Acetic acid induced writhing method

The analgesic activity of the extracts of *Balanites Roxburghii* was done on albino mice.

Materials and Method

Albino mice weighing 22-35 g of Swiss strain.

Acetic acid (0.6%).

Standard drug (Acetyl salicylic acid).

Stop clock.

Tween-80 solution (0.1%).

Colony bred albino mice (Swiss strain) of either sex weighing 22-35 gm were used to evaluate analgesic activity²⁸. Analgesic activity was determined as described by the method based on acetic acid induced writhing in mice²⁹⁻³⁸. To study the response of various extracts of *Balanites Roxburghii* for analgesic activity, 24 mice were selected and divided into 6 groups comprising of 4 animals each. All the animals received 0.6% acetic acid solution (10 ml/kg body weight) intraperitoneally and number of writhing were recorded after 5 minutes for a period of 20 minutes. These groups served as control group. The same groups of animals were used next day for evaluating analgesic activity of various extracts of the plant.

First group received of Tween-80 (0.1%, 0.5 ml) and served as control, second group received the suspension of acetyl salicylic acid (150 mg/kg body weight) in Tween-80 (0.1%, 5 ml) orally and served as standard. The animals in III, IV, V and VI groups received various extracts of the fruits of *Balanites Roxburghii* at a dose of 200 mg/kg suspended in Tween-80 (0.1%). After one hour, all the animals received 10 ml/kg body weight of acetic acid (0.6%) intraperitoneally, and number of writhing were recorded after 5 min. for the duration of 20 min. The results are recorded in the **Table-4.11**. The photographs showing analgesic activity are represented in **Figure-4.17** and **Figure-4.18**.

The percentage protection was calculated by using the formula

$$1 - \frac{V_t}{V_c} \times 100$$

Where, V_t = Mean number of writhing in test animals

V_c = Mean number of writhing in control.

Table-4.11 Analgesic activity of the extracts of *Balanites Roxburghii*.

Group	Compound	Dose (mg/kg)	Mean No. of writhing		Percentage protection
			Without the administration of the drug $\pm$ SEM	With the administration of the drug $\pm$ SEM	
I	Control	-	45.83 $\pm$ 1.28	45.83 $\pm$ 1.28	-
II	Acetyl salicylic acid	150	47.00 $\pm$ 2.50	11.80 $\pm$ 1.27	74.9
III	Petroleum ether extract	600	20.50 $\pm$ 1.58	07.83 $\pm$ 1.79	61.8
IV	Chloroform extract	600	40.00 $\pm$ 2.73	14.33 $\pm$ 3.88	64.2
V	Ethanol extract	600	28.76 $\pm$ 2.17	12.18 $\pm$ 1.06	57.6
VI	Water extract	600	27.34 $\pm$ 1.56	17.67 $\pm$ 1.89	35.4

Figure-4.17 Photograph showing the mice being injected with acetic acid for inducing writhing.

Figure-4.18 Photograph showing the mice exhibiting writhing.



FIGURE-4.17

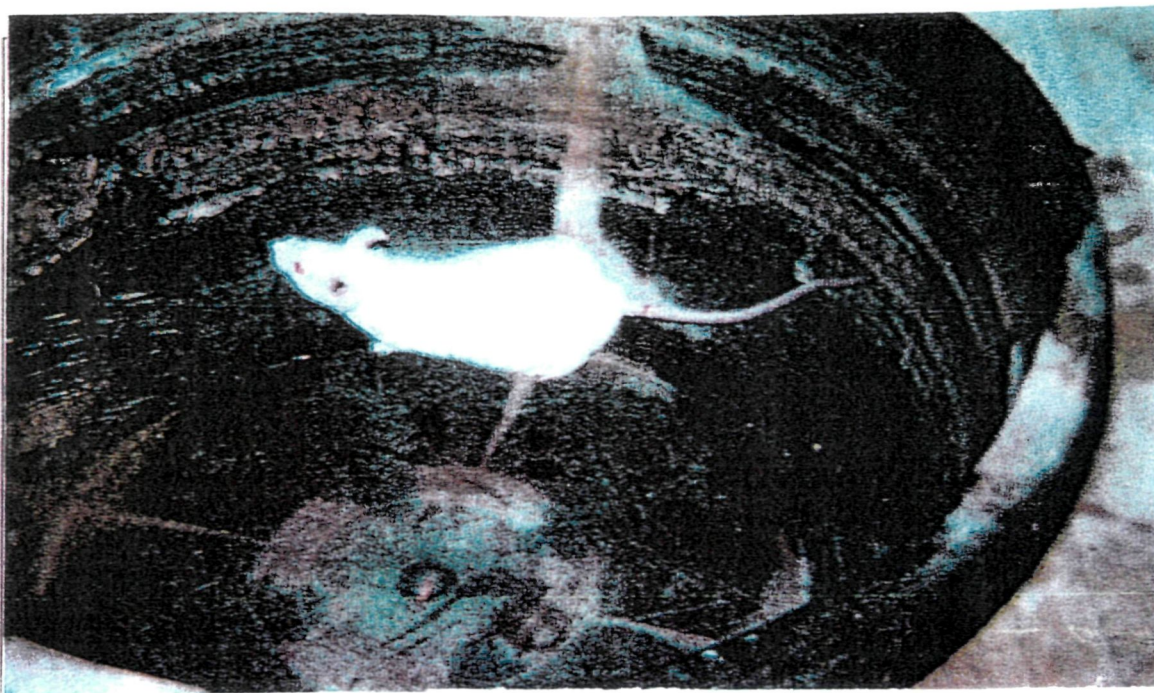


FIGURE-4.18

Index for analgesic activity.

Method	:	Acetic acid induced writhing
Animals	:	Swiss albino mice
Number of animals per group	:	4
Route of administration	:	Oral
Standard	:	Acetyl salicylic acid
Control	:	Tween-80 (0.1%)
S.E.M.	:	Standard Error Mean.

Results

The observations revealed that the petroleum ether, chloroform and ethanolic extract exhibited considerable analgesic activity when compared with that of standard drug and water extract showed poor analgesic effect. Among all the extracts, the chloroform extract was found to exhibit maximum analgesic activity.

Discussion

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nerves system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only rise the threshold of pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. The petroleum ether, chloroform and ethanolic extracts of the fruits of *Balanites Roxburghii* exhibited significant analgesic activity when compared with acetyl salicylic acid.

4.G Diuretic activity

Introduction

Diuretics are class of drugs that increase the rate of urine flow, however clinically useful diuretics also increase the rate of excretion of Na^+ and an accompanying anion, usually, Cl^- . Sodium chloride in the body is the major determinant of extracellular fluid volume and most clinical applications of diuretics are directed towards reducing the extracellular fluid volume by decreasing the total sodium chloride content of the body. A sustained imbalance between the dietary sodium loss is incompatible with the life. A sustained positive sodium balance would result in volume overload with pulmonary edema and a sustained negative sodium balance would result in volume depletion and cardiovascular collapse. Although, continued administration of a diuretic causes sustained net deficit in total body sodium, the time course of natriuresis was finite as renal compensatory mechanisms bring sodium excretion in line with sodium intake, a phenomenon known as diuretic braking. Frusemide is a standard drug, used to evaluate diuretic activity.

Materials and Method

Albino rats weighing 150-200 g of (Wistar strain).

Tween-80 solution (0.1%).

Stop clock.

Standard drug (Frusemide).

Metabolic cage.

Measuring jar.

For evaluation of diuretic activity on albino rats (Wistar strain) the method adopted by Lipschitz³⁹ was employed. Rats of either sex, weighing between 100-200 g were divided into 6 groups each containing 4 animals. Group one served as control and received 5 ml of 0.1% Tween-80 in distilled water orally. Group two received 40 mg/kg body weight of frusemide in 0.1% Tween-80 orally and served as standard. Group 3, 4, 5 and 6 received the extracts at the dose of 600 mg/kg body weight suspended in 0.1% Tween-80 orally. Each group of animals was kept in different metabolic cages provided with a wire mesh at the bottom and a funnel to collect urine. Sieves made up of stainless steel were placed on the funnel to retain feces. The rats were fed with standard diet and water *ad libitum*. Food and water were withdrawn 24 hr prior to the experiment. Urine excretion was collected after 5 hr and the values were tabulated in Table-4.12. The Figure-4.19 shows the rats in metabolic cage.

Table-4.12 Diuretic activity of the extracts of *Balanites Roxburghii*.

Group	Compound	Dose (mg/kg)	Volume of urine collected after 5 hr.	T/S (Lipschitz values)
I	Control	600	08	0.27
II	Standard	600	29	1.00
III	Petroleum ether extract	600	14	0.55
IV	Chloroform extract	600	17	0.58
V	Ethanol extract	600	12	0.41
VI	Water extract	600	8	0.69

Figure-4.19 Photograph showing rats in metabolic cage.

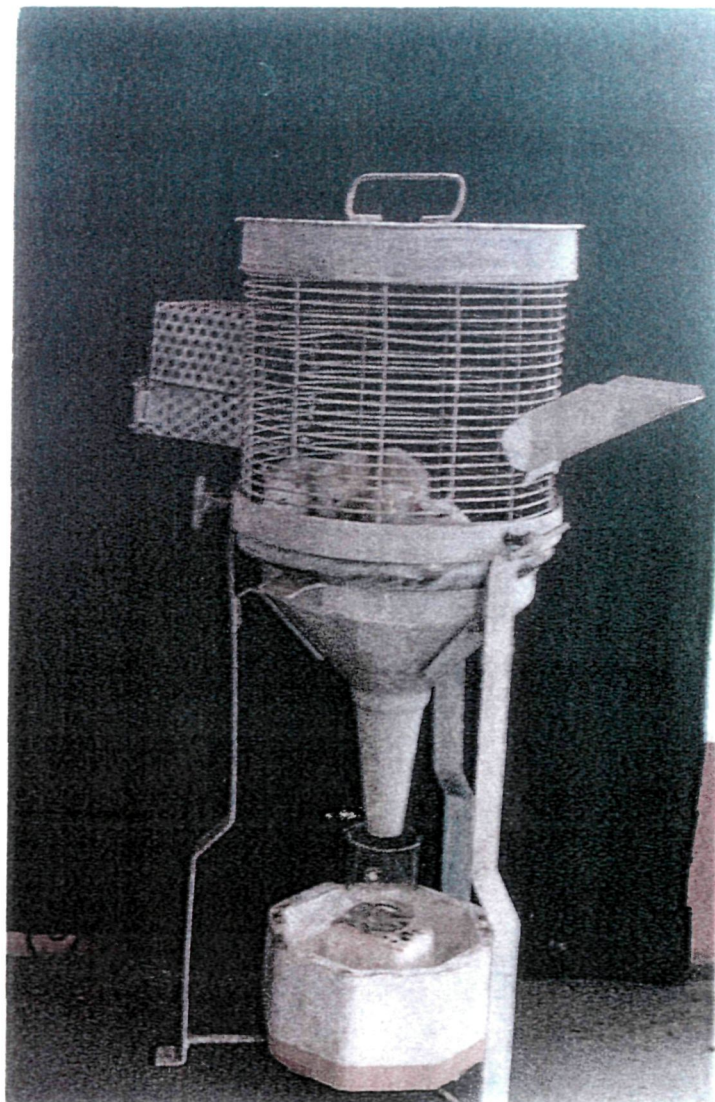


FIGURE-4.19

Index for diuretic activity.

Animals	:	Albino rats
N $\bar{o}$. of animals per group	:	4
Standard	:	Frusemide
T	:	Urine collected for extracts
S	:	Urine collected for standard drug
Control	:	Tween-80 (0.1%)

Results

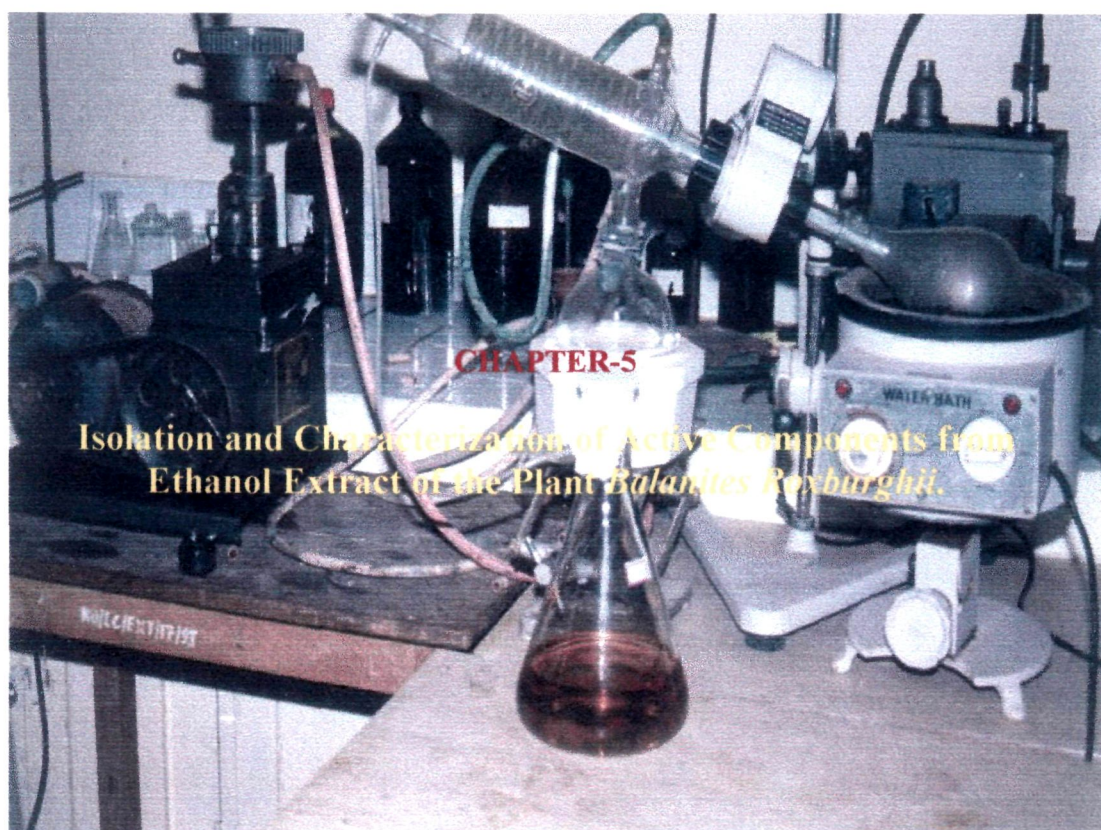
The Lipschitz value for the standard drug Frusemide is taken as 1. The tested extracts of *Balanites Roxburghii* showed less T/S value. Hence, only water extract exhibited diuretic property and the other extracts showed less diuretic activity.

References

1. Basak, "Fasc. Fl. India", **4**, 20 (1980).
2. George Watt, "The Economic Products of India", **1**, 363 (1972).
3. R.N. Chopra, S.L. Nayar and I.C. Chopra, "Glossary of Indian Medicinal Plants", **32**, 1992).
4. U. Khanna and R.R. Chaudhury, *Indian J. Med. Res.*, **56**, 1575 (1968).
5. M.N. Ghosh, In; "Fundamentals of Experimental Pharmacology", Second Edn., Scientific Book Agency, Calcutta, (1984).
6. D.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
7. C.A. Good, H. Cramer and M. Somogyi, *J. Biol. Chem.*, **100**, 485 (1956).
8. N.K. Lohiya and R.B. Goyal, *Ind. J. Exp. Biol.*, **30**, 1051 (1991).
9. B Shivarama Holla, Balakrishna Kalluraya and Suresh N. Shetty, *Indian J. Heterocyclic Chem.*, **2**, 61 (1992).
10. H.M. Vagdevi, K.P. Latha, V.P. Vaidya, M.L. Vijaya Kumar and K.S.R. Pai, *Indian J. Pharma. Sci.*, **63**(4), 286 (2001).
11. C. Fayard, Ascaridiose Ed Piperazine. Thesis, Paris (Quoted from Sem., Hop. Paris, 35: 1778 (1949).
12. K.N. Gaind, R.N. Dhar, B.N. Chopra and K.N. Kaul, *Indian J. Pharm.*, **27**, 198 (1963).
13. O.D. Standen, Chemotherapy of Anthelmintic Infections. In 'Experimental Chemotherapy' Vol 1 (Schnitzer R. J. and Hawking F, Eds) Academic Press Inc. New York. **3**, 701 (1963).
14. Balakrishna Kalluraya and S. Sreenivasa, *IL. Farmaco.*, **53**, 399 (1998).
15. Guruprasad, R.D. Patel and J.S. Biradar, *Asian J. Chem.*, **12**, 39 (2000).
16. C.A. Winter, E.A. Risley and G.W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
17. M.D. Rosa, J.P. Giroud, and D.A. Willoughby, *J. Path.*, **104**, 1, 15 (1971).

18. D.N. Kellet, *J. Pharmacol.*, **24**, 705 (1965).
19. M.N. Ghosh and H. Singh, *Brit. J. Pharmacol.*, **51**, 503 (1974).
20. S. Rashad, A. Hemingway and K. Rinford, "Effect of Nonsteroidal Anti-inflammatory Drugs on the course of Osteoarthritis", *Lancet*, **2:1**, 519 (1989).
21. G. Wilhlmi and R.D. Domenjoz, *Ann. Univ. Sarauiensis. Med.*, **1**, 317 (1953).
22. P. Sirodia and Rao, Symposium on "C.N.S. Drugs", Regional Research Laboratory, Hyderabad, **1**, 238 (1966).
23. K.O. Rainford, (Ed) "*Inflammation mechanisms and actions of Traditional Drugs*" I, Anti-inflammatory and Anti-rheumatic drugs (R C Press Boca. Raton, FL, 1985a).
24. G. Bagavant, S.R. Gole, V.W. Joshi and S.B. Soni, *Indian J. Pharm. Sci.*, **56(3)**, 80 (1994).
25. A.M. Sobottka, W. Werner, G. Blaschke, W. Kiefer, U. Nowe, G. Dannhardt, E.E. Schapoval and G.K. Scriba, *Arch. Pharm. (Weinheim)*, **333(7)**, 205 (2000).
26. H.K. Hsieh, L.T. Tsao, J.P. Wang and C.N. Lin, *J. Pharm. Pharmacol.*, **52(2)**, 163 (2000).
27. S.L. Belagali and M. Himaja, *Indian J. Chem.*, **348**, 505 (1999).
28. J. Weijlard, P.D. Orahovats, A.P. Sullivan, Jr., G. Purdue, F.K. Heath and K. Pfisher, III. *J. Am. Chem. Soc.*, **78**, 2342 (1956).
29. K. Fukava, O. Kawano, M. Hibi, N. Misaki, S. Onba and T. Hatunaka, *J. Pharmacol. Meth.*, **4**, 251 (1980).
30. T.N. Bhalla and K.P. Bhargava, *J. Pharmacol. Meth.*, **3**, 9 (1980).
31. G. Hayashi and A.E. Takemori, *Eur. J. Pharmac.*, **19**, 63 (1971).
32. J.R. Vane, "Inhibition of Prostaglandin Synthesis as a Mechanism of action of Aspirin like Drugs", *Nature New Biol.*, **231**, 232 (1971).
33. M.M. Baddi and C.S. Mahajan Shetty, *Indian J. Chem.*, **36B**, 1074 (1997).
34. O.H. Hishmat, H.I. El-Diwani, F.R. Melek and H.M. El-Sahrawi, *Indian J. Chem.*, **35B**, 30 (1996).

35. T. Naqvi, V.C. Dhawan, W. Haq, R. Raghydir, G.H. Patnaik, K.B. Mathur and B.N. Dhawan, *Indian J. Chem.*, **33B**, 445 (1994).
36. S.M. Sondhi, Nidhi Singhal and R.P. Verma, *Indian J. Chem.*, **36B**, 620 (1997).
37. A.R. Saundane, K. Rudresh, N.D. Satyanarayana and S.P. Hiremath, *Indian J. Pharm. Sci.*, **60(6)**, 379 (1998).
38. Yogendra K. Shukla, *Indian J. Chem.*, **33B**, 799 (1994).
39. W.L. Lipschitz, Z. Hadidian and A. Kerpesar, *J. Pharmacol. Exp. Ther.*, **79**, 97 (1943).



5.A Isolation and characterization of active components from ethanolic extract of fruits of *Balanites Roxburghii*

Ethanolic extract of the fruits of *Balanites Roxburghii* exhibited considerable abortifacient and estrogenic activities in Wistar strain female albino rats. It also exhibited good anti-inflammatory, analgesic and anthelmintic activities. In view of these findings, isolation and characterization of components present in ethanol extract was carried out.

Isolation of pure components

The isolation of pure components involved the following steps:

1. Chromatographic separation using silica gel (100-200 mesh)
2. Chromatographic separation using silica gel (60-120 mesh)
3. High performance liquid chromatography (HPLC)

5.A.1 Chromatographic separation using silica gel (100-200 mesh)

Ethanol extract, which was obtained as dark brown colored gummy mass (80 g), was stirred with ethyl acetate (500 ml) to remove residual amount of chlorophyll. The remaining residue was stirred with n-butanol (500 ml) and left overnight. The supernatant liquid was collected and the solvent was removed by using rotary flash evaporator, which yielded brown colored pasty mass (8 g) and it was labeled as butanolic-fraction. The residue, which was obtained as dark brown paste, was labeled as ethanolic-fraction.

The ethanolic-fraction (20 g) was chromatographed over silica gel (100-200 mesh) on column of 52 cm length and 6 cm diameter. Elution was carried out with solvents and solvent mixtures of increasing polarities. Fractions were collected in 100 ml portions and monitored on TLC (silica gel G as adsorbent, solvent system-carbon tetrachloride:ethyl acetate, 60:40) and the fractions showing similar spots were pooled together. The chromatography details are given in **Table-5.1**. Elution with ethyl acetate

and methanol (60:40) gave yellowish hygroscopic solid (2.5 g) and was named as **IBR-1**. Similarly elution with ethyl acetate and methanol (30:70) yielded light brown colored crystalline solid (6.5 g) and it was named as **IBR-2**. Elution with pure methanol produced dark brown colored gummy mass (1.5 g) and was labeled as **IBR-3**.

The butanolic fraction, which was obtained as pasty mass, was further purified by column chromatography using silica gel (100-200 mesh). The elution of the column with ethyl acetate and methanol (50:50) produced dark brown colored gummy mass (2.5 g) and it was named as **IBR-4**.

The details of the column chromatography are given in **Table-5A.1** and **Table-5A.2**.

Table-5.A.1 Column chromatography of the ethanolic fraction over silica gel (100-200 mesh) (each fraction 100 ml).

Number of fractions	Eluent	Residue on evaporation	Yield in g
1-7	Petroleum ether	No residue	-
	Petroleum ether : Chloroform		
8-15	90 :10	„	-
16-22	80:20	„	-
23-28	70:30	„	-
29-34	60:40	„	-
35-40	50:50	„	-
41-46	40:60	„	-
47-52	30:70	„	-
53-58	20:80	„	-
59-64	10:90	„	-
65-70	Chloroform	„	-
	Chloroform : Ethyl acetate		
71-76	90 :10	„	-
77-82	80:20	„	-
83-88	70:30	„	-
89-94	60:40	„	-
95-100	50:50	„	-
101-106	40:60	„	-
107-112	30:70	„	-
113-118	20:80	„	-
119-124	10:90	„	-
125-130	Ethyl acetate	„	-
	Ethyl acetate : Methanol		
131-136	90 :10	„	-
137-142	80:20	„	-
143-154	70:30	„	-
155-169	60:40	Yellowish white solid (IBR-1)	2.5 g
170-185	50:50	No residue	-
186-210	40:60	No residue	-
211-225	30:70	Light brown crystalline solid (IBR-2)	6.5 g
226-242	20:80	No residue	-
243-265	10:90	No residue	-
266-272	Methanol	Dark brown gummy mass (IBR-3)	1.5 g

Table-5.A.2 Column chromatography of the butanolic fraction over silica gel (100-200 mesh) (Each fraction 100 ml).

Number of fractions	Eluent	Residue on evaporation	Yield in g
100 ml	Petroleum ether	No residue	-
	Petroleum ether : Chloroform		
1-7	90 :10	„	-
8-15	80:20	„	-
16-21	70:30	„	-
23-27	60:40	„	-
28-33	50:50	„	-
34-40	40:60	„	-
41-47	30:70	„	-
48-51	20:80	„	-
52-57	10:90	„	-
58-63	Chloroform	„	-
	Chloroform : Ethyl acetate		
64-70	90 :10	„	-
71-75	80:20	„	-
76-81	70:30	„	-
82-87	60:40	„	-
88-93	50:50	„	-
94-99	40:60	„	-
10-106	30:70	„	-
107-112	20:80	„	-
113-116	10:90	„	-
117-121	Ethyl acetate	„	-
	Ethyl acetate : Methanol		
122-127	90 :10	„	-
128-140	80:20	„	-
141-149	70:30	„	-
150-156	60:40	„	-
157-180	50:50	Dark brown gummy mass (IBR-4)	2.5 g
181-185	40:60	No residue	-
186-190	30:70	No residue	-
191-196	20:80	No residue	-
197-200	10:90	No residue	-
201-204	Methanol	No residue	-

5.A.2 Purification of IBR-2 by column chromatographic separation using silica gel (60 –120 mesh)

The fractions **IBR-1**, **IBR-2**, **IBR-3** and **IBR-4** were subjected for screening for antifertility activity and other biological activities as described in **Chapter-4**. It was observed that **IBR-2** exhibited considerable antifertility activity (described in **Section-B**), whereas remaining fractions showed relatively less activity. Hence it was thought of interest to purify and isolate the compounds present in **IBR-2**.

The **IBR-2** was found to be impure and it was contemplated to purify it further. Thus **IBR-2** (6 g) was chromatographed over silica gel (60-120 mesh) on column of 15 cm length and 3 cm diameter. Elution was carried out with solvents and solvent mixtures of increasing polarities. Fractions were collected (25 ml portions) and monitored on TLC (silica gel G as adsorbent, solvent system-ethyl acetate:methanol 60:40) and the fractions showing similar spots were combined. The chromatography details are given in the **Table-5.A.3**.

Table-5.A.3 Purification of IBR-2

Number of fractions	Eluent	Residue on evaporation	Yield in gm
	Ethyl acetate : Methanol	Light brown solid	Negligible
1-10	90 :10	Light brown solid	Negligible
11-18	80:20	Light brown solid	Negligible
19-26	70:30	Light brown solid	Negligible
27-34	60:40	Light brown solid	Negligible
35-47	50:50	Light brown crystalline solid	4 g

The last fraction yielded a light brown-colored solid showing a single spot on TLC with slight tailing. **IBR-2** showed the absence of nitrogen and sulphur in elemental analysis. This ruled out the possibility of the presence of any alkaloid or protein or any

other nitrogenous compounds. **IBR-2** also showed the absence of steroid compounds as indicated by negative Libermann sterol test. It failed to give positive Shinoda test, confirming the absence of 5-hydroxy flavanoids.

5.A.3 High performance liquid chromatography

In a classical liquid chromatography, the mobile liquid phase flows slowly through the column by means of gravity. This method is characterized by low column efficiency and long separation time. Since 1969, there has been a marked revival of interest in the technique of liquid chromatography because of the introduction of High performance liquid chromatography (HPLC) by Kirkland and Huber. HPLC systems are capable of operating at pressure of 1000-6000 psi. The columns have small diameter of 1-3 mm with support particle size in the region of 30 μm . The eluent is pumped through the column at high flow rate. The efficiency is nearly 100 times more than that of conventional liquid chromatography.

The apparatus of HPLC

The modern high performance liquid chromatograph comprises of the following four basic components:

1. Solvent delivery system (mobile phase)
2. Sample injection system
3. Chromatographic column (stationary phase)
4. Detector and recording system

1. Solvent delivery system (including pump)

The mobile phase is pumped under pressure from reservoir and flows through the column at a constant rate. It is always advisable to use doubly distilled HPLC grade solvents after deaeration.

The choice of mobile phase is very important in HPLC. The eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of sample components. For normal phase separation, eluting power increases with increasing polarity of the solvent, but for reverse phase separations, eluting power decreases with increasing polarity of the solvent. Optimum separating conditions can often be achieved by making use of a mixture of two solvents.

Another important component of HPLC is the pump, because its performance directly affects the retention time, detector sensitivity and reproducibility. For analytical applications in which column of 25-50 cm in length, packed with particles of 5-10 μm , the pump must be capable of delivering mobile phase at flow rate of 1-10 ml min^{-1} and at nearly 5000 psi of pressure.

2. Sample injection system

There are two important ways of introducing the sample into the injection port, viz.,

- Valve injection
- On column injection

There are two types of valve injectors, the one in which a fixed volume is introduced by making use of a fixed volume loop injector and the other in which a variable volume can be introduced by making use of an injection valve.

In on column injection, variable volume can be introduced by means of a syringe through a septum. The most widely used devices, in currently marketed commercial HPLC instruments, are the micro volume sampling valves which enable samples to be introduced reproducibly into pressurized columns without significant interruption of the mobile phase flow. The sample, in solution form, is first loaded into an external loop in the valve and then introduced into the mobile phase by rotating the valve.

3. The chromatographic column

The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure, having length of 10-50 cm narrow tubes and filled with stationary particles of diameters of 25 μ m or less. The column packing material is generally silica or modified silica particles.

4. Detectors

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. An ultraviolet photometer operating at wavelength of 215-280 nm is one of the most widely used detectors for HPLC, because of its relatively low cost and high sensitivity to change in temperature, flow rate and mobile phase composition.

In the present investigation, careful examination of TLC indicated that the **IBR-2** contained more than one component in it. But, it was not possible to isolate the components by conventional chromatography. Hence, it was envisaged to make use of more effective HPLC to accomplish desired separation and isolation of pure compounds from **IBR-2**.

The HPLC instrument of Shimadzu with ultraviolet detector having micro volume sampling valves as sample injection system was used in the present work. The C-R 5 A chromatopac recorder was used to obtain corresponding chromatogram.

To start with, analytical work was carried out by using Hypersil Analytical Column, to find out the exact number of compounds present in the impure sample and also to fix the proper solvent system. Reverse phase chromatography was used in which mixture of solvents i.e., methanol and water (70:30) was found to be most suitable combination for separation. Double distilled HPLC grade methanol was used and it was deaerated by using sonicator. Similarly, water was also double distilled and deaerated before using as mobile phase. The analytical work was carried out by using both 254 nm and 215 nm wavelengths in the detector. The sample was prepared by dissolving 5 mg of the impure sample in 5 ml of solvent system used for mobile phase. Each time 1 ml of the solution was injected maintaining a flow rate of 10 ml min^{-1} . Even after repeated trials, effective separation could not be achieved. Hence, it was contemplated to use Videx Protein and Peptide (C-18) column. Even though the separation was more effective than Hypersil Analytical Column, it was not found to be suitable for semi-preparative or preparative work.

This necessitated the use of more effective, though expensive, Spherisorb ODS analytical column. Effective separation was obtained using methanol-water (70:30), mobile phase, having concentration of 1 mg/ml of sample. The chromatogram recorded showed as many as five peaks (**Figure-5.1**). Careful examination of peak areas revealed that the impure sample contained mainly one compound and remaining four being in negligible quantity.

ANALYSIS PARAMETER FILE 0

WIDTH 5
 DRIFT 0
 T.DBL 0
 ATEN 4
 METHOD\$ 41
 SPL.WT 100
 SLOPE 250
 MIN.AREA 10000
 STOP.TM 655
 SPEED 10
 FORMAT\$ 0
 IS.WT 1

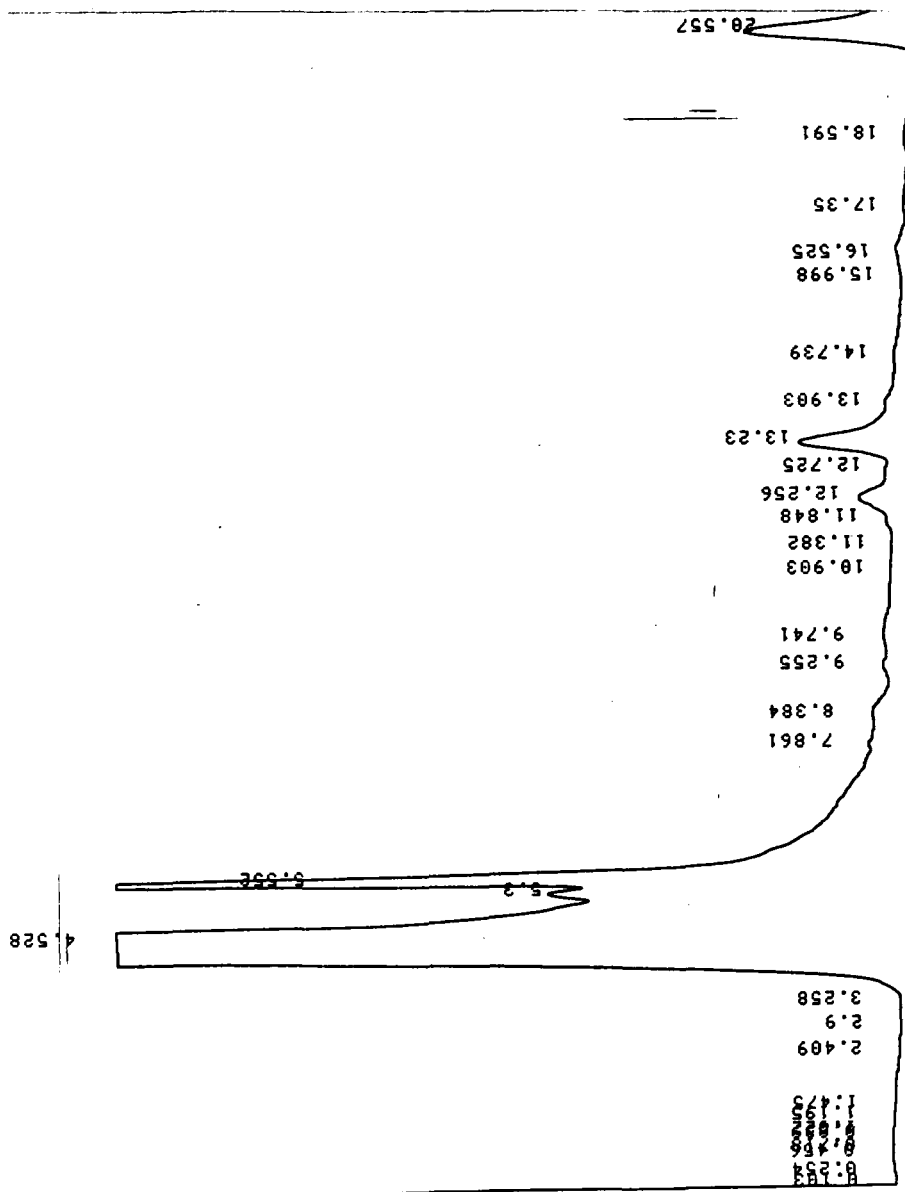


FIGURE-5.1

CHANNEL NO 1
 SAMPLE NO 0
 REPORT NO 1
 PKNO TIME AREA MK IDNO CONC NAME
 FILE METHOD 0 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.528	1475610	V		61.7461	
2	5.3	97838	V		4.0938	
3	5.552	461649	SV		19.3175	
4	12.256	18547	V		0.7261	
5	13.23	50488	SV		2.1126	
6	20.557	137707			5.7623	
7	30.642	13749			0.0753	
8	31.235	79228	V		3.8151	
9	32.267	10090	V		0.4222	
10	32.957	25721	V		1.0784	
11	33.515	19135	V		0.8007	
TOTAL						100
2389802						

Thus, it was planned to scale up the work of separation by using preparative column. For this purpose 250/20 mm ID preparative column was used.

The separation was carried out by using methanol and water (70:30) as mobile phase and keeping the detector at 254 nm wavelength. The impure sample (50 mg) dissolved in mobile phase (10 ml) was injected into the column. The fraction having the retention time 4.528 was collected. Evaporation of the solvent using rotary film evaporator (Buchi) at reduced pressure and controlled temperature, afforded 12 mg of pure compound designated as **Compound-1**. The pure compound was stored under vacuum and anhydrous condition.

5.A.4 Characterization of pure compound

The pure compound **compound-1** was characterized by different physical parameters and spectral studies.

Melting point

Melting point was recorded in an open capillary tube and is uncorrected.

Ultraviolet spectrum

The UV spectrum was measured in aldehyde free spectral grade methanol using JASCO module UVIDEK-610 double beam spectrophotometer and UV-160A from Shimadzu Corporation, Japan.

Infrared spectrum

The infrared spectrum of the compound was recorded in Nujol mull in the range of 4000-600 cm^{-1} on Unicam FTIR (Research spectrometer series).

Nuclear magnetic resonance spectrum

The nuclear magnetic resonance spectrum was recorded on Jeol GSX 27 FT NMR spectrophotometer (300 MHz) using TMS as an internal standard. Sample was prepared by dissolving the compound in DMSO-d₆. Chemical shifts are expressed in δ ppm.

Mass spectrum

The mass spectrum was recorded on Bruker Apex-II mass spectrometer at 70 eV ionization energy with direct inlet system.

Characterization of compound-1**Solvent system**

The **compound-1** was isolated from **IBR-2** using HPLC and methanol-water (70:30) as a solvent system.

Melting point

Melting point of the **compound-1** was found to be 180-186⁰C.

Homogeneity

The homogeneity of **compound-1** was proved by a single iodine staining (and also UV absorbing) spot in TLC using silica gel G as adsorbent at varying polarity solvent systems.

Color reactions

The **compound-1** exhibited a negative ferric chloride test indicating the absence of phenolic hydroxyl groups.

Spectral properties of compound-1

Ultraviolet spectrum

The ultraviolet spectrum (**Figure-5.2**) of **compound-1** was studied to determine the nature of chromographic groups. The UV spectrum recorded in methanol indicated the presence of chromophoric group with an extended conjugation. A bathochromic shift was observed when the spectrum was recorded in presence of sodium methoxide in the same solvent. These observations support the presence of a cyclic ketonic group with extended conjugation.

UV $\lambda_{\max}$ (MeOH) : 358 nm, 300 nm

(NaOMe) : 372 nm, 316 nm

Infrared spectrum:

In the IR spectrum (**Figure-5.3**) of **compound-1** exhibited predominant absorption band at 1680 cm^{-1} .

IR $\bar{\lambda}$ (Nujol) : 1680 cm^{-1}

Proton magnetic resonance spectrum

The ^1H NMR spectrum (**Figure-5.4**) exhibited two multiplets centered at δ 7.90 and δ 7.52 integrating for two and three protons respectively. Two doublets appeared at δ 6.60 and δ 6.41 integrating for one proton each. A singlet was observed at δ 6.7 integrating for one proton, which may be due to a proton situated adjacent to carbonyl group and hence experiencing a deshielding effect. In addition, two singlets at upfield of δ 3.98 and δ 3.93 were observed integrating for three protons each.

^1H NMR (300 MHz, CDCl_3)

δ 7.90 (2H, m)

δ 7.52 (3H, m)

δ 6.71 (1H, s)

δ 6.60 (1H, d)

δ 6.41 (1H, d)

δ 3.98 (3H, s)

δ 3.93 (3H, s)

Mass spectrum

The mass spectrum (**Figure-5.5**) exhibited the molecular ion peak at m/z 282 (100%). In addition, other peaks indicating the fragmentation pattern were observed at ,

m/z 253 (40%)

236 (32%)

209 (22%)

150 (18%)

127 (15%)

5.A.5 Conclusions drawn from the spectral data of compound-1

The UV spectrum of **compound-1** indicated the presence of chromophoric group with an extended conjugation. The bathochromic shift was observed when the spectrum was recorded in presence of sodium methoxide. These observations support the presence of a cyclic ketonic group with extended conjugation¹.

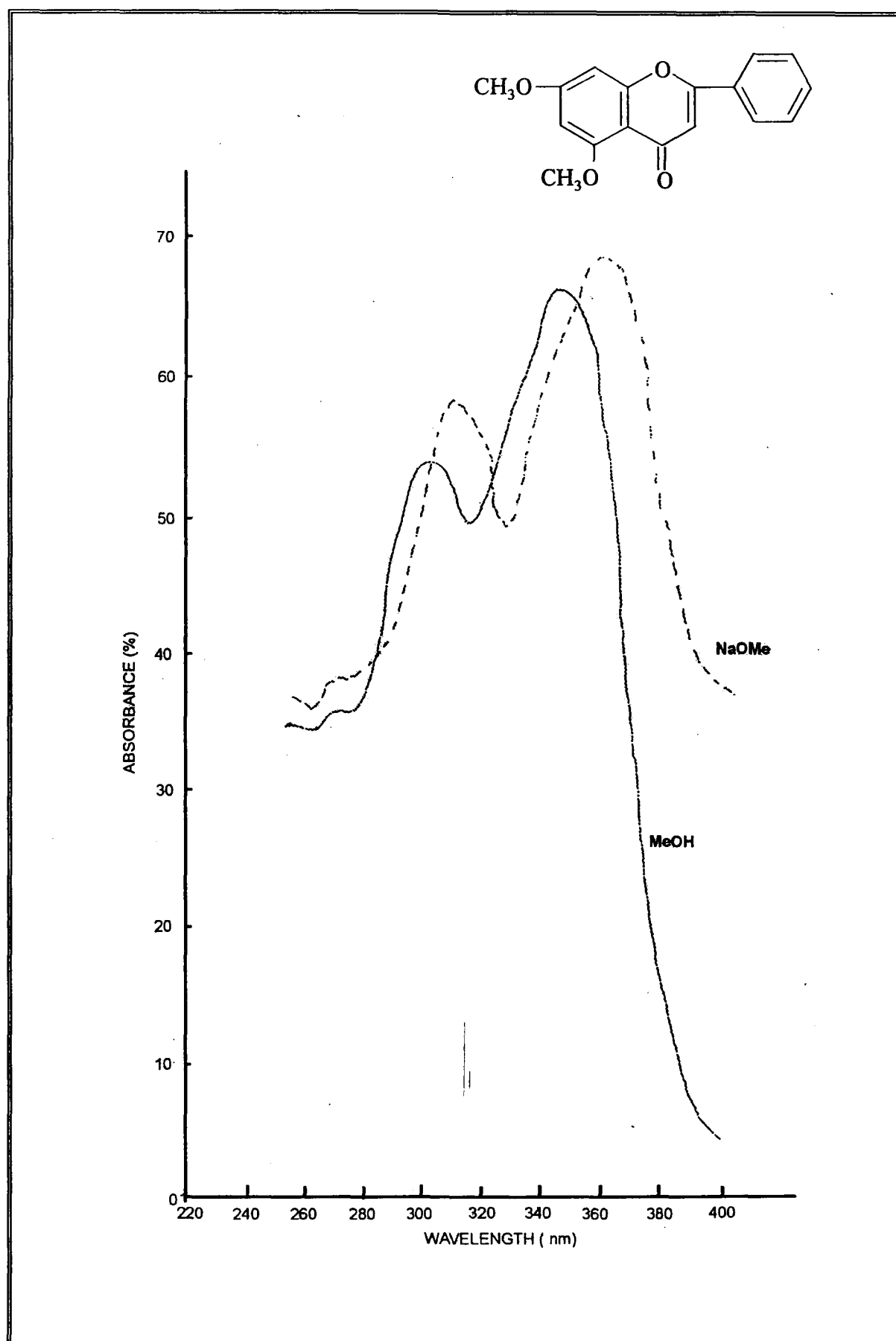


FIGURE-5.2

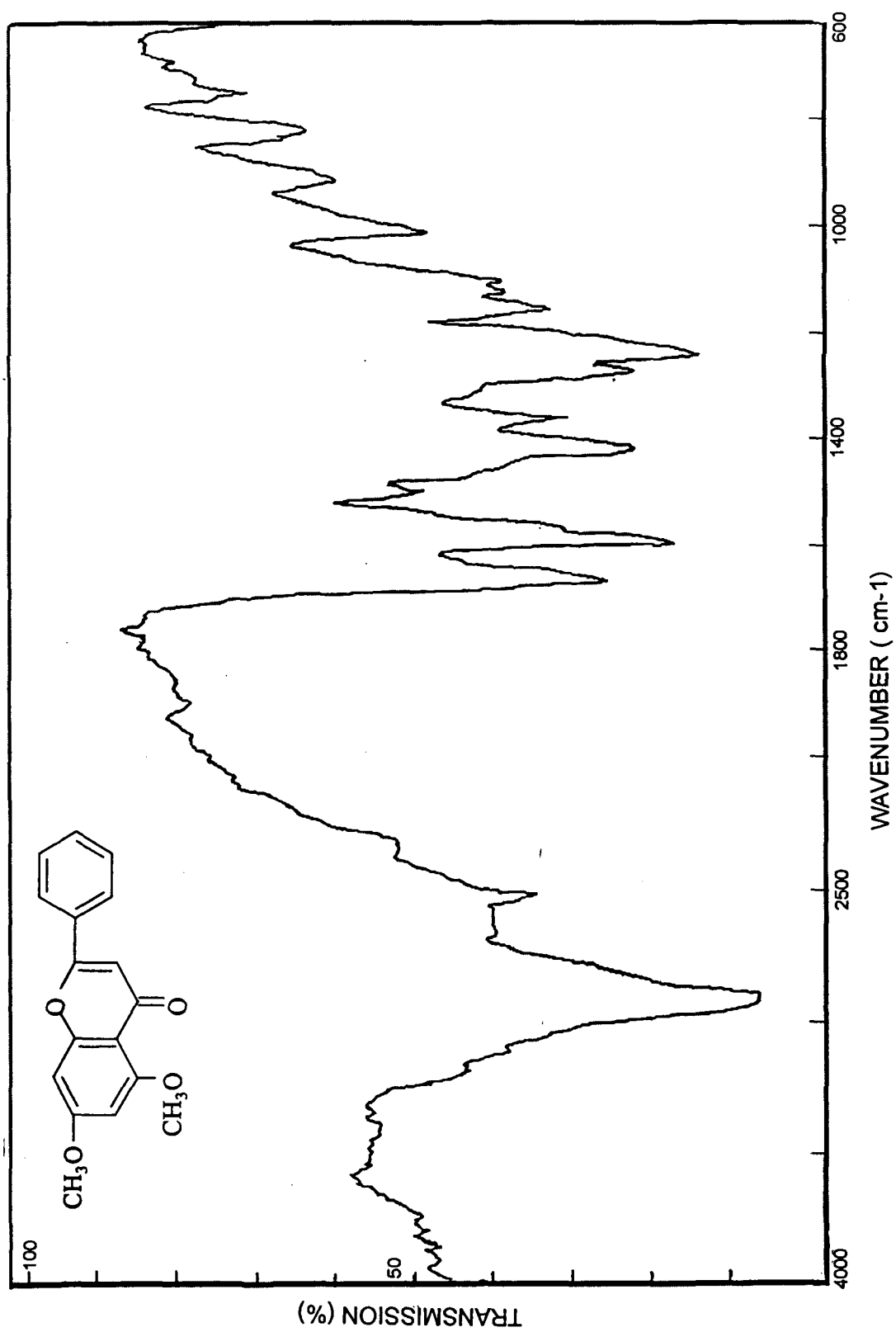


FIGURE-5.3

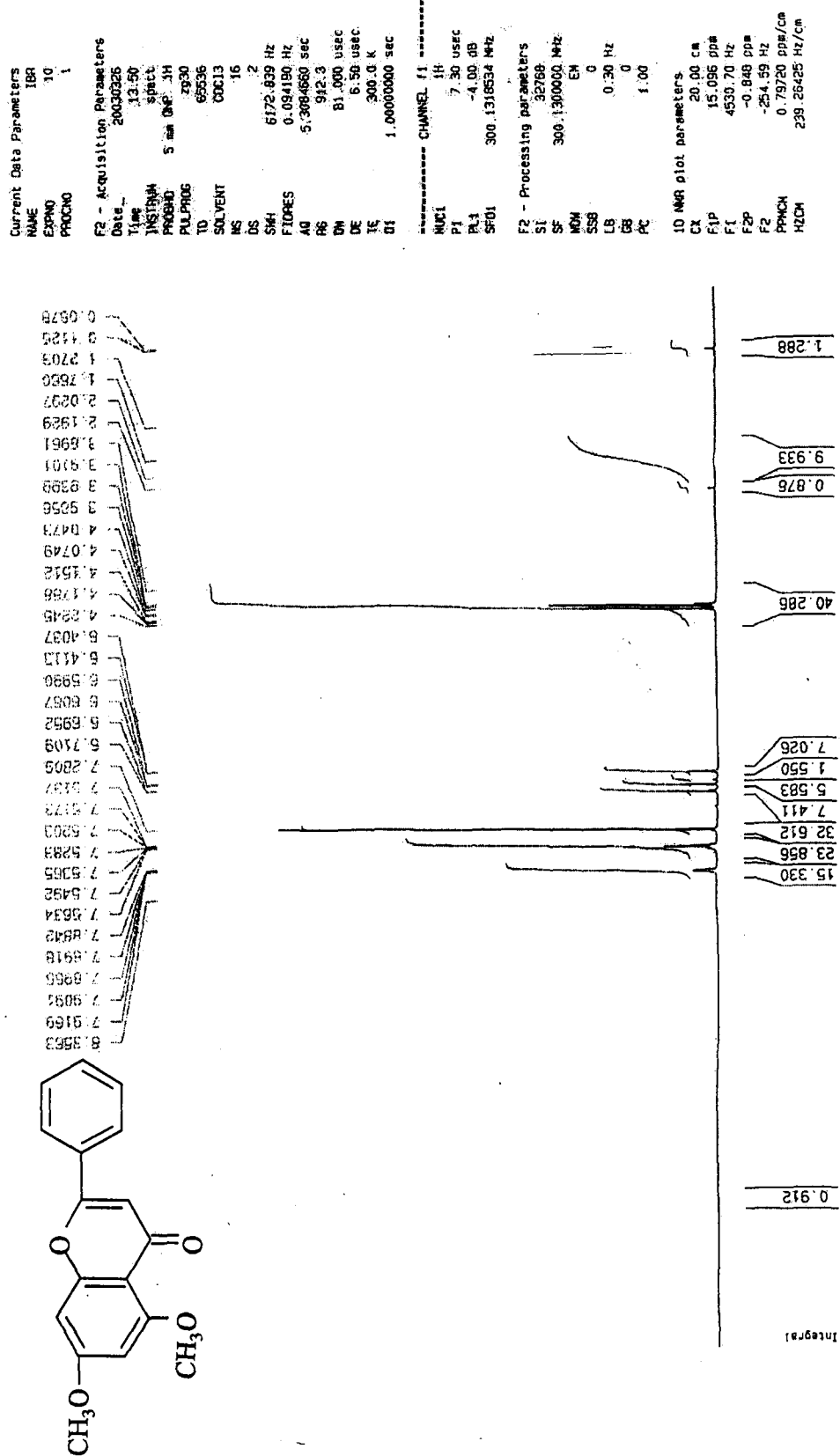


FIGURE-5.4

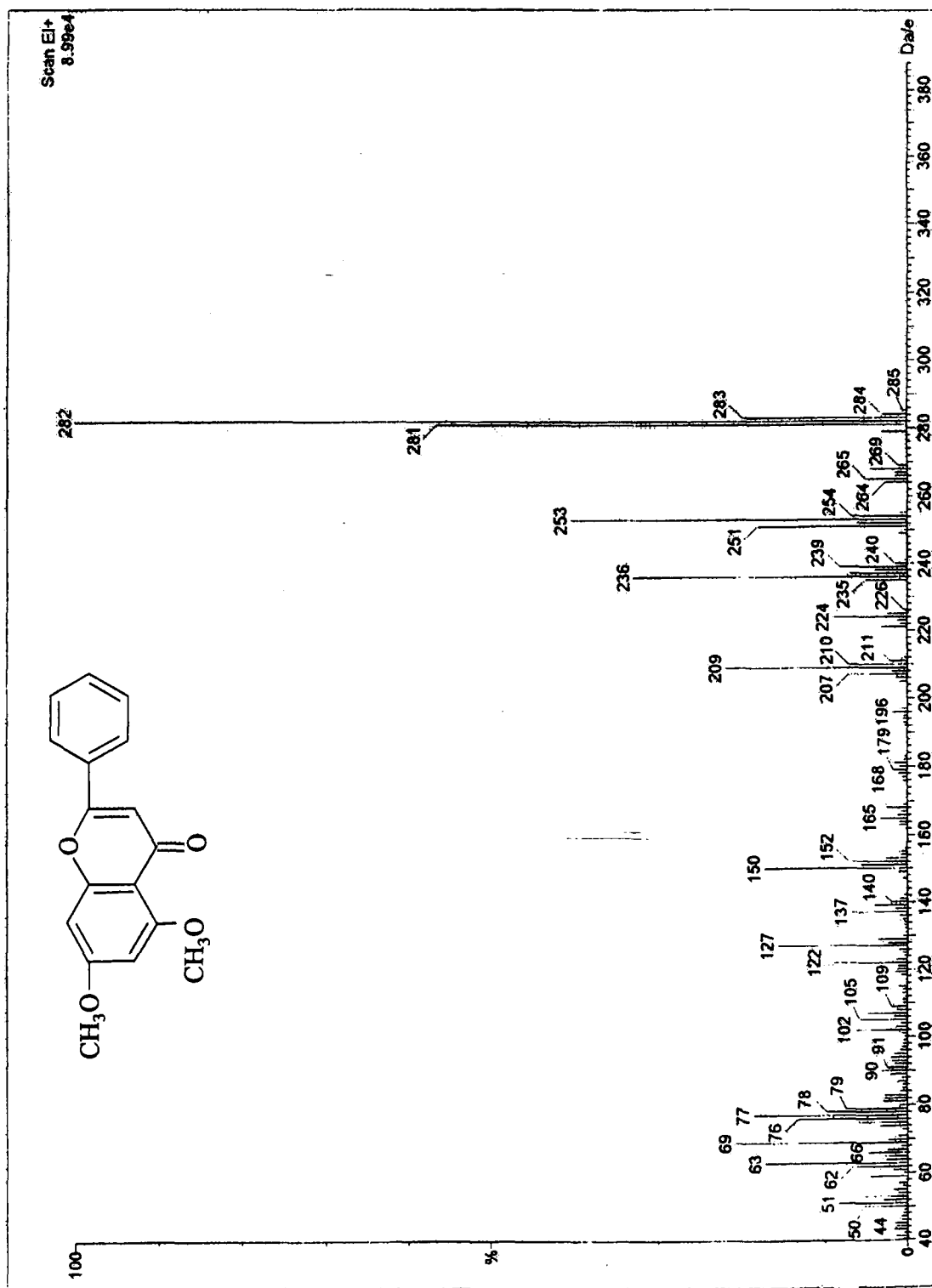


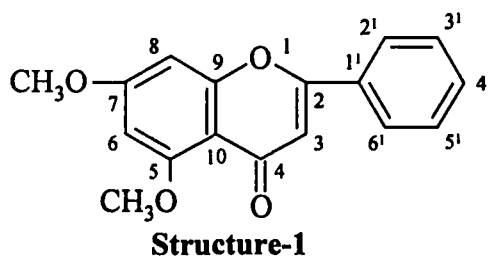
FIGURE-5.5

The methanol spectrum of flavones² exhibit two major absorption bands, commonly referred as Band-I (300-380 nm) and Band-II (240-300 nm). Band-I is considered to be associated with absorption due to B-ring cinnamoyl system and Band-II with absorption involving A-ring benzyl system. The UV spectrum of **compound-1** also exhibited two bands characteristic of flavones.

The IR spectrum of **compound-1** exhibited a predominant absorption band at 1680 cm^{-1} . It may be assigned for the absorption of α , β -unsaturated carbonyl group. This observation supports the conclusion drawn in the study of UV spectrum of **compound-1**.

The ^1H NMR spectrum of **compound-1** exhibited two multiplets at δ 7.90 and δ 7.52. This is the indicative of aromatic protons in **compound-1**. The integration of these multiplets for 5 protons may be attributed to aromatic system in **compound-1**. A singlet appearing at δ 6.71 may be assigned to the methine proton adjacent to the carbonyl group experiencing a deshielding effect. Two more doublets were observed at δ 6.60 and δ 6.41 integrating for one proton each, may be attributed to another set of aromatic protons. These doublets have coupling constant of 7 Hz. Appearance of two signals as singlets at δ 3.98 and δ 3.93 integrating for 3 protons each may be assigned to the protons of two methyl groups. As these peaks appeared at considerable downfield as compared with simple methyl groups, these may be assigned to methyl groups attached to oxygen atom. These observations indicated that the **compound-1** contains two methoxy groups.

On the basis of above spectral data, the **compound-1** under investigation may be assigned flavanoid nucleus having the following structure,



This structure accounts for the extended conjugated ketone as observed in UV and IR spectra.

A multiplet appearing at δ 7.90 for two protons can be assigned to $2'$ and $6'$ protons. The multiplet observed at δ 7.52 for three protons can be attributed to $3'$, $4'$ and $5'$ protons of benzene nucleus attached at position-2. The absorption peak at δ 6.71 for one proton may be due to methine proton at position-3 of chromone nucleus. The downfield shift of peak can be justified due to its location adjacent to the carbonyl group. A doublet appearing at δ 6.60 ($\Delta J = 8$ Hz) can be attributed to proton at position-8. The appearance of this signal at relatively downfield may be due to its conjugation with carbonyl group. Another doublet observed at δ 6.41 can be assigned to a proton at position-6. As discussed earlier two singlets were assigned to the protons of two methoxy groups, the singlet appearing at δ 3.98, relatively at downfield, may be assigned to the protons of OCH_3 group at position-7, which is in conjugation with the carbonyl group. The signal appearing at δ 3.93 can be assigned to the protons of OCH_3 group at position-5. The assignments made are in analogy with the observations reported earlier³⁻⁵.

The **structure-1** assigned to the **compound-1** was further supported by its mass spectrum.. The molecular ion peak was observed at m/z 282 (M^+ , 100%), which is equivalent to the molecular weight of the compound. The fragment ion peak appearing at m/z 253 (40%) accounts for the elimination of two methyl groups. The fragment ion at m/z 209 (22%) accounts for the removal of CO_2 molecule. Further loss of phenyl group resulted in the appearance of peak at m/z 150 (18%) followed by the loss of CO molecule as indicated by the peak at m/z 127 (15%).

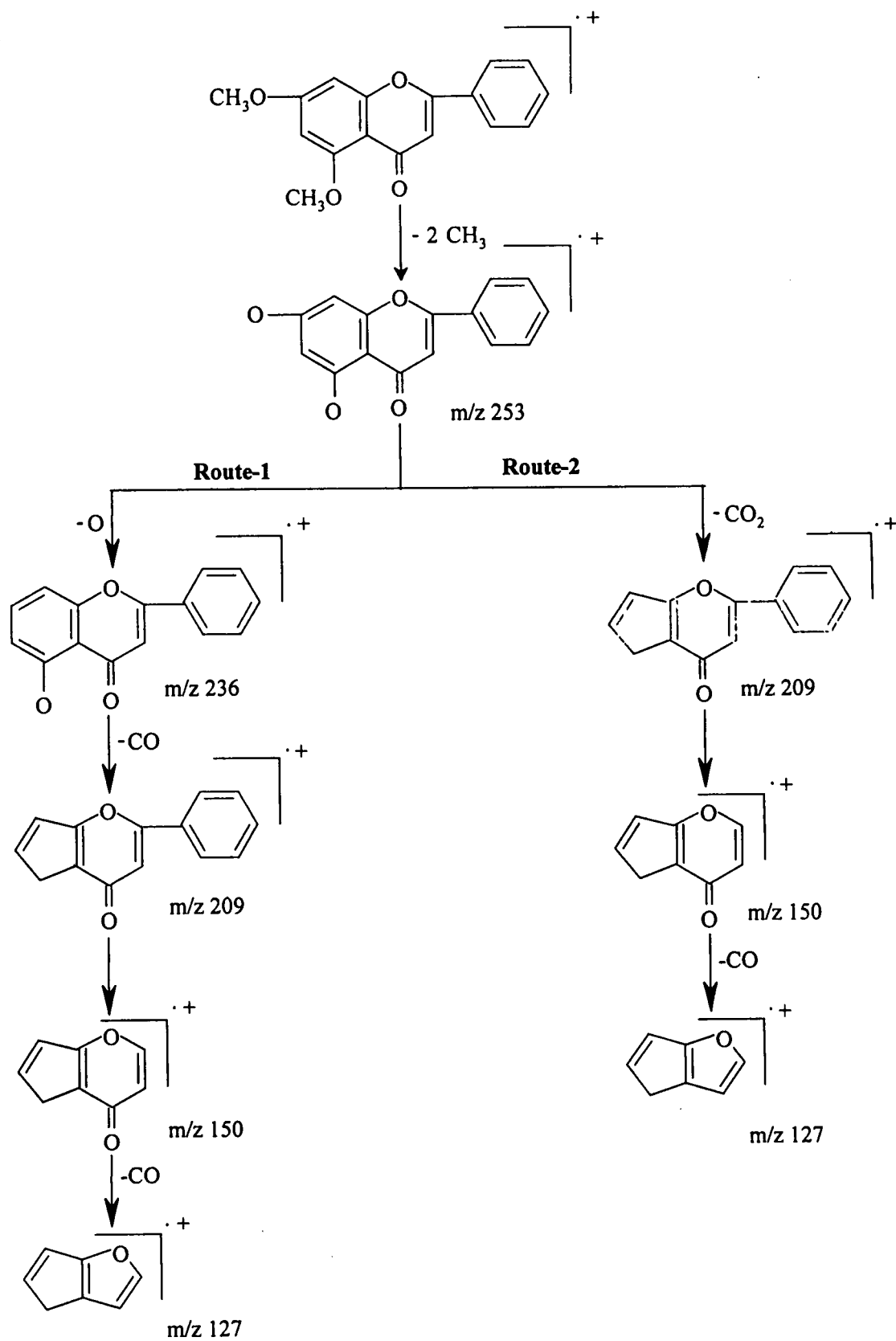
The appearance of other peaks in the mass spectrum of **compound-1**, results due to the cleavage of **compound-1** in another route. The fragmentation pattern **compound-1** is shown in **Scheme-5.1**.

All the above physical and spectral data are in agreement with the **structure-1** i.e. 5,7-dimethoxy flavone, which is assigned to **compound-1**.

Thus in the present investigation, isolation and identification of **compound-1** is reported for the first time from the plant *Balanites Roxburghii*.

The antifertility activity of the fruits of *Balanites Roxburghii* may be due to the presence of 5,7-dimethoxy flavone, because flavones are known to exert antifertility effect⁶⁻⁸ in both males and females.

Scheme-5.1



5.B Antifertility activity of IBR-2

The antifertility activity of **IBR-2** obtained from ethanol extract has been divided into two parts.

- ◆ Antifertility activity in females
- ◆ Antifertility activity in males

5.B.1 Antifertility activity in female albino rats

Antifertility effect of **IBR-2** in female is studied in following two parameters.

- 1) Post-coital antifertility activity in matured female rats
- 2) Estrogenic and antiestrogenic activity in young female rats.

5.B.1.1 Abortifacient activity

Post-coital antifertility testing was carried out in female Wistar rats as per the method described by Khanna and Chaudhury⁹. Wistar strain female albino rats weighing between 150-200 g of proven fertility and with regular estrous cycle were selected for this experiment. These female rats were monitored daily for different stages of estrous cycle. The rats, which were in proestrous stage were separated and mated with male rats that had sired litters before, in the ratio of 2:1 in separate cages. Every morning the vaginal smears were examined for the presence of lumps of spermatozoa. The day when spermatozoa were detected in the vaginal smear was considered as day 1 of pregnancy. Then the pregnant rats were isolated and divided into two groups of 6 rats each. The rats in group I were administered with the suspension of **IBR-2** in 0.1% Tween-80 solution at the dose of 60 mg/kg through intragastric catheter from day 1 to day 7 of pregnancy and both the horns of the uterus were observed for number, color and size of implants. Simultaneously, the number of corpora lutea were also noted. The abdomen was sutured

and the pregnancy was allowed to go to full term. After the delivery, the litters were counted and examined for evidence of teratogenicity. The observations made are presented in Table-5.B.4.

Table-5.B.1 Abortifacient activity of the **IBR-2** isolated from ethanol fraction.

Treatment	Dose mg/kg body weight	Group	No. of rats with implantation sites on day 10	Mean No. of implants on day 10	Mean No. of litters delivered	Percentage of abortifacient activity
Control	-	I	6	12.00	11.00	-
IBR-2	60 mg	II	6	11.23.	3.24	71.14

5.B.1.2 Estrogenic and antiestrogenic activity

For studying estrogenic and antiestrogenic activity, young female rats (21-23 days) weighing between 35-40 g were bilaterally ovariectomised under light ether anesthesia and sterile conditions. They are divided into 4 groups consisting of 6 rats each. The first group served as the control and received vehicle only (Tween-80, 1%, 5 ml/kg). The second group received ethinyl estradiol in olive oil (1 µg/rat/day) subcutaneously. The third group received the suspension of **IBR-2** at the dose of 60 mg/kg. The fourth group correspondingly received the suspension of **IBR-2** at the same dose along with ethinyl estradiol, at the dose of 1µg/rat/day subcutaneously. All the above treatments were given for 7 days. Vagina and vaginal smears were examined in all the animals in the treated groups for 7 days of treatment. On day 8, the rats were sacrificed by decapitation, the uteri were dissected out, weighed and fixed in Bouin's fluid for 25 hr for histological studies. The diameter of the uterus, thickness of endometrium and the height of

endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer. The statistical analysis was carried out by using student's 't' test. The results are expressed as mean $\pm$ SEM. The findings are summarized in Table-5.B.2 and Table-5.B.3.

Table-5.B.2 Estrogenic activity of the IBR-2 isolated from ethanol fraction.

Group	Treatment (Dose/day)	Uterine weight in mg/100mg	Vaginal cornification
I	Control	70.62 $\pm$ 4.64	nucleated and cornified cells
II	Ethinyl estradiol (1 μ g/rat)	322.43 $\pm$ 7.81*	cornified cells
III	IBR-2 (60 mg/kg)	87.13 $\pm$ 5.59*	nucleated and cornified cells
IV	Ethinyl estradiol (1 μ g/rat) + IBR-2 (60 mg/kg)	346.54 $\pm$ 16.28*	cornified cells

*p < 0.001 vs control

Table-5.B.3 Histological changes in the uterus.

Group	Treatment (Dose/day)	Diameter of the uterus (μ m) $\pm$ SEM	Thickness of the endometrium (μ m) $\pm$ SEM	Height of the endometrial epithelium (μ m) $\pm$ SEM
I	Control	348.61 $\pm$ 12.72	48.06 $\pm$ 1.16	26.49 $\pm$ 5.43
II	Ethinyl estradiol (1 μ g/rat)	832.14 $\pm$ 9.63	236.72 $\pm$ 17.08*	44.21 $\pm$ 6.64
III	IBR-2 (60 mg/kg)	612.45 $\pm$ 1.22*	81.94 $\pm$ 5.67*	29.45 $\pm$ 3.58**
IV	Ethinyl estradiol (1 μ g/rat) + IBR-2 (60 mg/kg)	894.32 $\pm$ 6.38*	254.84 $\pm$ 8.36*	49.54 $\pm$ 13.12*

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

The compound **IBR-2** was evaluated for post coital antifertility activity at the dose of 60 mg/kg body weight. No toxic effects were observed either by gross visual examination or in the weight of rats. After discontinuation of the treatment, all the rats were mated. This resulted in pregnancy and delivery of normal litters, indicating that the action of **IBR-2** is reversible.

Since the number of corpora lutea and number of litters born were equal, it clearly indicate that **IBR-2** does not possess antiimplantation activity. However, it exhibited abortifacient activity of 71.14% at the dose of 60 mg/kg body weight. This observation revealed that all the implants have been absorbed by the uterus.

Oral administration of the **IBR-2** caused significant increase in uterine weight. This suggested the mild estrogenic activity of the **IBR-2**. This fact was well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium and height of endometrial epithelium. It was also observed that the **IBR-2** potentiated the action of ethinyl estradiol when administered together. This fact was well indicated by the significant increase in diameter of uterus, thickness of endometrium and height of the endometrial epithelium.

Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

Hence, it was concluded that this fraction is mainly responsible for antifertility activity of the ethanol extract of the fruits of *Balanites Rroxburghii*.

5.B.2 Antifertility activity in male albino rats

Encouraged by the antiandrogenic property of the ethanol extract of the fruits of *Balanites Roxburghii*, the compound **IBR-2**, obtained by the isolation of ethanol extract was also investigated for androgenic/antiandrogenic activity.

Androgenic and antiandrogenic effect of compound IBR-2.

The androgenic/antiandrogenic activity has been carried out by following the method as described by Lohiya and Goyal¹⁰.

Materials and Method

Colony bred immature male albino rats, (Wistar strain) 22 days old and weighing between 28-32 g, were used for evaluation of androgenic or antiandrogenic activity. They were divided into four groups, each group comprising of six animals. The first group served as control and received the vehicle (Tween-80, 0.1%). The second group received testosterone suspension in 0.1% Tween-80 solution at the dose of 1 mg/kg subcutaneously. The third group received a suspension of the **IBR-2** in 1% Tween-80 at 60 mg/kg body weight. The fourth group received, in addition to testosterone, **IBR-2** at 30 mg/kg body weight. All the treatments were given for seven days. On eighth day of the experiment, all the animals were sacrificed by decapitation. The testes, epididymes, vas deferens, seminal vesicle and ventral prostate were dissected out, freed from surrounding tissues and weighed quickly on a sensitive balance. Gravimetric changes were recorded and shown in **Table-5.B.4** and **Table-5.B.5**.

Table-5.B.4 Effect of IBR-2 on male reproductive organs when fed orally to immature male rats for seven days.

Group	Treatment dose (mg/kg)	Testes ± SEM	Seminal vesicle ± SEM	Ventral prostate ± SEM	Vas deferens ± SEM	Epididymes ± SEM
I	Control	638.44 ± 4.81	42.68 ± 15.30	49.54 ± 12.10	28.12 ± 23.51	76.65 ± 10.84
II	Testosterone (1 mg/kg)	679.80 ± 16.73*	71.32 ± 1.03*	82.62 ± 0.86*	40.28 ± 2.89*	84.38 ± 7.94*
III	IBR-2 (60 mg/kg)	627.61 ± 11.21**	39.93 ± 2.09*	42.17 ± 9.62*	26.37 ± 9.72*	72.29 ± 0.27**
IV	IBR-2 (60 mg/kg) + testosterone (1 mg/rat)	664.52 ± 3.02*	63.04 ± 0.08**	70.16 ± 4.01*	32.66 ± 2.28*	80.41 ± 4.04*

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

Table-5.B.5 Changes in the testis due to oral administration of IBR-2 on immature male rats for seven days.

Group	Treatment (Dose)	Diameter of the testes (µm) ± SEM	Diameter of the seminiferous tubules (µm) ± SEM
I	Control	39.58 ± 0.86	130.2 ± 4.1
II	Testosterone (1 mg/kg)	43.19 ± 9.44**	146.78 ± 2.9*
III	Ethanol extract (600 mg/kg)	40.35 ± 3.62*	127.52 ± 8.3**
IV	Testosterone (1 mg/kg) + Ethanol extract (600 mg/kg)	43.10 ± 1.16*	142.82 ± 2.06*

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

Results

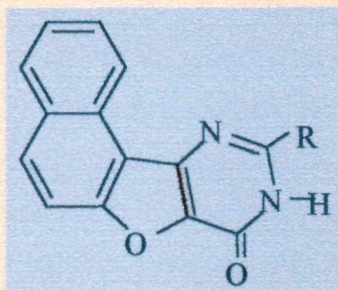
The administration of the fraction **IBR-2** obtained from ethanol extract of the fruits of *Balanites Roxburghii* to prepubertal male rats for seven days has reduced the weights of testes, epididymes, seminal vesicle and ventral prostate when compared with the rats of control and ethinyl estradiol group. A significant increase in the weights of testes and other accessory organs is observed when testosterone was administered alone, but androgenic effect of testosterone was decreased with simultaneous administration of **IBR-2** and testosterone, which was indicated by the reduction in the weights of all the organs when compared to testosterone-administered animals. Considerable reduction in weights of seminal vesicle and ventral prostate were observed, thus indicating moderate antiandrogenic activity of **IBR-2**.

References

1. A.D. Patil and V.H. Deshpande, *Indian J. Chem.*, **22 B**, 109 (1983).
2. L. Jurd, In; "The Chemistry of Flavanoid Compounds" (ed. By T.A. Geissmann), Oxford, Pergman Press, 155 (1962).
3. H.K.S. Swamy, Ph.D. Thesis, Gulbarga University, Gulbarga, (1997).
4. S.M. Badami, Ph.D. Thesis, Gulbarga University, Gulbarga, (1999).
5. K. Rudresh, Ph.D. Thesis, Gulbarga University, Gulbarga, (2000).
6. S.P. Hiremath, H.K.S. Swamy, S.M. Badami, S.B. Patil and R.L. Londonkar, *Int. J. Pharmacog.*, **34(1)**, 1 (1996).
7. S.P. Hiremath and S. Hanumantha Rao, *Contraception*, **42**, 467 (1990).
8. S.P. Hiremath, H.K.S. Swamy, S.M. Badami and M.G. Purohit, *Fitoterapia*, **65**, 372 (1994).
9. U. Khanna and R.R. Chaudhury, *Indian J. Med. Res.*, **56**, 1575 (1968).
10. N.K. Lohiya and R.B. Goyal, *Ind. J. Exp. Biol.*, **30**, 1051 (1991).

CHAPTER-6

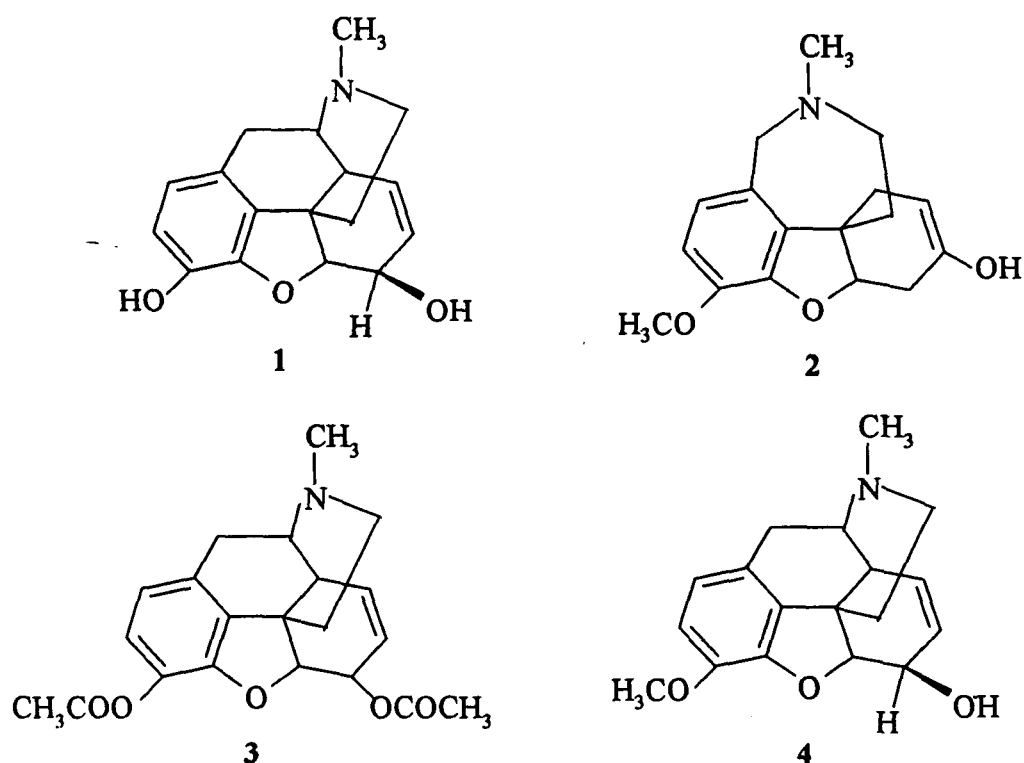
Synthesis of Naphtho[2,1-b]furo[3,2-d]pyrimidine Derivatives



INTRUDUCTION

The compounds encompassing furan nucleus are widely distributed in nature, particularly amongst the plant kingdom. Many of these compounds are well known for their useful physiological and pharmacological properties. This generated considerable interest in the investigation of compounds containing furan moiety for pharmacological activities. As a result of such investigations 5-nitrofurans and other furan derivatives were brought to light and were found to possess interesting biological activities. Since then, many research groups are involved in the synthesis of new heterocyclic compounds containing furan nucleus and evaluating them for various biological activities. A number of synthetic benzofuran derivatives have been found to possess wide range of biological activities such as antimicrobial, analgesic, anti-inflammatory, analeptic and antiviral. This research has resulted in the accumulation of voluminous work on benzofurans and several monographs^{1,5} devoted to the study of benzofurans have appeared in literature from time to time.

Furan nucleus is often found fused with oxygen heterocycles rather than nitrogen heterocycles in nature. Although the latter type of compounds are very less in number, they have occupied a prominent place in medicinal chemistry. Morphine **1**, galanthamine **2**, heroin **3**, codeine **4** and related alkaloids are the examples of such compounds in which furan nucleus is condensed with nitrogen heterocycle.

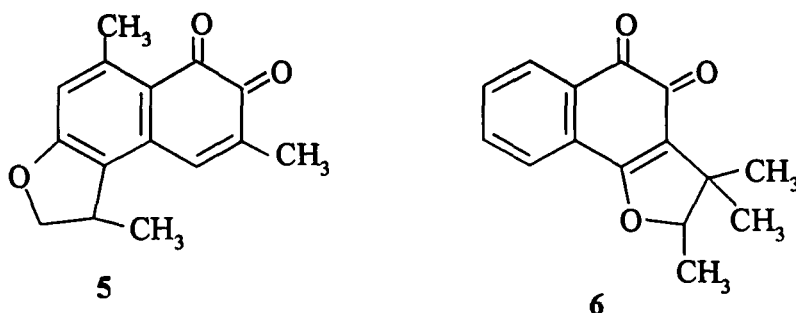


These compounds are well known for their analgesic activity and further research work has indicated that furan ring is an essential part of the structures of these molecules for medicinal properties⁶.

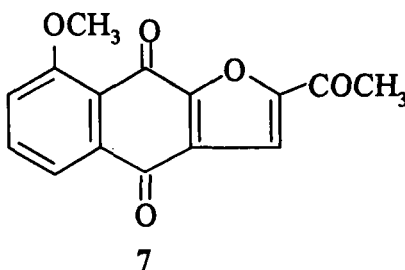
During last few years, interest in this area was hence focused on synthesis and investigation of biological activities of various benzofurans fused with nitrogen heterocycles such as pyrimidine, pyridine, diazepine, triazepine, oxazine, pyridazine, thiazole, pyrazole, benzoxazole, quinoline, indole, benzimidazole, benzothiazole, quinoxaline etc⁷⁻¹⁷.

In contrast with furan and benzofuran derivatives, naphthofuran and its derivatives are very rarely found in nature. Only few of them are reported to occur in some plants in the form of naphthofuroquinones. Mansonone D was found to be present in the extracts of leaves of *Mansonia altissima* belonging to Stuculiaceae family. This

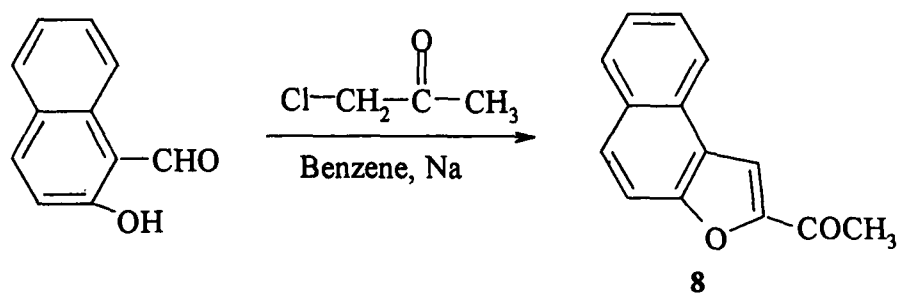
compound was identified as 3,4,8-trimethyl-2,3-dihydronaphtho[2,1-b]furan-5,6-dione¹⁸
 5. Price^{19,20} et al., isolated a new compound named as Dunnione 6 from the leaves of *Streptocarpus dunnii* and later assigned the structure 2,3,3-trimethylnaphtho[1,2-b]furan-4,5-dione on the basis of spectral studies.



Heartwood of *Tabebuia pentaphylla* was found to contain many derivatives of 2-acetylnaphtho[2,3-b]furan-4,9-diones 7²¹⁻²⁴.



Encouraged by these reports some researchers attempted the synthesis of some naphthofuran derivatives in the laboratory. First report in this connection appeared in the literature way back in 1897 by Stoermer²⁵. He reported the synthesis of parent heterocycle, naphtho[2,1-b]furan from 1-naphthoxyacetaldehyde. Same author could synthesize 2-acetylnaphtho[2,1-b]furan 8 by reacting 2-hydroxy-1-naphthaldehyde with chloroacetone in presence of dry acetone and metallic sodium²⁶.



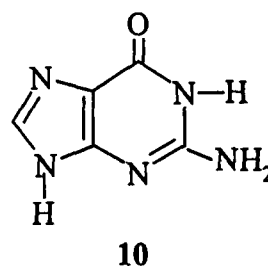
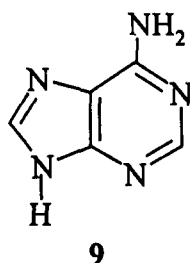
Since then Emmott²⁷ et al., Loader²⁸ et al., Chatterjee²⁹ et al., Weillthevorrot³⁰ et al., Gilotdelhalle³¹ et al., Arrault³² et al., and few other researchers have synthesized some derivatives of naphtho[2,1-b]furan. Some of the compounds were found to possess mutagenic and carcinogenic activities.

Receiving impetus from these reports, synthesis of naphthofuran derivatives especially coupled or fused or bridged with nitrogen heterocycle was initiated in our laboratory. Several research papers describing the synthesis, characterization and biological activities of naphthofuran compounds have been published from our laboratory³³⁻⁴⁰. These derivatives are found to possess various biological and pharmacological activities such as antibacterial, antifungal, analgesic, anti-inflammatory anthelmintic and diuretic activities.

In the present investigation, our interest is to search a new non-steroidal drug possessing wide spectrum of pharmacological activities including antifertility activity. Thus, the importance of the less explored naphthofuran and biologically active nitrogen heterocycles encouraged us to take up the work of annulating suitable nitrogen heterocycle on easily accessible naphtho[2,1-b]furan derivatives. The obvious choice of the nitrogen heterocycle was pyrimidine, which is devoted with the important biodynamic properties. Pyrimidines have been explored to a maximum extent by various chemists and

biochemists and this has resulted in the accumulation of voluminous work in this field of research and numerous research papers have appeared in literature. Detailed discussion of the work carried out on pyrimidines is not within the scope of this thesis. However to point out the importance of pyrimidine derivatives, only few recent reports in this regard have been discussed in the following pages.

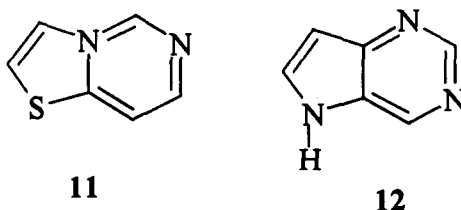
Pyrimidine nucleus occurs in biologically important products such as nucleic acids, vitamins, coenzymes and pharmacologically useful natural products of plant origin. The major interest in fused pyrimidines is mainly due to purine based components of nucleic acids. The two purine bases adenine **9** and guanine **10**, which are imidazo pyrimidine derivatives, are most important components of DNA, RNA and ultimately the genetic materials.



Several synthetic analogues of the natural nucleosides, adenosine and guanosine are prepared by modifying the base component for different purposes such as exploration of antiviral and anticancer agents. Such modified base involved in the fusion of pyrimidine with various nitrogen heterocycles like pyrrole, pyrazole, triazole, thiazole etc. Hence fusion of pyrimidine ring on different heterocyclic systems resulted in novel compounds possessing one or the other biological or pharmacological activities⁴¹⁻⁵¹.

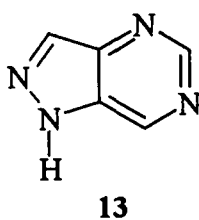
Some derivatives of thiazolo[3,2-c]pyrimidines **11** have been synthesized and tested for antimicrobial activity and anthelmintic activity on a number of strains⁵².

Itoh et al., reported the synthesis of pyrrolo[3,2-d]pyrimidines **12** and studied the effect of these compounds on dihydrofolate reductase⁵³.



In search for novel agrochemicals with high activity and low toxicity, a series of diheterocyclic compounds containing 1,2,4-triazolo[1,5-a]pyrimidine rings were designed and synthesized by a four step synthetic route. When preliminary bioassay against herbicidal activity was carried out, it was observed that some of the compounds displayed activity at the low concentration of 100 ppm⁵⁴.

Moukha et al., have synthesized some pyrazolo[3,4-d]pyrimidine **13** derivatives and evaluated for their inhibitory effects against the replication of HIV-1 (IIIB), HIV-2 (ROD), various DNA viruses, tumor cell lines and tuberculosis⁵⁵.



Pyrimido[2,1-b]benzothiazole and benzothiazolo[2,3-b]quinazoline derivatives have been synthesised and tested for their antitumor and antiviral activities. Some of the compounds showed potential activity against Herpes simplex type-1 (HSV-1)⁵⁶.

Pyrrolo[3,4-e][1,2,3]-triazolo[1,5-a]pyrimidine and Pyrrolo[3,4-d][1,2,3]-triazolo[1,5-a]pyrimidine, new tricyclic systems of biological interest have been reported by Lauria et al⁵⁷.

New polycyclic pyrimidine derivatives have been synthesized and investigated for their biological activities. 5-Pyrrolidino-2-methyl-thiobenzopyrano[4,3-d]pyrimidine was found to fulfill the chemical requirements to exhibit antiplatelet activity with gastro protective effect⁵⁸.

Biagi et al., have synthesized some 6-substituted pyrazolo[3,4-d]pyrimidine derivatives and tested as inhibitors of the xanthine oxidase enzyme⁵⁹.

Tricyclic conformationally restricted tetrahydropyrido annulated furo[3,2-d]pyrimidines have been synthesized and are reported as non classical antifolate inhibitors of dihydrofolate reductase⁶⁰.

This fact revealed the wide scope for systematic investigation in this area of heterocyclic chemistry. This led Agasimundin et al., to initiate an exhaustive research programme devoted to the synthesis and biological evaluation of benzofuro[3,2-d]pyrimidines. The results of such investigations are published in a series of research papers⁶¹⁻⁶⁵. However there are no reports of fusion of pyrimidine ring with naphthofuran moiety in literature.

Present work

The initial work was devoted to the general synthetic approaches for this ring system and later it was directed towards the synthesis of various substituted naphtho[2,1-b]furo[3,2-d]pyrimidines. Invitro screening of these compounds has shown that many of these compounds possess wide spectrum of biological activities⁶⁶.

As a logical extension of earlier work, it was thought worthwhile to take up the study of synthesis of naphtho[2,1-b]furo[3,2-d]pyrimidines and evaluation for various pharmacological activity in addition to antifertility activity.

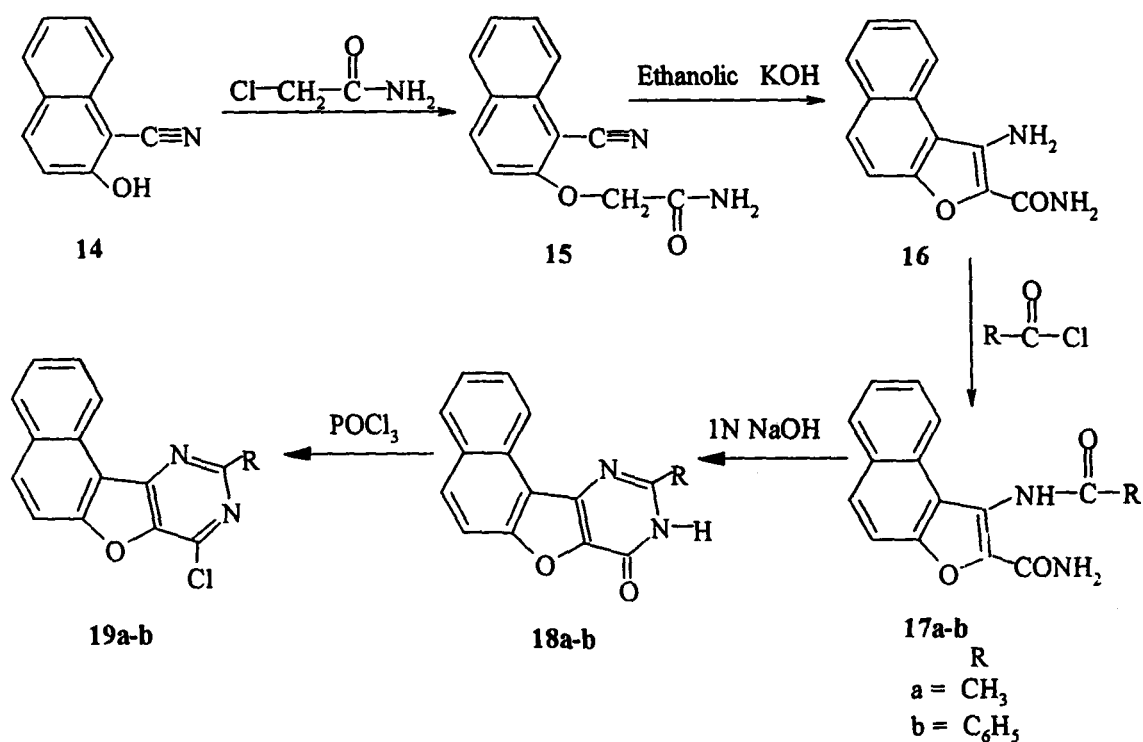
In the present work, logically there appears to be three different synthetic strategies for the construction of pyrimidine nucleus with naphtho[2,1-b]furan.

- a) The desired nitrogen heterocyclic ring system may be fused on a preformed naphthofurans.
- b) Naphthofuran ring system may be fused on a preformed nitrogen heterocycle with suitable functionalities.
- c) The two preformed naphthofuran and nitrogen heterocycles may be fused together.

In the present investigation, the synthetic strategy “a” involving the construction of desired nitrogen heterocycle onto a preformed naphthofuran is followed for the synthesis of condensed naphthofurans with nitrogen heterocycle, because of easy availability of appropriately substituted naphthofuran derivatives through convenient synthetic methods.

One of the methods for the construction of pyrimidine nucleus requires o-amino carboxamide system. For building pyrimidine moiety on naphtho[2,1-b]furan nucleus, such a functionality in 2- and 3- positions was necessary. This necessitated the synthesis of 2-hydroxy-1-naphthonitrile, which was subsequently converted into 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines by a sequence of reactions as shown in the **Scheme-1**.

Scheme-1



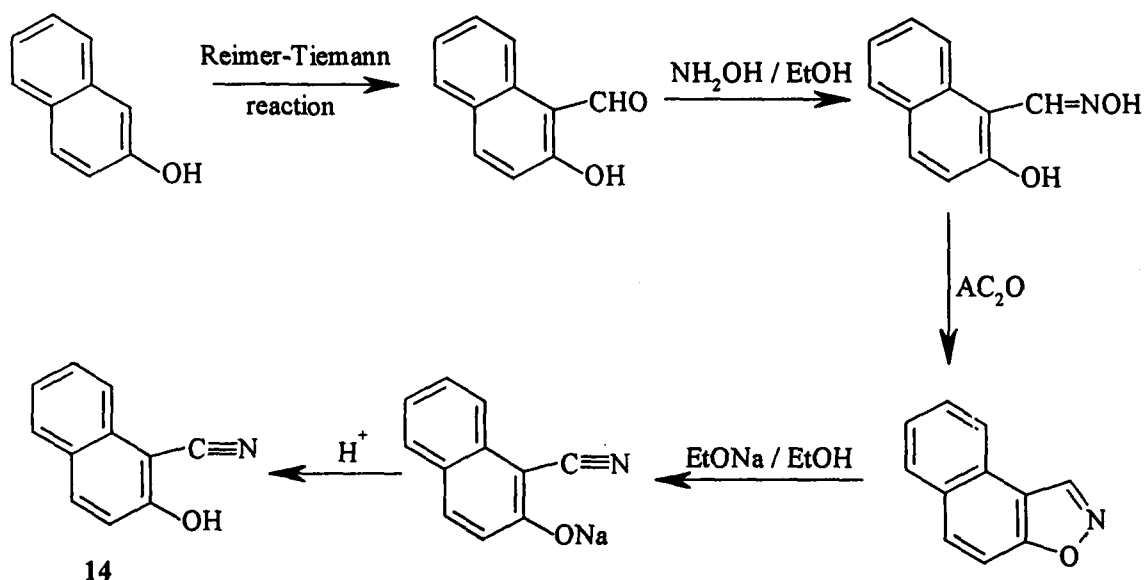
The various steps involved in the synthetic strategy are as follows,

- 1) Synthesis of 2-hydroxy-1-naphthonitrile
- 2) Condensation of 2-hydroxy-1-naphthonitrile with chloroacetamide followed by cyclization to obtain 3-aminonaphtho[2,1-b]furan-2-carboxamide.
- 3) Conversion of 3-aminonaphtho[2,1-b]furan-2-carboxamide into 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines.
- 4) Nucleophilic substitution reactions of 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines to get the title compounds.

1. Synthesis of 2-hydroxy-1-naphthonitrile

2-Hydroxy-1-naphthonitrile **14** was synthesized by traditional method of dehydration of the corresponding oxime by a well-established procedure in our laboratory. The required 2-hydroxy-1-naphthaldehyde was prepared from 2-naphthol by Reimer-Tiemann reaction and then converted into its oxime by adopting literature procedure. The treatment of oxime with acetic anhydride formed initially naphthisoxazole, which without isolation was converted into the desired 2-hydroxy-1-naphthonitrile by treatment with sodium ethoxide followed by acidification (**Scheme-2**).

Scheme-2

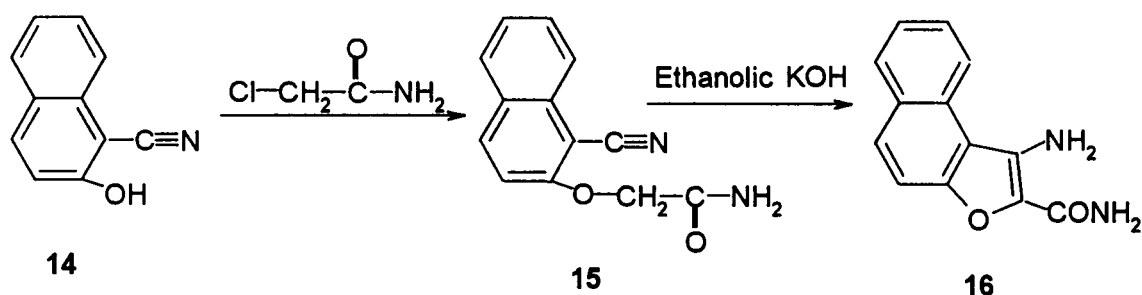


The structure of 2-hydroxy-1-naphthonitrile thus synthesized was ascertained by superimposable IR and ^1H NMR with that of authentic sample and by mixed melting point.

2. Condensation of 2-hydroxy-1-naphthonitrile with chloroacetamide followed by cyclization to obtain 3-aminonaphtho[2,1-b]furan-2-carboxamide.

2-Hydroxy-1-naphthonitrile **14** was condensed with chloroacetamide in presence of anhydrous potassium carbonate and dry acetone, to obtain the desired condensed product 1-cyano-2-naphthoxyacetamide **15**.

1-Cyano-2-naphthoxyacetamide **15** underwent Thorpe-Ziegler cyclization smoothly on refluxing with ethanolic potassium hydroxide and produced 3-amino naphtho[2,1-b]furan-2-carboxamide in good yield.



The IR spectrum (Figure-6.1) of **15** exhibited absorption bands at 3451 cm^{-1} , 2220 cm^{-1} and 1683 cm^{-1} due to $-\text{NH}_2$, $-\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ stretching frequencies. The structure assigned was further supported by its ^1H NMR spectrum (Figure-6.2). It exhibited a singlet at δ 4.6 integrating for two protons of CH_2 and a broad singlet at δ 5.8 (D_2O exchangeable) also integrating for two protons of NH_2 group. In addition, it also showed a multiplet at δ 7.0 - δ 8.4 integrating for six aromatic protons.

The proposed structure was confirmed by recording its mass spectrum (Figure-6.3). It exhibited a molecular ion peak at m/z 226 corresponding to its molecular weight. The other peaks found in the mass spectrum correspond with the fragmentation pattern as depicted in Scheme-3.

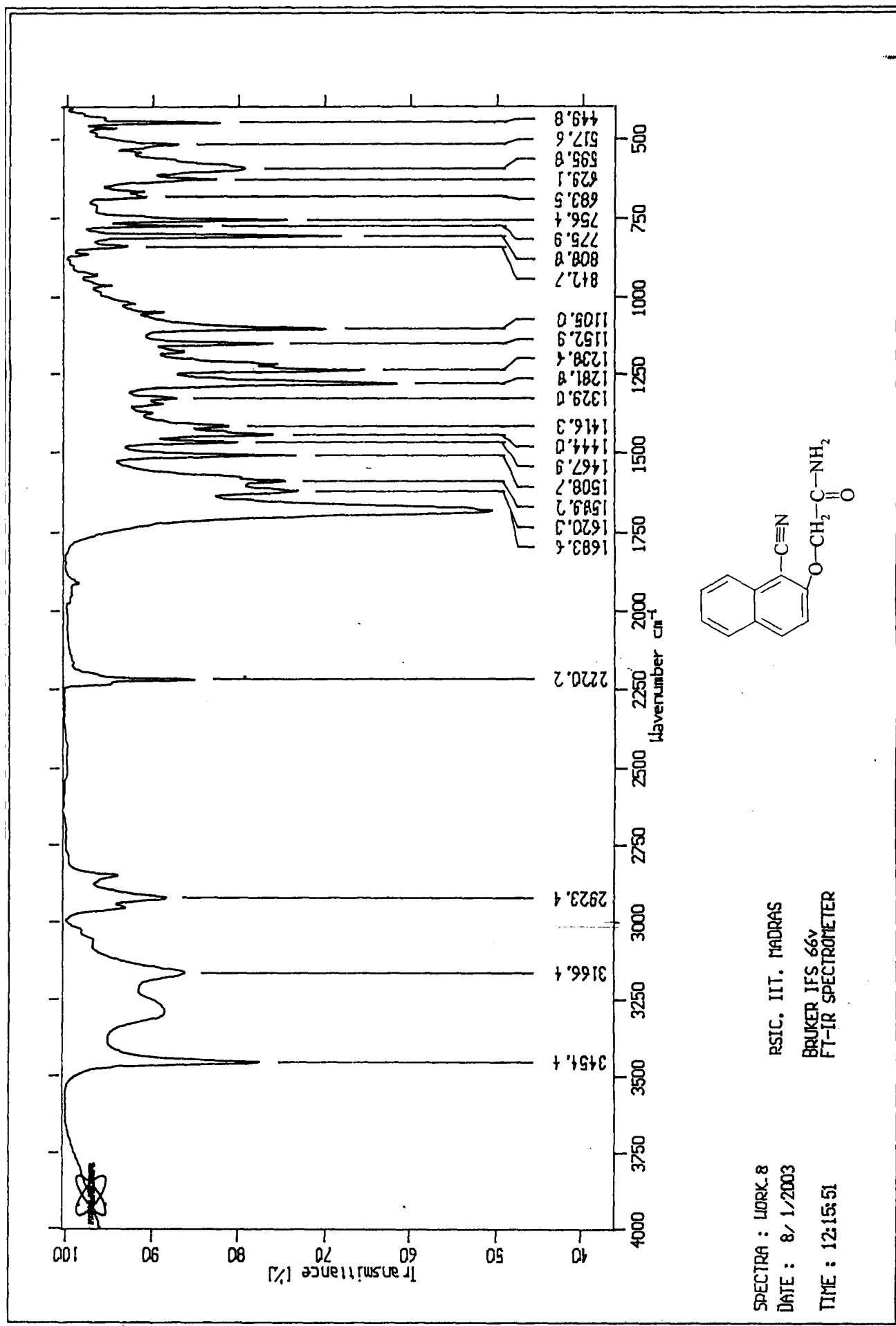
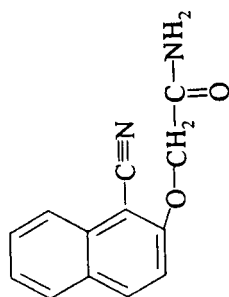


FIGURE-6.1

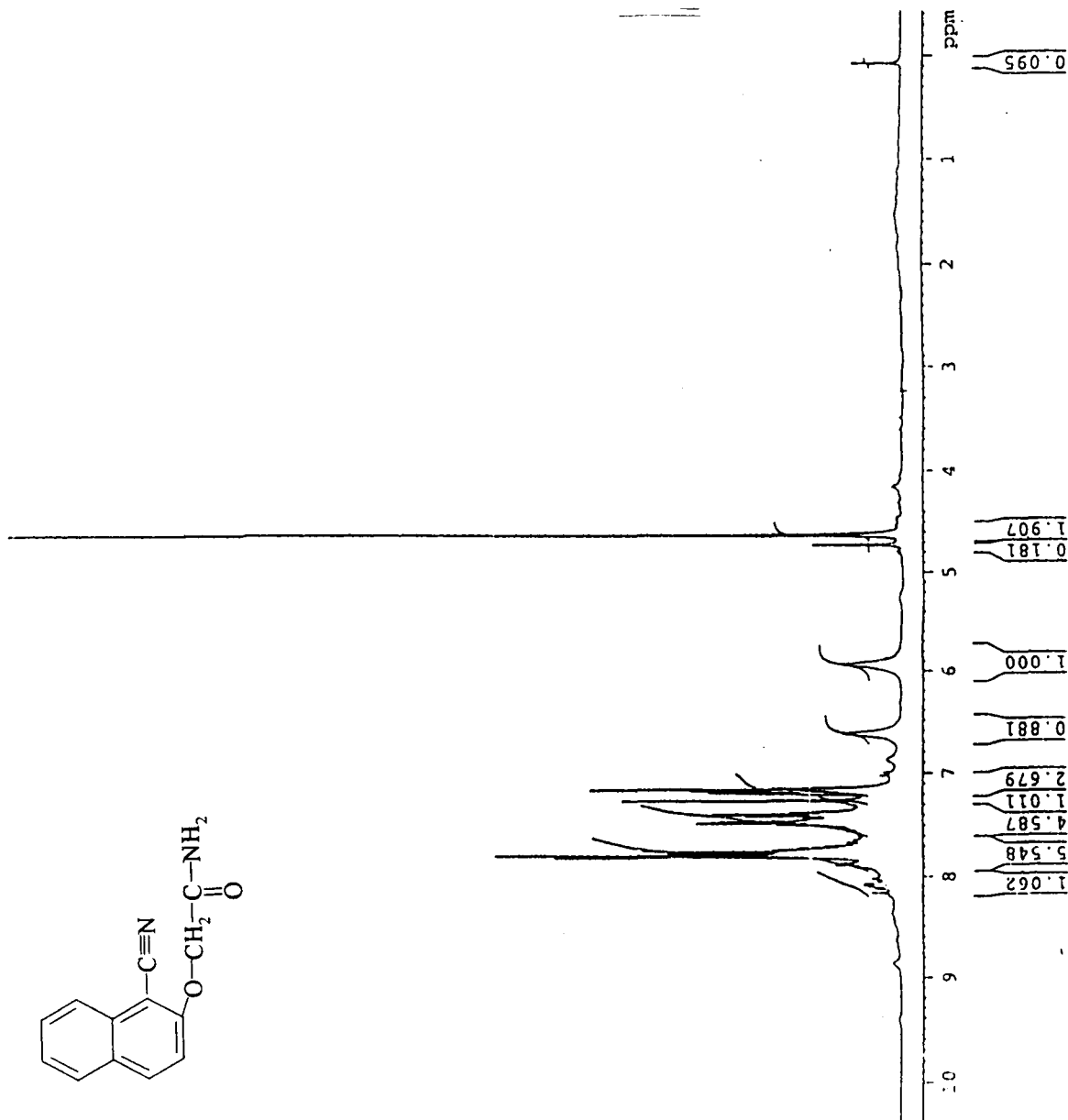
sample i



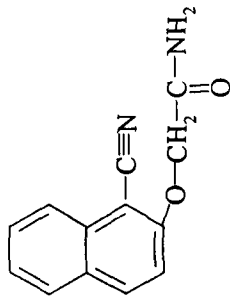
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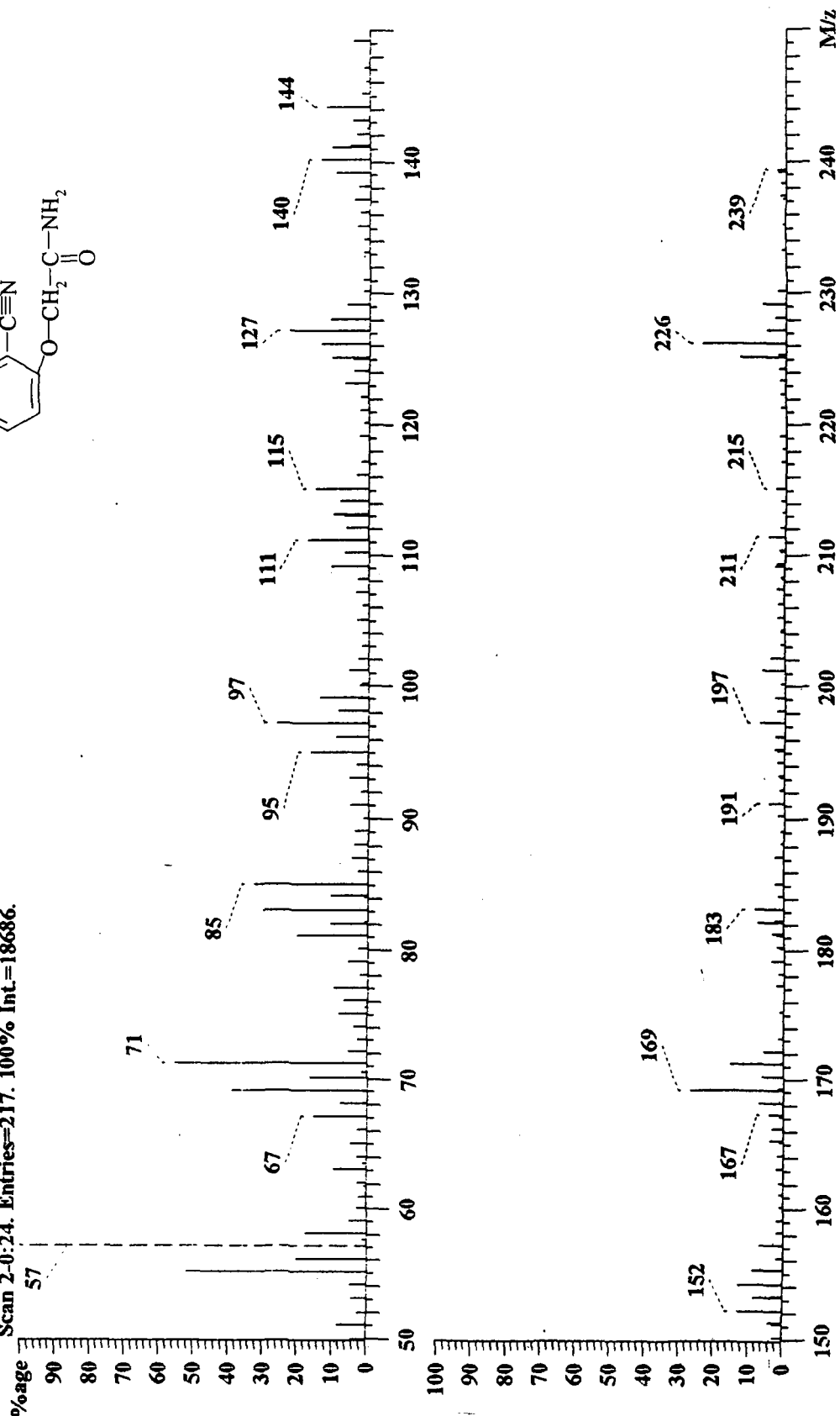
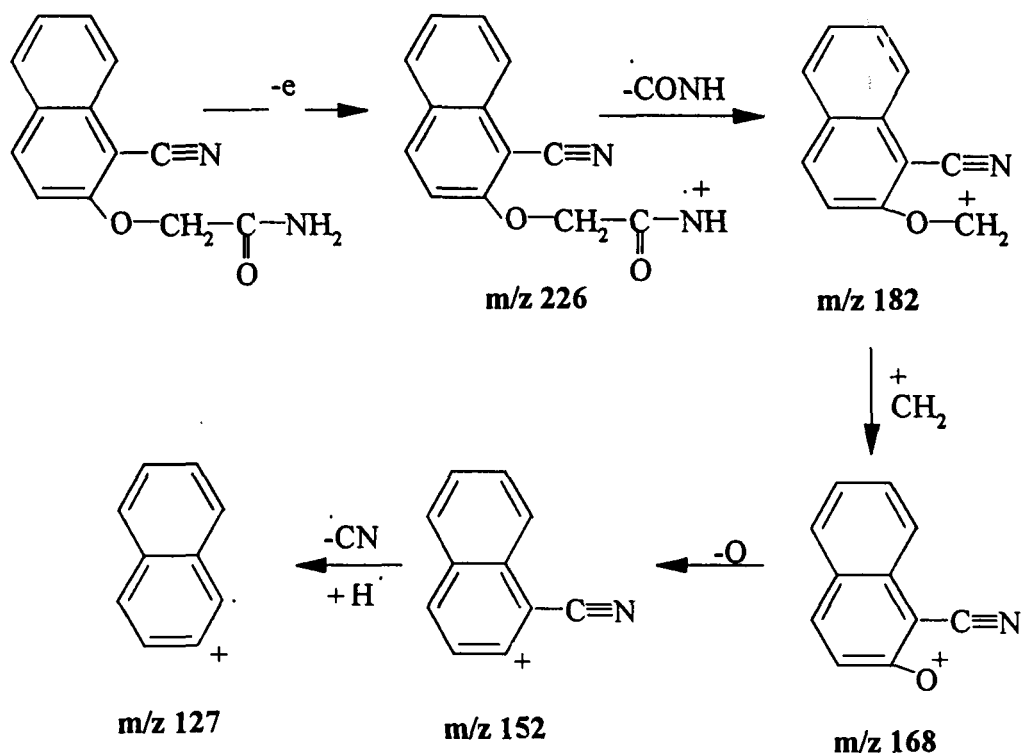
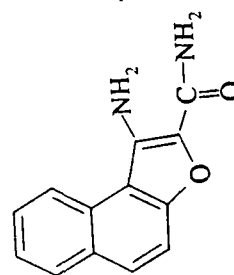
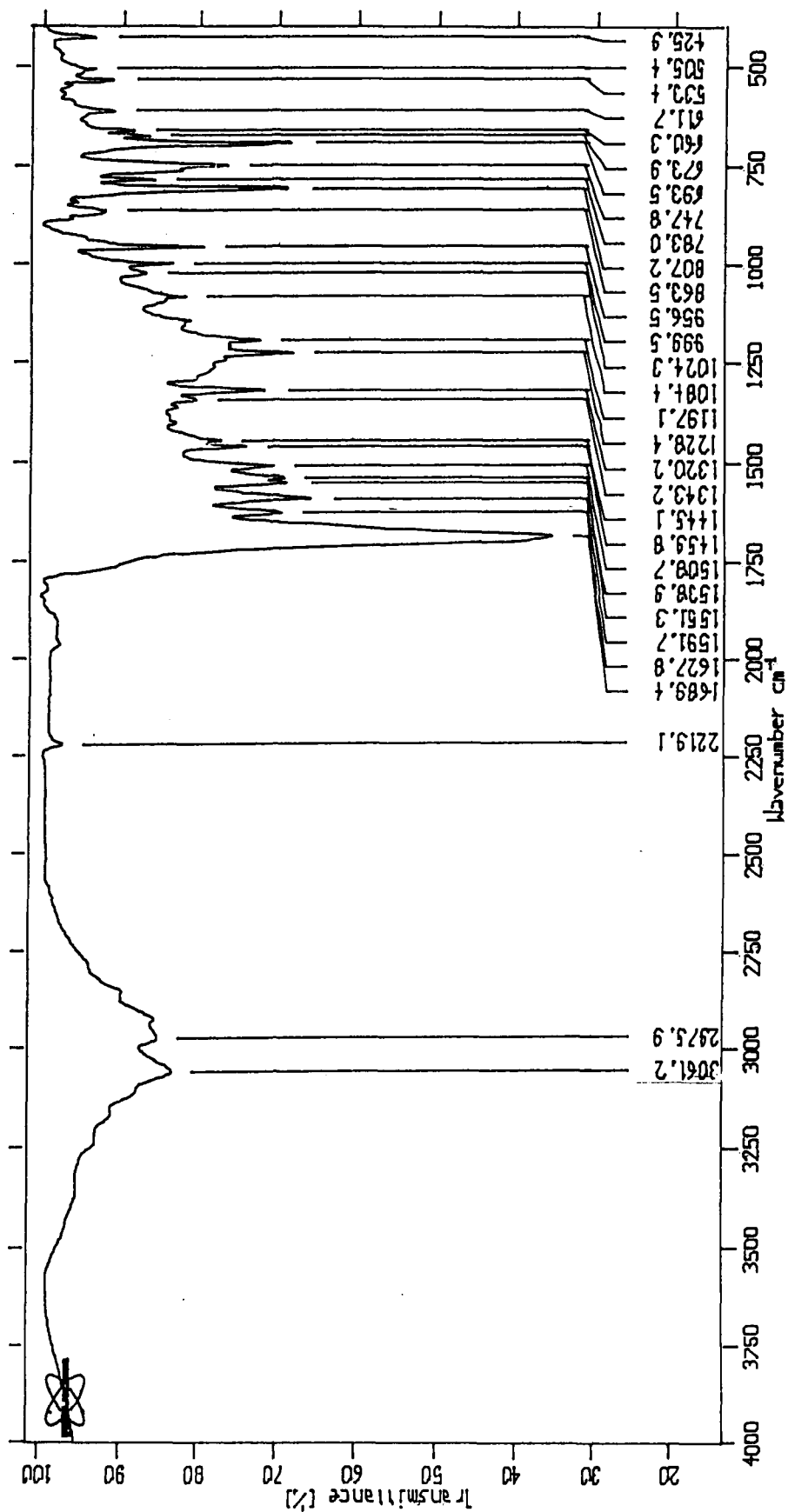


FIGURE 6 3

Scheme-3

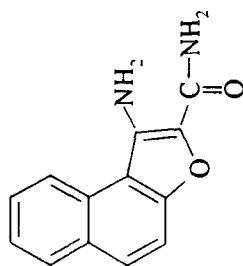


The IR spectrum (Figure-6.4) of 16 was conspicuous by the absence of peak at 2220 cm^{-1} due to $-\text{C}\equiv\text{N}$. As expected the absorption band due to $-\text{C}=\text{O}$ group appeared at 1689 cm^{-1} . The absorption at this frequency may be attributed to the involvement of carbonyl group in intramolecular hydrogen bonding with $-\text{NH}_2$ group on adjacent carbon atom. In the ^1H NMR spectrum (Figure-6.5) of compound 16, the peak due to $-\text{CH}_2$ group at δ 4.6, which was observed in its precursor, was conspicuously absent. This supported the involvement of $-\text{CH}_2$ group in Thorpe-Ziegler cyclization. The ^1H NMR spectrum exhibited a singlet at δ 3.9 and another broad singlet at δ 6.0 (D_2O exchangeable), which were attributed to $-\text{NH}_2$ and $-\text{CONH}_2$ protons. Six aromatic protons appeared as a multiplet at δ 7.0 - δ 8.2. To obtain further evidence for the structure assigned to compound 3, its mass spectrum was recorded (Figure-6.6). It exhibited a



SPECTRA : WORK.3
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 RSIC, IIT, MADRAS
 BRUKER IFS 66V
 FT-IR SPECTROMETER

FIGURE 6.4



sample 2

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 HL1 1 dB
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 SFO1 400.1364282 MHz
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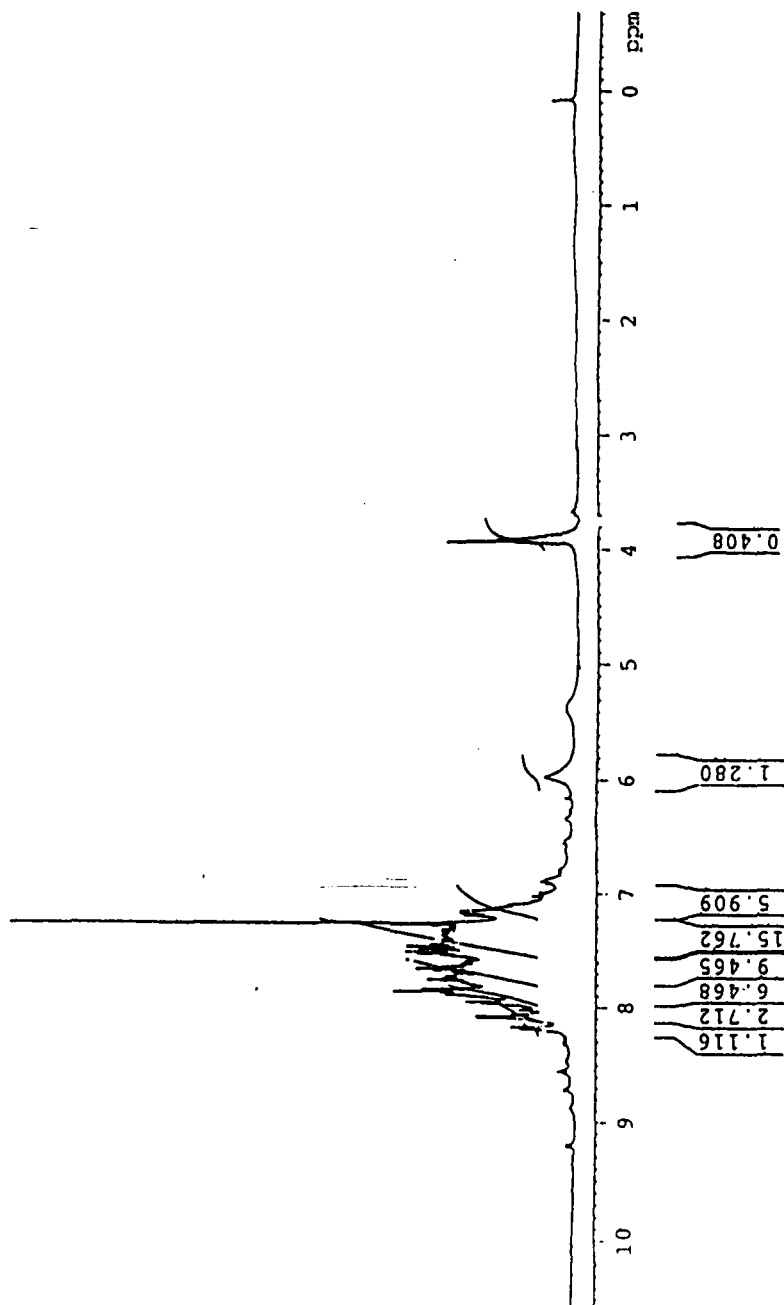


FIGURE-6.5

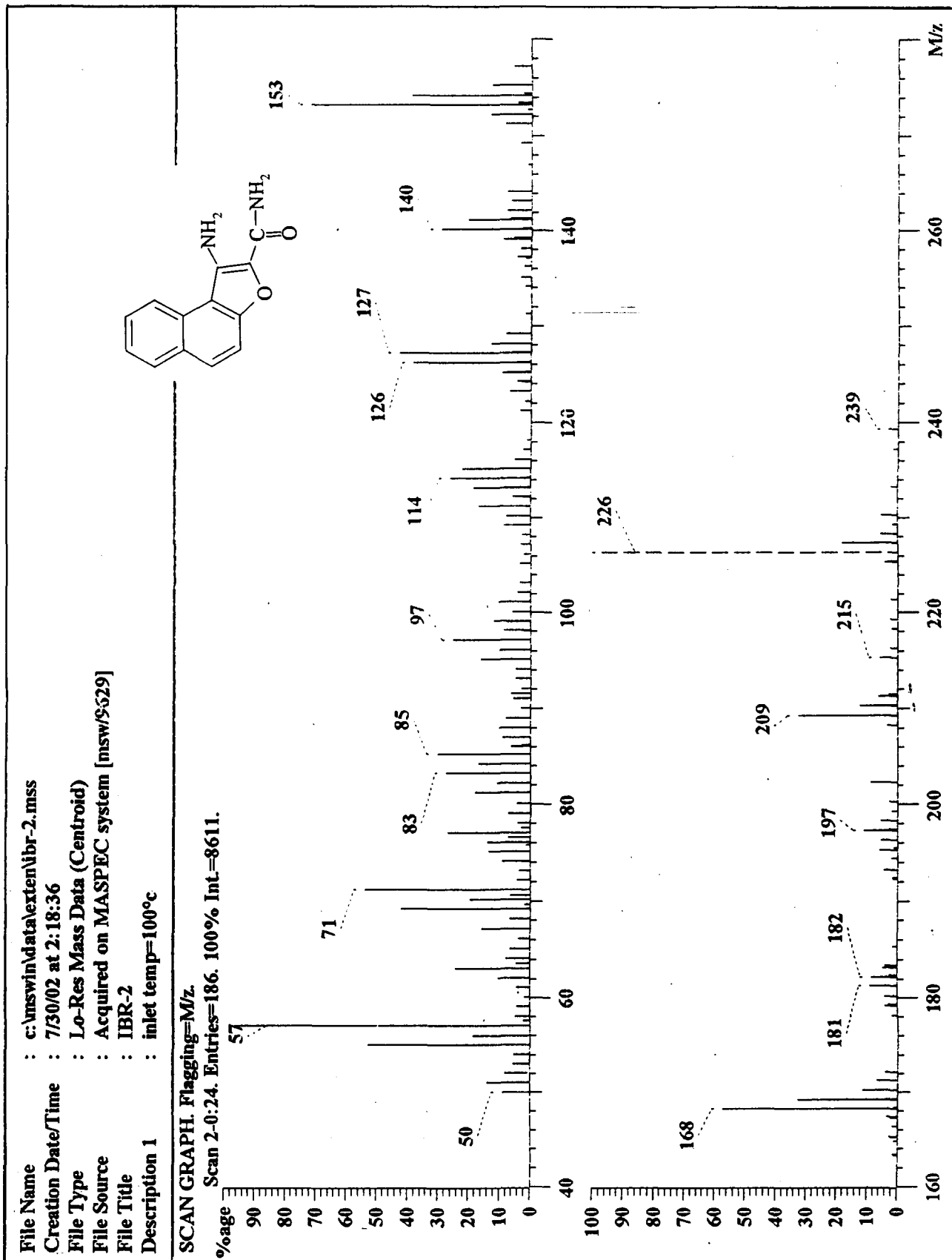
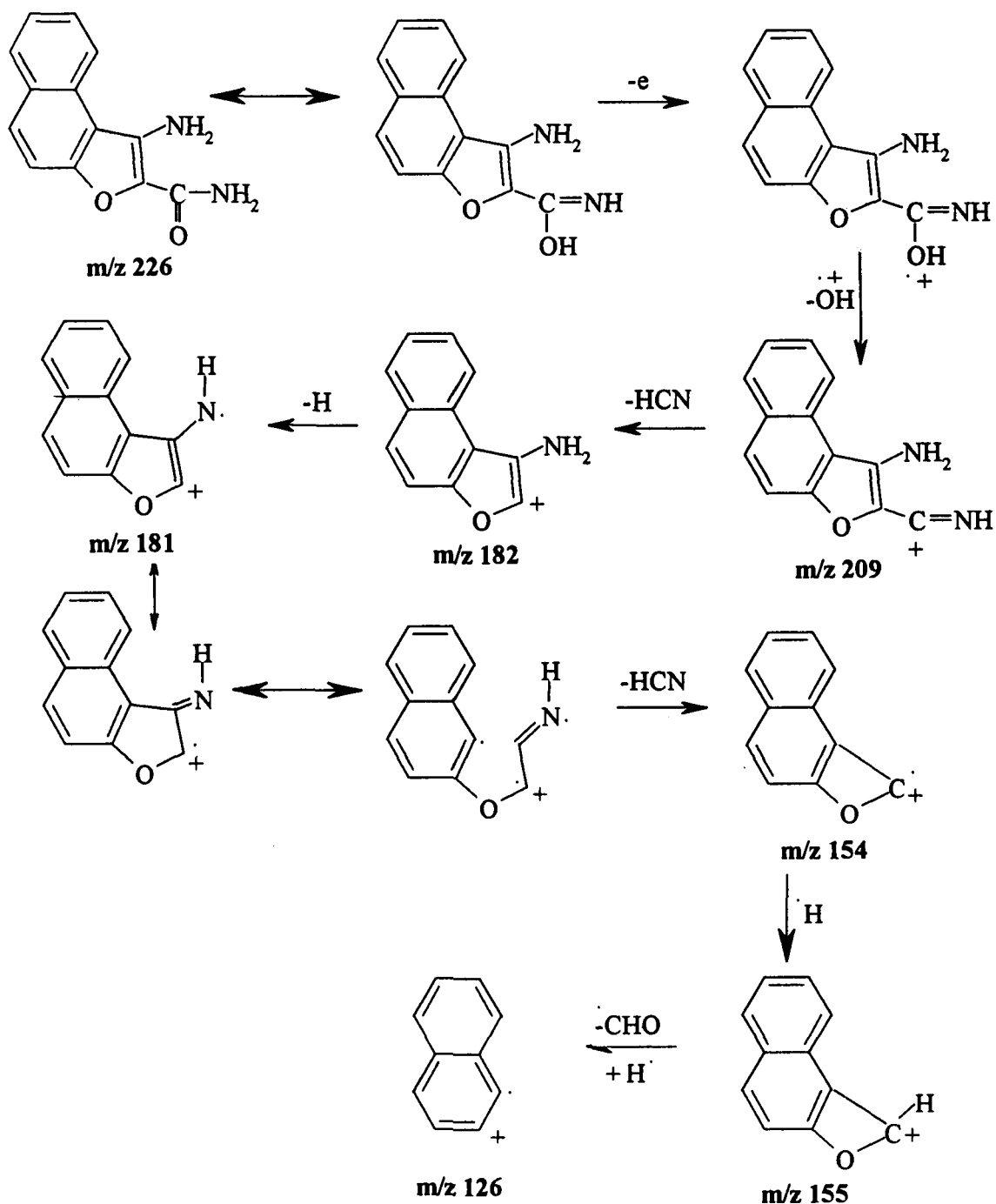


FIGURE-6.6

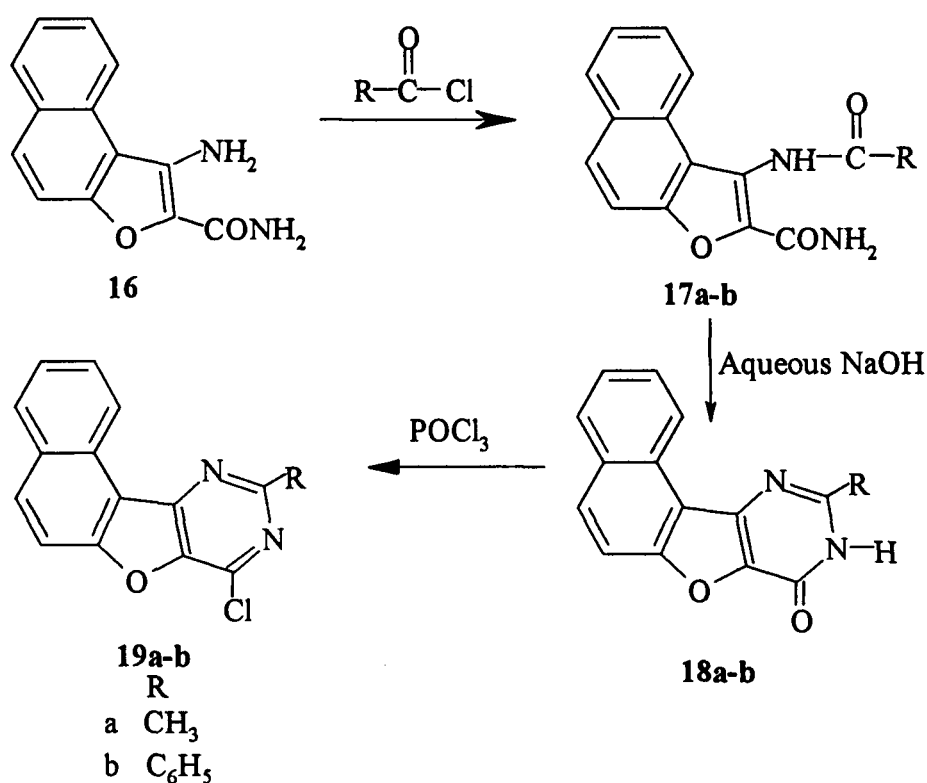
molecular ion peak at m/z 226 coinciding with its molecular weight. Even though the molecular weights of compounds **15** and **16** are same, the mass spectra were different as regards the fragmentation pattern. The fragmentation of compound **16** followed the pattern as depicted in Scheme-4.

Scheme-4

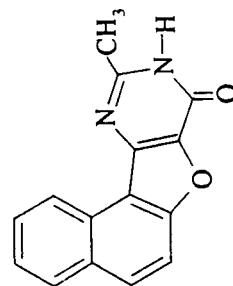
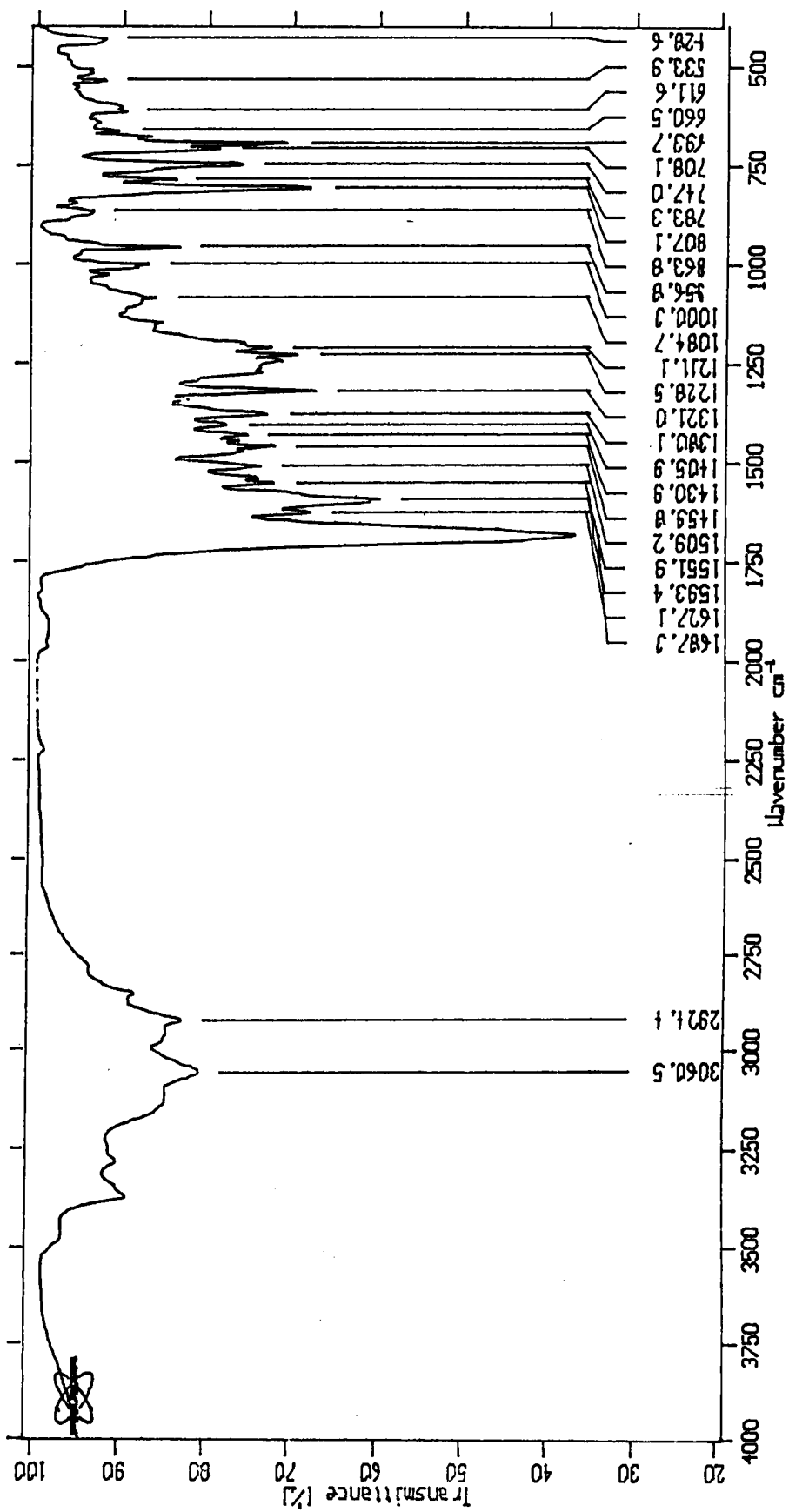


3. Conversion of 3-aminonaphtho[2,1-b]furan-2-carboxamide into 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines.

3-Aminonaphtho[2,1-b]furan-2-carboxamide **16** was converted into corresponding acyl derivatives by treatment with acetyl chloride and benzoyl chloride in presence of alkali. These acylated compounds underwent smooth ring closure on reaction with aqueous sodium hydroxide and resulted in the formation of 2-substituted-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidines.



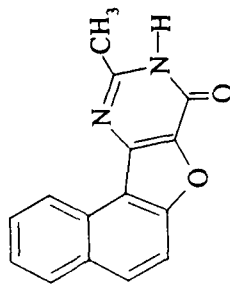
The structure of 2-methyl-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine **18a** was established by recording various spectra. The IR spectrum (Figure-6.7) exhibited carbonyl stretching frequency at 1687 cm^{-1} . The 1H NMR spectrum (Figure-6.8) exhibited a singlet at δ 2.5 due to three protons of methyl group. A multiplet was



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 DATE : 8/ 1/2003
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RSIC, IIT, MADRAS
 BRUKER IFS 66V
 FT-IR SPECTROMETER

FIGURE-6.7



sample 4

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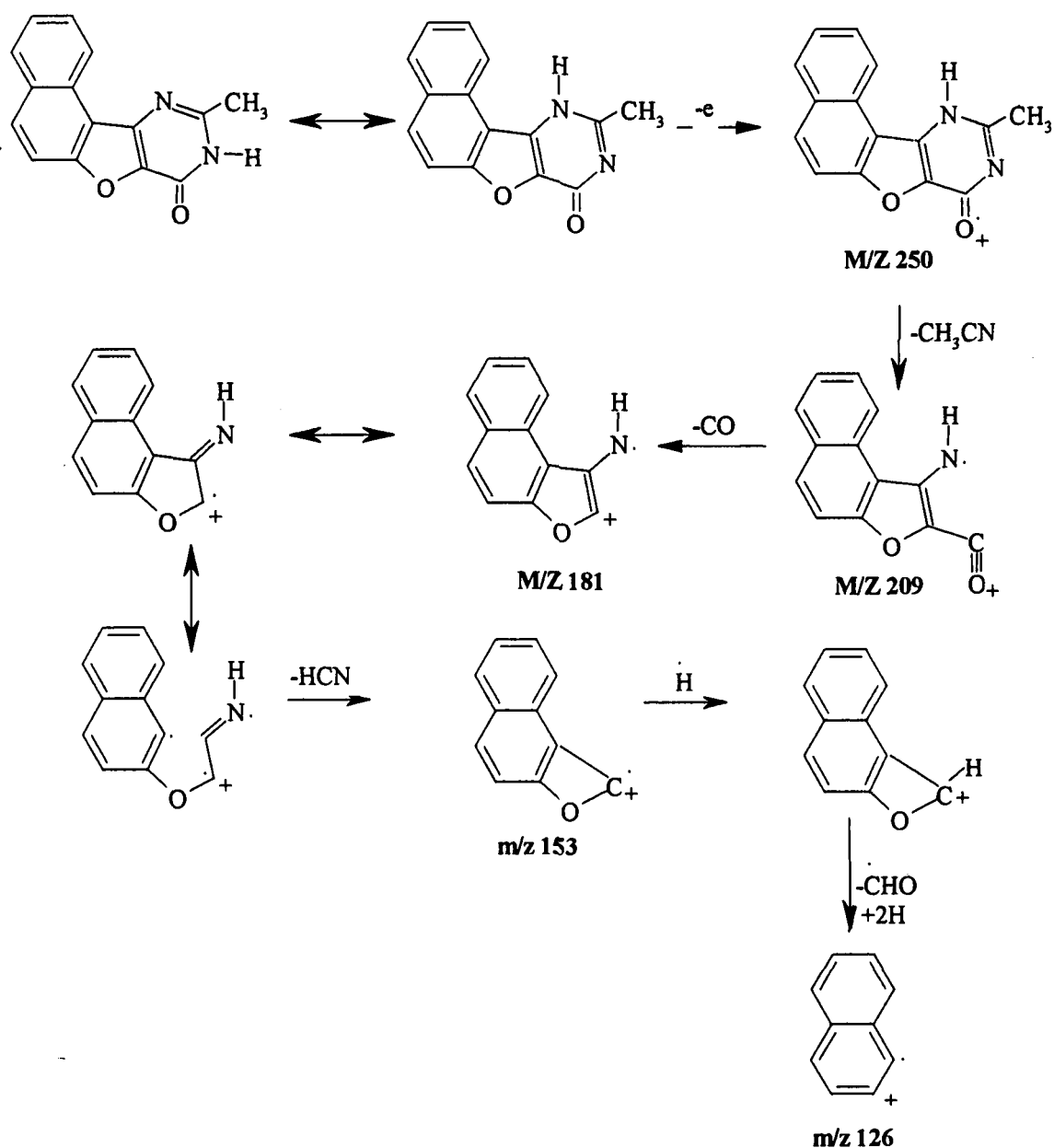
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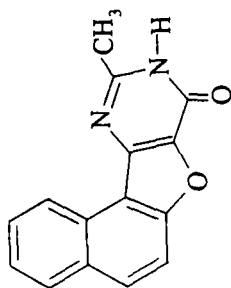
FIGURE-6.8

observed at δ 7.2 - δ 8.2 integrating for six protons was assignable to aromatic protons. The NH proton appeared as a singlet (D_2O exchangeable) at δ 9.3. The mass spectrum (Figure-6.9) showed a molecular ion peak at m/z 250 corresponding to its molecular weight. The fragment ions appearing at m/z 209, 181, 153 and 127 were in accordance with the fragmentation pattern as depicted in Scheme-6.

Scheme-6



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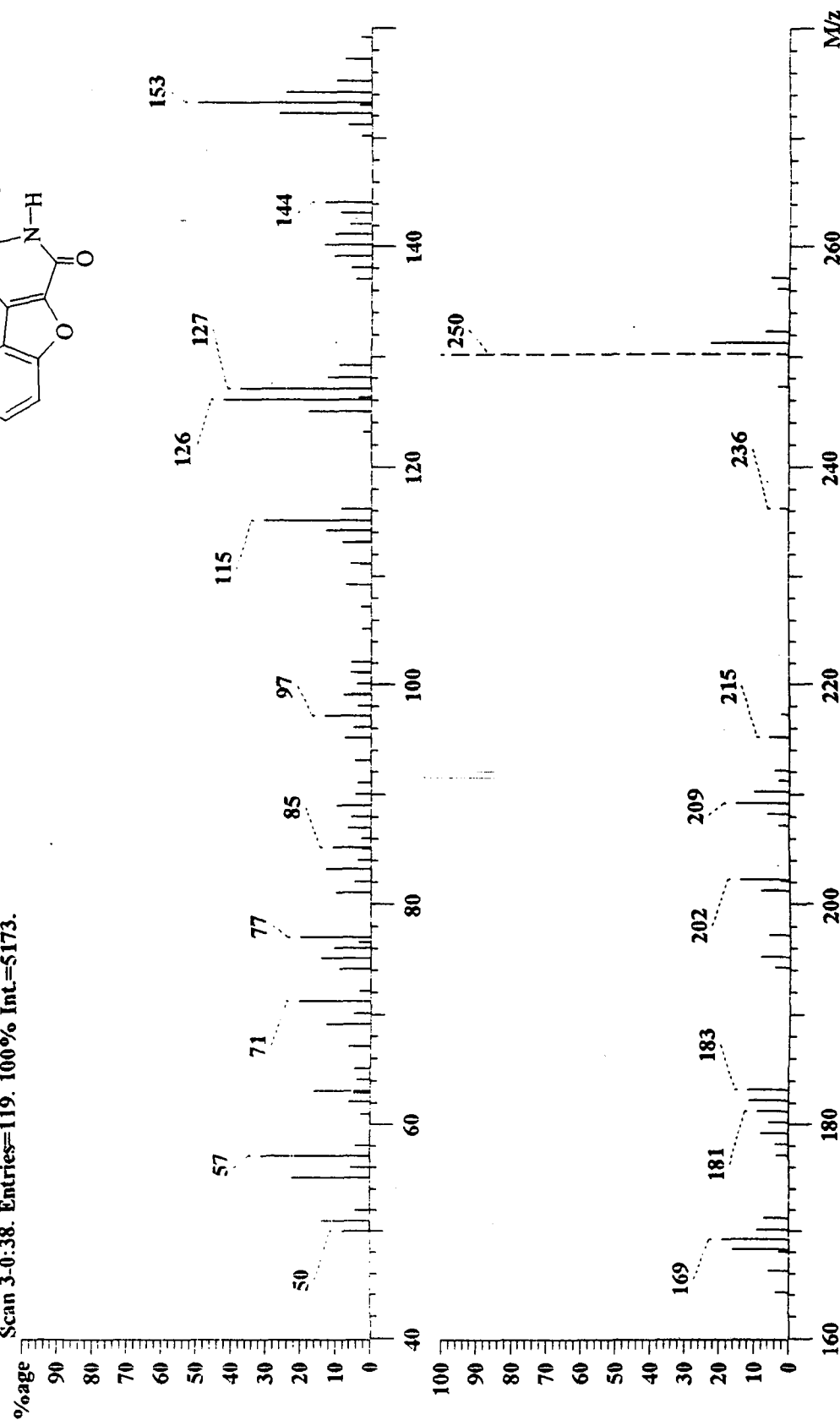


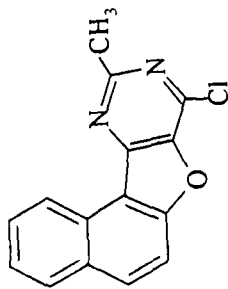
FIGURE-6.9

It is well-established fact that the change in substitutions at position 2 and 4 will have marked effect on biological properties of compounds⁶⁸⁻⁷⁰. Hence it was thought of interest to synthesize various substituted naphtho[2,1-b]furo[3,2-d]pyrimidines and investigate their biological activities with special reference to the effect of substituents.

With this view, 2-alkyl/aryl-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidines were converted into 4-chloro derivatives by refluxing with POCl₃. The formation of 4-chloro compounds was confirmed by their IR spectra, which showed absence of absorption bands due to both -C=O and -NH stretching frequencies. The structure of 2-methyl-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidine **19a** was further confirmed by recording ¹H NMR spectrum (Figure-6.10) which exhibited a singlet at δ 2.5 due to three protons of methyl group. A multiplet observed at δ 7.2 - δ 8.2 integrating for six protons was assignable to aromatic protons.

4. Nucleophilic substitution reactions of 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines to get the title compounds.

2-Alkyl/aryl-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines **19a-b** were subjected for substitution reactions with different nucleophilic reagents in order to obtain various 4-substituted compounds. The main purpose of carrying out these reactions was to investigate the effect of different kinds of substituents on biological activities. Thus the compounds **19a-b** was heated with various alcohols, phenols and amines to obtain 2-substituted-4-alkoxynaphtho[2,1-b]furo[3,2-d]pyrimidines **20a-d**, 2-substituted-4-aryloxynaphtho[2,1-b]furo[3,2-d]pyrimidines **21a-h** and 2-substituted-4-alkyl/aryl-amino naphtho[2,1-b]furo[3,2-d]pyrimidines **22a-t** respectively. Various amines selected in the



Sample15

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 EXPNO 6
 PROCNO 1

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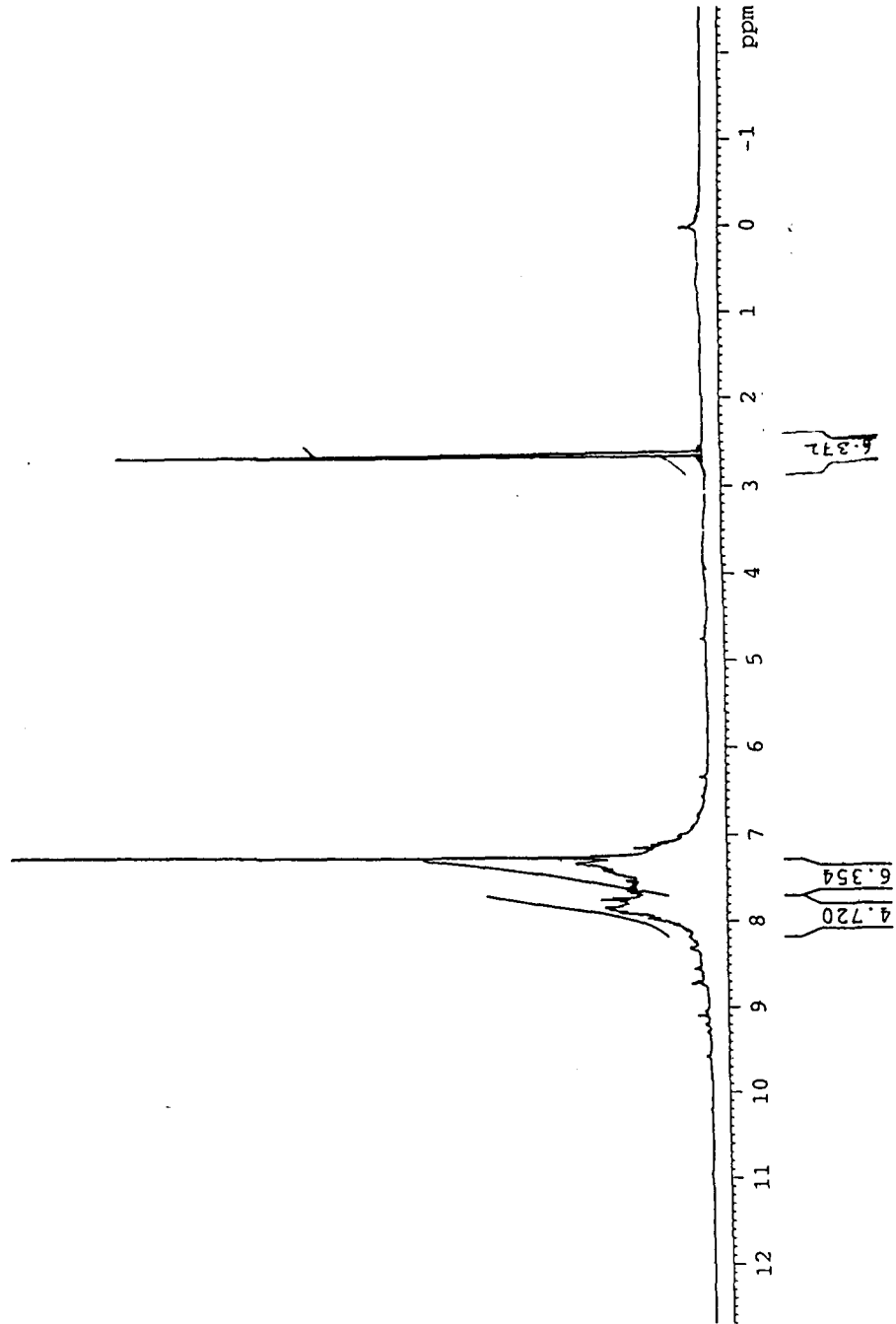
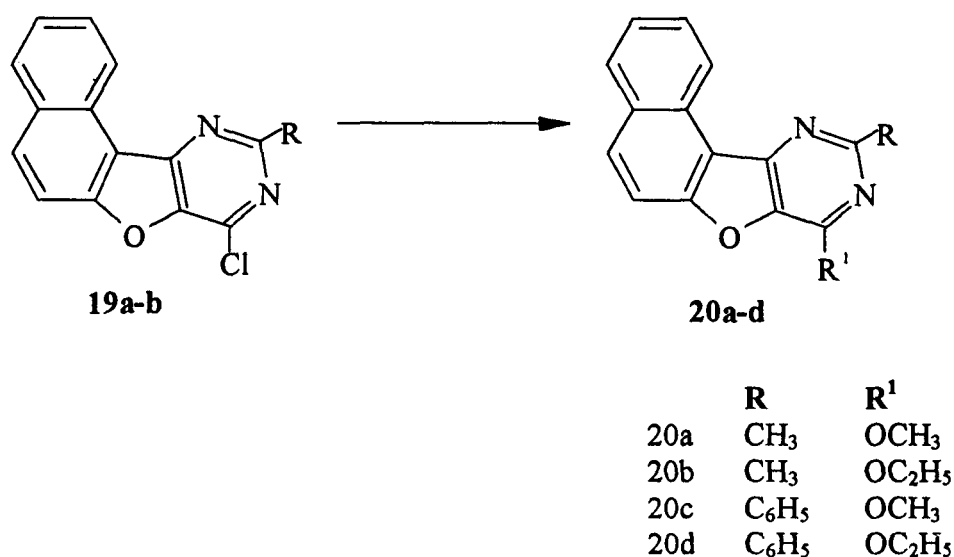


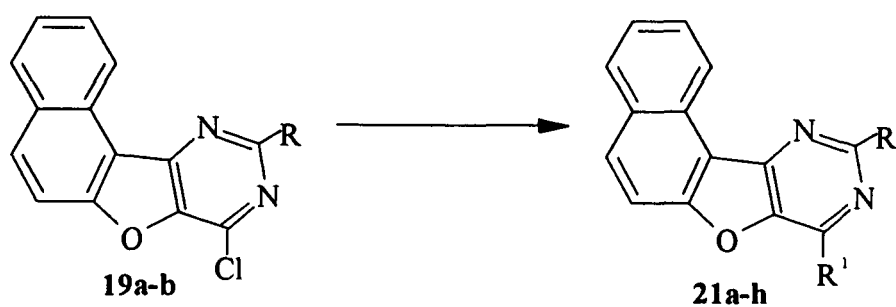
FIGURE-6.10

present study contained either electron donating groups or electron withdrawing groups in different positions of the aromatic system.

The displacement of chlorine atom by alkoxide ions was effected by heating the compound **19a-b** with sodium alkoxide in respective alcohols. There was separation of sodium chloride in the reaction. The IR spectral data of some selected compounds is shown in **Table-6.1**.

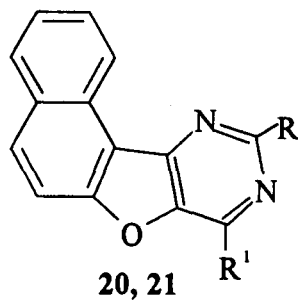


On the other hand 2-substituted-4-aryloxynaphtho[2,1-b]furo[3,2-d]pyrimidines were synthesized by heating corresponding phenol with ammonium carbonate and subsequently worked up by basification to get the compounds **21a-h** in considerably good yield. The IR spectral data of some selected compounds is shown in **Table-6.1**.



	R	R¹
21a	CH ₃	OC ₆ H ₅
21b	CH ₃	OC ₆ H ₄ -2-CH ₃
21c	CH ₃	OC ₆ H ₄ -3-CH ₃
21d	CH ₃	OC ₆ H ₄ -4-CH ₃
21e	C ₆ H ₅	OC ₆ H ₅
21f	C ₆ H ₅	OC ₆ H ₄ -2-CH ₃
21g	C ₆ H ₅	OC ₆ H ₄ -3-CH ₃
21h	C ₆ H ₅	OC ₆ H ₄ -4-CH ₃

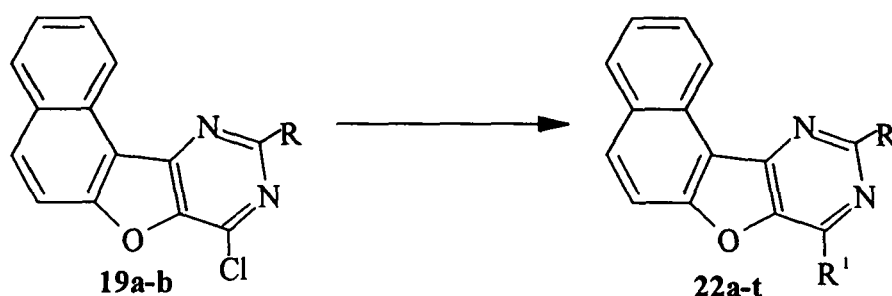
Table-6.1 IR spectral data of 2-substituted-4-alkoxy/aryloxynaphtho[2,1-b]furo[3,2-d]pyrimidines



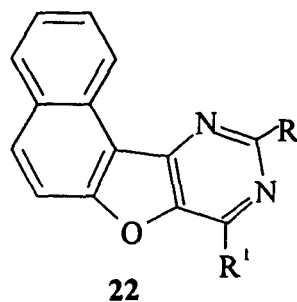
Compound	R	R ¹	IR (KBr) cm ⁻¹
			C=N
20a	CH ₃	OCH ₃	1582
20b	CH ₃	OC ₂ H ₅	1586
20c	C ₆ H ₅	OCH ₃	1574
20d	C ₆ H ₅	OC ₂ H ₅	1578
21a	CH ₃	OC ₆ H ₅	1570
21b	CH ₃	OC ₆ H ₄ -2-CH ₃	1576
21e	C ₆ H ₅	OC ₆ H ₅	1566
21f	C ₆ H ₅	OC ₆ H ₄ -2-CH ₃	1569

A series of 2-substituted-4-alkyl/arylamino naphtho[2,1-b]furo[3,2-d]pyrimidines were prepared by the reaction of **19a-b** with various primary and secondary aliphatic and aromatic amines. The amines such as ethylamine, which is commercially available in aqueous solution, was condensed with **19a-b** in aqueous solution of the amine itself. The condensation with aniline, 4-methoxyaniline, 4-bromoaniline, m-toluidine, p-toluidine, 4-aminobenzoic acid, 3-nitroaniline, 4-nitroaniline and 4-hydroxyaniline was carried out in an ethanolic solution.

The structures of all these products were routinely checked by recording IR spectra of representative compounds in the series. The IR spectral data of some selected compounds is shown in **Table-6.2**



	R	R ¹		R	R ¹
22a	CH ₃	NHC ₂ H ₅	22k	C ₆ H ₅	NHC ₂ H ₅
22b	CH ₃	NHC ₆ H ₅	22l	C ₆ H ₅	NHC ₆ H ₅
22c	CH ₃	NHC ₆ H ₄ -4-OCH ₃	22m	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃
22d	CH ₃	NHC ₆ H ₄ -4-Br	22n	C ₆ H ₅	NHC ₆ H ₄ -4-Br
22e	CH ₃	NHC ₆ H ₄ -3-CH ₃	22o	C ₆ H ₅	NHC ₆ H ₄ -3-CH ₃
22f	CH ₃	NHC ₆ H ₄ -4-CH ₃	22p	C ₆ H ₅	NHC ₆ H ₄ -4-CH ₃
22g	CH ₃	NHC ₆ H ₄ -4-COOH	22q	C ₆ H ₅	NHC ₆ H ₄ -4-COOH
22h	CH ₃	NHC ₆ H ₄ -3-NO ₂	22r	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂
22i	CH ₃	NHC ₆ H ₄ -4-NO ₂	22s	C ₆ H ₅	NHC ₆ H ₄ -4-NO ₂
22j	CH ₃	NHC ₆ H ₄ -4-OH	22t	C ₆ H ₅	NHC ₆ H ₄ -4-OH

Table-6.2 IR spectral data of 2-substituted-4-alkylamino/arylaminonaphtho[2,1-b]furo[3,2-d]pyrimidines

Compound	R	R ¹	IR(KBr) cm ⁻¹	
			C=N	NH
22a	CH ₃	NHC ₂ H ₅	1578	3400
22b	CH ₃	NHC ₆ H ₅	1576	3380
22c	CH ₃	NHC ₆ H ₄ -4-OCH ₃	1582	3382
22d	CH ₃	NHC ₆ H ₄ -4-Br	1578	3376
22e	CH ₃	NHC ₆ H ₄ -3-CH ₃	1584	3380
22g	CH ₃	NHC ₆ H ₄ -4-COOH	1566	3360
22h	CH ₃	NHC ₆ H ₄ -3-NO ₂	1569	3365
22j	CH ₃	NHC ₆ H ₄ -4-OH	1582	3390
22k	C ₆ H ₅	NHC ₂ H ₅	1574	3400
22l	C ₆ H ₅	NHC ₆ H ₅	1570	3296
22m	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃	1568	3300
22n	C ₆ H ₅	NHC ₆ H ₄ -4-Br	1562	3292
22o	C ₆ H ₅	NHC ₆ H ₄ -3-CH ₃	1565	3300
22q	C ₆ H ₅	NHC ₆ H ₄ -4-COOH	1550	3290
22r	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂	1562	3300
22t	C ₆ H ₅	NHC ₆ H ₄ -4-OH	1578	3360

Experimental

1-Cyano-2-naphthoxyacetamide 15

To a solution of 2-hydroxy-1-naphthonitrile **14** (16 g, 0.07 mol) in dry acetone (175 ml), 2-chloroacetamide (7 g, 0.074 mol) and anhydrous potassium carbonate (30 g) were added and refluxed for 8 hr. The reaction mixture was filtered and the filtrate on evaporation of the solvent gave **15** as light yellow colored solid. Some amount of **15** was recovered from the potassium salts by suspending in large excess of water and collecting the insoluble material. The combined yield was 96%. It was recrystallized from ethanol.

Yield, 96% ; m.p. 107⁰C ; Found : C, 69.00; H, 4.40; N, 12.26 ; C₁₃H₁₀N₂O₂ required : C, 69.02; H, 4.42; N, 12.38%.

3-Aminonaphtho[2,1-b]furan-2-carboxamide 16

The compound **15** (12 g, 0.05 mol), in a solution of potassium hydroxide (8 g, 0.05 mol) in ethanol (160 ml) was refluxed for 2 hr on a water bath. The reaction mixture was diluted with water and cooled in ice and neutralized. The brown colored solid thus separated was collected, dried and recrystallized from aqueous ethanol.

Yield, 86% ; m.p. 96⁰C ; Found : C, 68.97; H, 4.38; N, 12.28 ; C₁₃H₁₀N₂O₂ required : C, 69.02; H, 4.42; N, 12.38%.

3-Acetylamino-naphtho[2,1-b]furan-2-carboxamide 17a

A mixture of **16** (5 g, 0.01 mol) and acetyl chloride (25 ml) was warmed on water-bath for 15-20 min and then decomposed in ice water to get a crystalline solid of **17a**. It was recrystallized from aqueous ethanol.

Yield, 74% ; m.p. 148⁰C ; Found : C, 67.00; H, 4.41; N, 10.37 ; C₁₅H₁₂N₂O₃ required : C, 67.16; H, 4.47; N, 10.44%.

3-Benzoylaminonaphtho[2,1-b]furan-2-carboxamide 17b

The compound 16 (5 g, 0.01 mol) was warmed on water-bath for 20 min with benzoyl chloride (4 ml) in presence of aqueous sodium hydroxide (2N, 2 ml). The product 17b thus separated as solid was collected and recrystallized from aqueous ethanol.

Yield, 70% ; m.p. 152⁰C ; Found : C, 72.64; H, 4.14; N, 8.40 ; C₂₀H₁₄N₂O₃ required : C, 72.72; H, 4.24; N, 8.48%.

2-Methyl-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine 18a

Method A - Forgoing acetyl compound 17a (5 g, 0.02 mol) was suspended in aqueous sodium hydroxide (1N, 500 ml) and warmed on water-bath for 20 min. The resulting solution when acidified with dilute hydrochloric acid gave 18a as solid, which was recrystallized from aqueous ethanol.

Yield, 64% ; m.p. 160⁰C ; Found : C, 71.88; H, 4.00; N, 11.03 ; C₁₅H₁₀N₂O₂ required : C, 72.00; H, 4.00; N, 11.20%.

Method B - To a mixture of hydrogen peroxide (3 %, 20 ml) and aqueous potassium hydroxide (10 %, 15 ml), the acetyl compound 17a (1.5 g, 0.006 mol) was added and the mixture was heated at 70-75⁰ for about 30 min. The resulting solution when acidified with glacial acetic acid furnished 18a as light brown colored crystals, which was identical with the sample obtained in method (a).

2-Phenyl-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine 18b

The benzoyl derivative 17b (5 g, 0.015 mol) was suspended in aqueous sodium hydroxide (1N, 500 ml) and warmed on water-bath for 2 hr. The resulting solution when

acidified with dilute hydrochloric acid gave **18b** as solid, which was recrystallized from aqueous ethanol

Yield, 61% ; m.p. 144⁰C ; Found : C, 76.84; H, 3.72; N, 8.78 ; C₂₀H₁₂N₂O₂ required : C, 76.92; H, 3.84; N, 8.97%.

2-Substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines 19a-b

Compound **18a** (5 g, 0.02 mol) in freshly distilled phosphorus oxychloride (50 ml) was heated under reflux until a clear solution was obtained. The resulting solution was cooled and poured into crushed ice with stirring. The chloro compound **19a** thus separated was collected and recrystallized from ethanol. Compound **19b** was prepared from **18b** in the similar way. The physical and analytical data of the compounds synthesized is presented in **Table-6.3**.

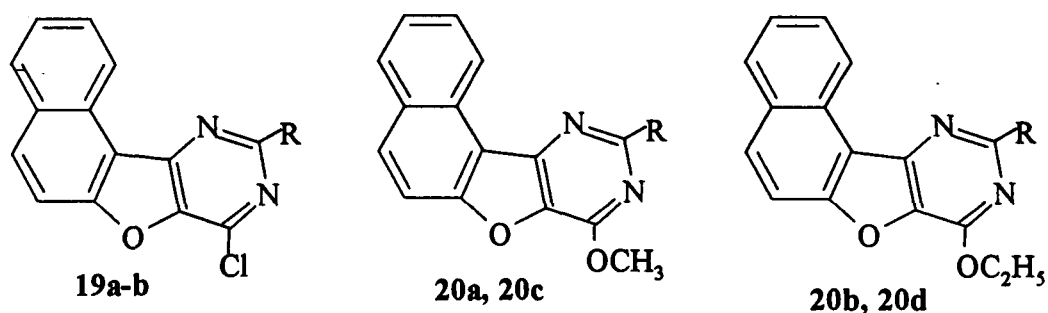
2-Substituted-4-methoxynaphtho[2,1-b]furo[3,2-d]pyrimidine 20a, 20c

To a solution of **19a** (0.6 g, 0.0022 mol) in absolute methanol (10 ml), a solution of sodium methoxide prepared from sodium metal (0.3 g) and absolute methanol (10 ml) was added. Immediately sodium chloride separated. The reaction mixture was warmed on water-bath for 30 min, cooled and diluted with water. **20a** was collected and recrystallized from methanol. Similarly **20c** was also prepared. The physical and analytical data of the compounds synthesized is presented in **Table-6.3**.

2-Substituted-4-ethoxynaphtho[2,1-b]furo[3,2-d]pyrimidine 20b, 20d

To a solution of **19a** (0.6 g, 0.0022 mol) in absolute ethanol (10 ml), a solution of sodium ethoxide prepared from sodium metal (0.3 g) and absolute ethanol (10 ml) was added. Immediately sodium chloride was separated. The reaction mixture was warmed on water-bath for 30 min, cooled and diluted with water. **20b** was collected and recrystallized from methanol. Similarly **20d** was also prepared. The physical and analytical data of the compounds synthesized is presented in Table-6.3.

Table-6.3

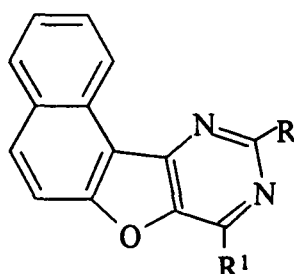


Compound	R	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
					C	H	N
19a	CH ₃	C ₁₅ H ₉ N ₂ OCl	76	168	67.04 (66.94)	3.35 (3.30)	10.42 (10.34)
19b	C ₆ H ₅	C ₂₀ H ₁₁ N ₂ OCl	72	122	72.61 (72.57)	3.32 (3.28)	8.47 (8.43)
20a	CH ₃	C ₁₆ H ₁₂ N ₂ O ₂	65	139	72.72 (72.70)	4.54 (4.54)	10.52 (10.50)
20c	C ₆ H ₅	C ₂₁ H ₁₄ N ₂ O ₂	67	134	77.30 (77.22)	4.29 (4.28)	8.58 (8.53)
20b	CH ₃	C ₁₇ H ₁₄ N ₂ O ₂	64	143	73.38 (73.40)	5.03 (4.95)	10.00 (10.00)
20d	C ₆ H ₅	C ₂₂ H ₁₆ N ₂ O ₂	65	140	77.64 (77.52)	4.70 (4.68)	8.21 (8.16)

2-Substituted-4-aryloxynaphtho[2,1-b]furo[3,2-d]pyrimidines 21a-h

A mixture of 19a (0.6 g, 0.0022 mol), freshly distilled phenol (6 g) and powdered ammonium carbonate (2 g) was initially heated gently followed by heating on water-bath for 40-50 min. Upon dilution with appropriate amount of aqueous sodium hydroxide solution (10%), the solid 21a-d that separated was filtered, washed with water and recrystallized from suitable solvent. Compounds 21e-h were also prepared in the same way. The physical and analytical data of the compounds synthesized is presented in Table-6.4.

Table-6.4



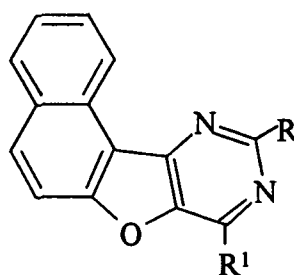
21a-h

Comp	R	R ¹	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
						C	H	N
21a	CH ₃	OC ₆ H ₅	C ₂₁ H ₁₄ N ₂ O ₂	58	150	77.30 (77.26)	4.29 (4.19)	8.58 (8.51)
21b	CH ₃	OC ₆ H ₄ -2-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	70	136	77.64 (77.62)	4.70 (4.55)	8.23 (7.98)
21c	CH ₃	OC ₆ H ₄ -3-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	64	153	77.64 (77.58)	4.70 (4.66)	8.23 (7.96)
21d	CH ₃	OC ₆ H ₄ -4-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	55	160	77.64 (77.60)	4.70 (4.74)	8.23 (8.02)
21e	C ₆ H ₅	OC ₆ H ₅	C ₂₆ H ₁₆ N ₂ O ₂	82	180	80.41 (80.32)	4.12 (4.10)	7.19 (7.12)
21f	C ₆ H ₅	OC ₆ H ₄ -2-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	80	210	80.59 (80.50)	4.47 (4.29)	6.96 (6.83)
21g	C ₆ H ₅	OC ₆ H ₄ -3-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	79	206	80.59 (80.53)	4.47 (4.34)	6.96 (6.87)
21h	C ₆ H ₅	OC ₆ H ₄ -4-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	86	218	80.59 (80.49)	4.47 (4.38)	6.96 (6.90)

2-Substituted-4-alkyl/arylamino-naphtho[2,1-b]furo[3,2-d]pyrimidines 22a-t

A mixture of 19a (0.6 g, 0.0022 mol) and appropriate amine (0.005 mol) in absolute ethanol (12 ml) was heated on a water-bath for 4-6 hr. After diluting with water, it was allowed to stand overnight. The solid product 22a-j was collected and recrystallized from suitable solvent. Compounds 22k-t were also prepared in the same way. The physical and analytical data of the compounds synthesized is presented in Table-6.5.

Table-6.5



22a-t

Comp	R	R¹	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
						C	H	N
22a	CH ₃	NHC ₂ H ₅	C ₁₇ H ₁₅ N ₃ O	42	129	73.64 (73.55)	5.41 (5.29)	15.16 (15.02)
22b	CH ₃	NHC ₆ H ₅	C ₂₁ H ₁₅ N ₃ O	58	136	77.54 (77.62)	4.60 (4.55)	12.92 (12.67)
22c	CH ₃	NHC ₆ H ₄ -4-OCH ₃	C ₂₂ H ₁₇ N ₃ O ₂	64	142	74.36 (74.20)	4.78 (4.66)	11.83 (11.80)
22d	CH ₃	NHC ₆ H ₄ -4-Br	C ₂₁ H ₁₄ N ₃ OBr	62	153	62.37 (62.19)	3.46 (3.29)	10.39 (10.19)
22e	CH ₃	NHC ₆ H ₄ -3-CH ₃	C ₂₂ H ₁₇ N ₃ O	70	169	77.87 (77.80)	5.01 (5.10)	12.38 (12.24)
22f	CH ₃	NHC ₆ H ₄ -4-CH ₃	C ₂₂ H ₁₇ N ₃ O	72	142	77.87 (77.68)	5.01 (4.89)	12.38 (12.19)
22g	CH ₃	NHC ₆ H ₄ -4-COOH	C ₂₂ H ₁₅ N ₃ O ₃	60	156	71.54 (71.42)	4.06 (4.00)	11.38 (11.32)
22h	CH ₃	NHC ₆ H ₄ -3-NO ₂	C ₂₁ H ₁₄ N ₄ O ₃	78	164	68.1 (68.19)	3.78 (3.67)	15.13 (15.10)

22i	CH ₃	NHC ₆ H ₄ -4-NO ₂	C ₂₁ H ₁₄ N ₄ O ₃	76	168	68.1 (68.01)	3.78 (3.62)	15.13 (15.00)
22j	CH ₃	NHC ₆ H ₄ -4-OH	C ₂₁ H ₁₅ N ₃ O ₃	58	140	73.90 (73.84)	4.39 (4.29)	12.31 (12.28)
22k	C ₆ H ₅	NHC ₂ H ₅	C ₂₂ H ₁₇ N ₃ O	70	195	77.87 (77.81)	5.01 (4.90)	12.38 (12.34)
22l	C ₆ H ₅	NHC ₆ H ₅	C ₂₆ H ₁₇ N ₃ O	80	200	80.62 (80.50)	4.39 (4.23)	10.88 (10.76)
22m	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃	C ₂₇ H ₁₉ N ₃ O ₂	68	168	77.69 (77.61)	4.55 (4.32)	10.07 (10.00)
22n	C ₆ H ₅	NHC ₆ H ₄ -4-Br	C ₂₆ H ₁₆ N ₃ OBr	74	175	66.95 (66.78)	3.43 (3.38)	9.01 (9.02)
22o	C ₆ H ₅	NHC ₆ H ₄ -3-CH ₃	C ₂₇ H ₁₉ N ₃ O	86	186	80.79 (80.80)	4.73 (4.59)	10.47 (10.34)
22p	C ₆ H ₅	NHC ₆ H ₄ -4-CH ₃	C ₂₇ H ₁₉ N ₃ O	79	190	80.79 (80.68)	4.73 (4.61)	10.47 (10.37)
22q	C ₆ H ₅	NHC ₆ H ₄ -4-COOH	C ₂₇ H ₁₇ N ₃ O ₃	76	238	75.17 (75.0)	3.94 (3.98)	9.74 (9.76)
22r	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂	C ₂₆ H ₁₆ N ₄ O ₃	64	192	72.22 (72.13)	3.70 (3.62)	12.66 (12.50)
22s	C ₆ H ₅	NHC ₆ H ₄ -4-NO ₂	C ₂₆ H ₁₆ N ₄ O ₃	69	189	72.22 (72.11)	3.70 (3.65)	12.66 (12.52)
22t	C ₆ H ₅	NHC ₆ H ₄ -4-OH	C ₂₆ H ₁₇ N ₃ O ₂	72	166	77.42 (77.35)	4.21 (3.98)	10.42 (10.43)

Some of the selected compounds have been evaluated for antibacterial, anthelmintic, anti-inflammatory and antifertility activities and the description of the pharmacological activities is discussed in **Chapter-8**.

References

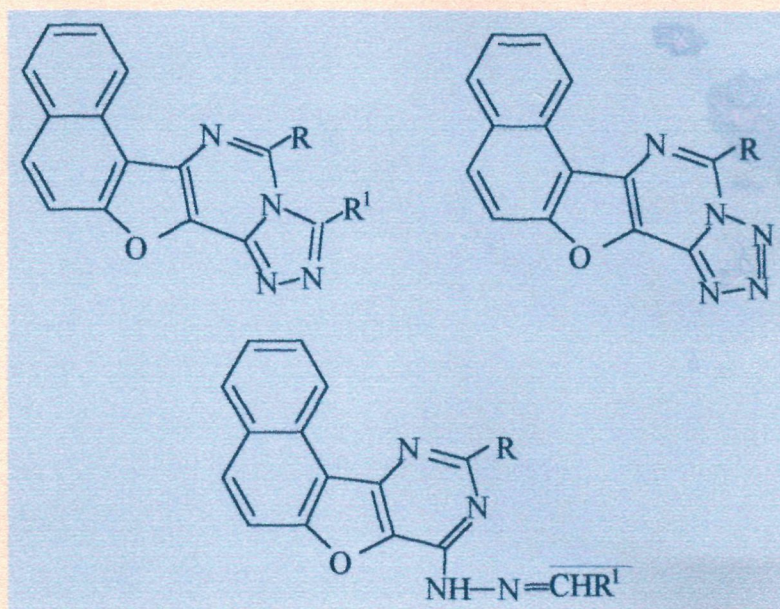
1. R. Dolique, "*Traite de Chimie Organique*", Vol. 18, p. 222-259, Masson, Paris, (1935).
2. V.B. Meyer, "Heterocyclic Compounds" (Ed. By Elderfield), Vol. 2, p. 1-65, Wiley, New York (1951).
3. T.S. Stevens, "Chemistry of Carbon Compounds" (Ed. By E.M. Rodd), Vol. IV A, p. 168-185, Elsevier Amsterdam (1957).
4. F.M. Dean, "Naturally Occurring Oxygen Ring Compounds", Butterworths and Co., London, (1963).
5. A. Mustafa, "Furofurans and Furofurones" (Ed. By Weissberger), Interscience Publisher, London, New York, (1967).
6. L.F. Small, N.B. Eddy, E. Moesetting and L.K. Himmelsbach, "Studies on Drug Addiction", Public Health Dept. Suppl., 138 (1938).
7. P.I. Abramenko, V.G. Zhiryakov, Z.A. Balykova and T.K. Ponomareva, *Khim. Geterosikl. Soedin.*, **6**, 796 (1974).
8. M. Robba and M. Cugnon de Sevracourt, *C.R. Hebd. Seance Acad. Sci. Ser. C.*, **280**, 583 (1975).
9. L.T. Bogolyubskaya and M.A. Al'perovich, *Zh. Obshch. Khim.*, **34**, 3119 (1964).
10. W.U. Malik, V.K. Mahesh and M. Raisinghani, *Indian J. Chem.*, **9**, 655 (1971).
11. D.J. Fry, E.G. Ficken and R.W. Burrows, *Brit.*, **1**, 168495 (1969); *Chem. Abstr.*, **72**, 68223 (1969).
12. B.A. Brady, J.A. Kennedy and W.I. O'Sullivan, *Tetrahedron*, **29**, 359 (1973).
13. C.R. Portal and A.R. Frasca, *An. Asos. Quim. Argent*, **59**, 69 (1971).
14. H. Gillman, P.T. Parker, J.C. Bailic and G.F. Brown, *J. Am. Chem. Soc.*, **61**, 2836 (1939).
15. J.W. Cornforth, G.K. Hughes, F. Lions and R.H. Harradence, *J. Proc. Roy. Soc.*, **71**, 475 (1938).
16. R.K. Anderson and G.W.H. Cheeseman, *J. Chem. Soc., Parkin Trans-I*, 129 (1974).

17. S.S. Sangapure and Y.S. Agasimundin, *Indian J. Chem.*, **14B**, 688 (1976).
18. R. Sivano, *Brit.*, 1,219,847 (1947); *Chem. Abstr.*, **74**, 141901 91971).
19. J.R. Price and R. Robinson, *J. Chem. Soc.*, 1522 (1939).
20. J.R. Price and R. Robinson, *J. Chem. Soc.*, 1493 (1940).
21. B.K. Rohatgi, R.B. Gupta, D. Roy and R.N. Khanna, *Indian J. Chem.*, **22B**, 886 (1983).
22. R.N. Khanna, P.K. Sharma and R.H. Thomson, *J. Chem. Soc., Perkin Trans-I*, 1882 (1987).
23. P.K. Sharma, P.K. Gupta, R.B. Khanna and B.K. Rohatgi, *Indian J. Chem.*, **24B**, 1070 (1985).
24. P.K. Sharma, R.B. Khanna B.K. Rohatgi and R.H. Thomson, *Phytochemistry*, **27(2)**, 633 91988).
25. R. Stoermer, *Chem. Ber.*, **30**, 1693 (1897).
26. R. Stoermer, *Annalen.*, **312**, 237 (1900).
27. P. Emmott and R. Livingstone, *J. Chem. Soc.*, 3144 (1957).
28. C.E. Loader and C.J. Timmons, *J. Chem. Soc.*, 1678 (1967).
29. J.N. Chattergiea Lal S. Jha, R.D.S. Udari and J. Carnduff, *J. Chem. Res. Synop.*, (11), 356 (1979).
30. N. Weillthevenet, J.P. Buisson, R. Royer and M. Hofnung, *Mutation Research*, **88(4)**, 355 (1981).
31. J. Gilotdelhalle, J. Moutschen and M. Moutschendahmen, *Environmental and exptl. Botany*, **34(4)**, 451 (1994).
32. A. Arrault, F. Touseau, G. Guillaunet and J.Y. Merour, *Synthesis*, **7**, 1241 (1999).
33. H.M. Vagdevi and V.P. Vaidya, *Indian J. Heterocycl. Chem.*, **10**, 253 (2001).
34. H.M. Vagdevi, K.P. Latha, V.P. Vaidya, M.L. Vijayakumar and K.S.R. Pai, *Indian J. Pharm. Sci.*, **63(4)**, 286 (2001).
35. K.P. Latha, V.P. Vaidya, J. Keshavayya and H.M. Vagdevi, *J. Indian Council Chem.*, **17(3)**, 31 (2001).

36. K.M. Mahadevan, Basavaraj Padmashali and V.P. Vaidya, *Indian J. Heterocycl. Chem.*, **11**, 15 (2001).
37. K.M. Mahadevan, and V.P. Vaidya, *J. Indian Concil Chem.*, **18(2)**, 78 (2001).
38. K.P. Latha, V.P. Vaidya and J. Keshavayya, *Nat. Acad. Sci. Lett.*, **25**, 586 (2002).
39. H.M. Vagdevi, V.P. Vaidya and K.M. Mahadevan, *Kuvempu Uni. Sci. J.*, **2(1)**, 13 (2002).
40. K.M. Mahadevan and V.P. Vaidya, *Indian J. Pharm. Sci.*, **11**, 15 (2001).
41. M.I. Crespo, L. Payes, A. Vega, V. Seggarra, M. Lopez, T. domenech, M. Miralpeix, J. Beleta, H. Ryder, J.M. Palacios, *J. Med. Chem.*, **41(21)**, 4035 (1998).
42. M.W. Partridge and M.G. Stevnts, *J. Chem. Soc.*, 1127 (1966).
43. J. Slouka, V. Bakarek and J. Kubata, *Monash Chem.*, **105**, 535 (1974).
44. J. Slouka, V. Bakarek and J. Kubata, *Acta. Univ. Polaki. Olomuc. Rac Reetrium Nature.*, **4a**, 217 (1976).
45. A. Gangjee, F. Mavandadi, S.F. Queener, *Adv. Exp. Med. Biol.*, **338**, 441 (1993).
46. J. Slouka and V. Bakarek, *Coll. Czech. Chem. Commun.*, **49**, 275 (1984).
47. C.G. Dave and M.C. Shukla, *Indian J. Chem.*, **39B**, 210 (2000).
48. G. Suma, R.H. Bahekar and A.R.R. Rao, *Org. Prep. Procd. Int.*, **32**, 99 (2000).
49. R.P. Mailavaram, R.R.R. Akkinapalli, S.R. Pamulaparthi and S.R. Kombu, *Synthesis*, **14**, 2610 (2001).
50. M.S. Manhas, V.V. Rao, P.A. Seetharaman, D. Succardi and J. Pazdera, *J. Chem. Soc.*, 1937 (1969).
51. A. Gangjee, E. Elzein, S.F. Queener, J.J. McGuire, *J. Med. Chem.*, **41(9)**, 1409 (1998).
52. M.Rinald, P. Pecorari, L. Constantino, A. Provvisionato, M. Molagoli and C. Cermelli, *Farmaco.*, **47(10)**, 1315 (1992).
53. F. Itoh, K. Yukishige, M. wajima, K. Ootsu and H. Akimoto, *Chemical and Pharmaceutical Bulletin*, **43(2)**, 230 (1995).

54. Liu Zumina, Yang Guangtu and Qin Xianghua, *Journal of Chemical Technology and Biotechnology*, **76(11)**, 1154 (2001).
55. O. Moukha Chafiq, M.L. Taha, H.B. Lazrek, J.J. Vasseur, C. Pannecouque, M. Witvrouw and E. De Clercq, *Nucleosides, Nucleotides and Nuclieic acids*, **20(11)**, 1811 (2001).
56. M.A. el Shaerbeny, *Arznelmittel forschung.*, **50(9)**, 848 (2000).
57. Lauria Antonino, Diana Patrizia, Barraja Paola, Almerico Anna Maria, Cirrincione Girolamo and Dattalo Gaetano, *J. Hetrocycl. Chem.*, **37(4)**, 747 (2000).
58. Bruno Olga, Schenone Silvia, Ranise Angelo, Bondavalli Fraasco, Barocelli Elisabetta, Ballabeni Vigilio, Chivarini Milena, Betoni Simona, Tognolini Massimiliano and Impicciatore Mariannina, *Biorg. Med. Chem.*, **9(3)**, 629 (2001).
59. G. Biagi, A. Constantino, L. Constantino, I. Giogi, O. Livi, P. Pecorari, M. Rinald and V. Scartoni, *J. Med. Chem.*, **39(13)**, 2529 (1996).
60. Gangiee Aleem,. Elzein Elfatih, F. Queener Sherry and J. Mc Guire, *J. Med. Chem.*, **41(9)**, 1409 (1998).
61. S.S. Sangapure and Y.S. Agasimundin, *Indian J. Chem.*, **14B**, 688 (1976).
62. S.S. Sangapure and Y.S. Agasimundin, *Indian J. Chem.*, **15B**, 485 (1977).
63. S.B. Mahajan and Y.S. Agasimundin, *J. Indian Chem. Soc.*, **LIV**, 965 (1977).
64. S.S. Sangapure and Y.S. Agasimundin, *Indian J. Chem.*, **16B**, 627 (1978).
65. S.B. Mahajan and Y.S. Agasimundin, *Indian J. Chem.*, **19B**, 402 (1980).
66. K.M. Mahadevan, Ph.D. Thesis, Kuvempu University, 2003.

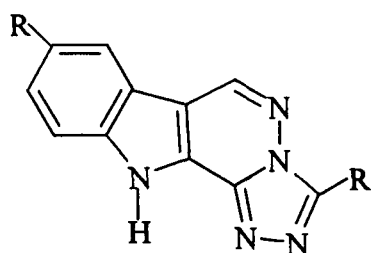
CHAPTER-7
Synthesis of some Angularly Fused
Pentacyclic Heterocycles



INTRODUCTION

A great deal of synthetic work describing the preparation of nitrogen containing 5-membered ring fused to different heterocyclic systems has appeared in literature. In this connection, there are several reports in which triazole and tetrazole moieties have been condensed with various heterocyclic moieties.

Several derivatives of 11H-1,2,4-triazolo[4,3-b]pyridazino[4,5-b]indoles **1a-f** have been synthesized and screened for antimicrobial activity against *E. coli*, *S. aureus* and *C. utilis*. Some of the compounds have been shown to exhibit considerable antimicrobial activity against these organisms^{1,2}.

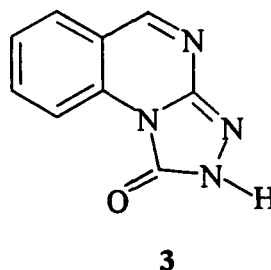
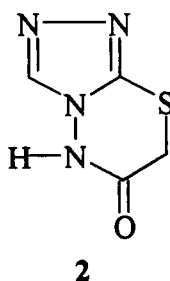


1a-f

	R	R ¹
a	CH ₃	CH ₃
b	OCH ₃	CH ₃
c	Br	CH ₃
d	CH ₃	C ₆ H ₅
e	OCH ₃	C ₆ H ₅
f	Br	C ₆ H ₅

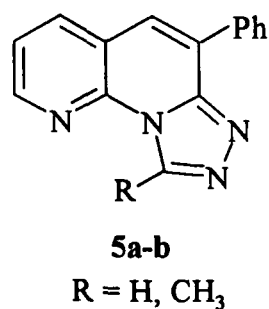
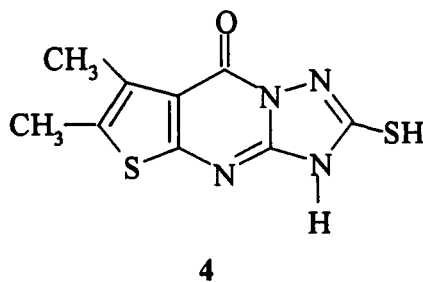
Talwar et al., carried out synthesis and antimicrobial activity of various s-triazolo[4,3-b][4,1,2]thiadiazinones **2** and observed that several of the newly synthesized compounds exhibited considerable antimicrobial activity against bacteria *E. coli* and *B. cirrrollagellosus* and fungi *C. albicans* and *A. niger*³.

An interesting report has appeared in literature in which triazole moiety has been constructed on quinazoline ring **3**. The compounds synthesized have demonstrated significant antifertility activity hormonal profile^{4,6}.



Pathak et al., have reported the synthesis of 1,2,4-triazolopyrimidines **4** for their biological importance⁷⁻¹⁰.

The synthesis of 1,2,4-triazolo[4,3-a][1,8]naphthyridines **5** has been carried out and the resulting compounds have been evaluated for antimicrobial activity by filter paper technique. Some of the compounds were found to be active against the fungi *F. oxysporum* and *C. lunata* and bacteria *E. coli* and *B. polymyxa*¹¹.



Michel et al., have synthesized polyazaheterocyclic derivatives containing triazole moiety and screened them for anti-inflammatory activity¹².

Several fused triazoles have been prepared as probes for retaining active sites of β -glycosides¹³.

Markosyan et al., reported the synthesis and psychotropic activity of triazoles and tetrazoles condensed with spirobenzo-2H-quinazoline-5,1'-cycloalkanes¹⁴.

Muscaranic activity of quinuclidine-3-yl-triazole and tetrazole derivatives were carried out by radio ligand binding assay using 3H-oxo-tremorine¹⁵. Potency and efficacy were observed in few compounds in which azole moiety was attached to the azo bicyclic ring.

Recently, Darias et al., reported the synthesis of 5H-s-triazolo[4,3-d][1,4]-benzodiazepines. The newly synthesized compounds were tested for acute toxicity and central nervous system effect in mice. Many of the compounds showed a clear CNS depressant activity. Some of the compounds presented a remarkable benzodiazepine like action with a pharmacological profile similar to that of chlordiazepoxide¹⁶.

Some 1,2,4-triazolo[4,3-c]quinazolines have been found to possess anti-inflammatory, anxiolytic and antitumor¹⁷⁻¹⁹ activities. Several pyrido[3,2-e]1,2,4-triazolo[4,3-c]pyrimidines have been synthesized for their analgesic, CNS depressant and antipyretic activities²⁰.

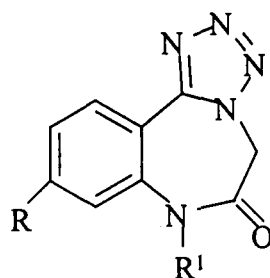
Similarly, fused tetrazoles such as pyrazolo[4,3-e]tetrazolo[1,5-c]pyrimidines and tetrazolo[5,1-c]purines have been evaluated for antimalarial activity and for inhibition of blood platelet aggregation^{21,22}.

New triazolo- and tetrazolo-pyrimidobenzodiazepines have been synthesized and screened as anti-HIV, anticancer and immunosuppressive agents²³. Some of the compounds showed immunosuppressive activity, similar to or stronger than that of cyclosporine A.

A convenient route for the synthesis of 1,2,3-selenadiazole, 1,2,3-thiadiazole, 1,2,4-triazolo[4,3-a]pyrimidine, tetrazolo[4,5-a]pyrimidine, benzimidazolo[1,2-a]pyrimidine and pyrazolo[3,4-b]pyridine derivatives has been reported, in which the naphthyl nucleus is incorporated. The preliminary results of antifungal testing indicated that some of the compounds showed considerable antifungal activity²⁴.

Maxwell et al., have synthesized a series of 5-aryl-2H-tetrazoles, 5-aryl-2H-tetrazole-2-acetic acids and (4-phenyl-5-aryl-4H-1,2,4-triazol-3-yl)-thioacetic acids and tested in vitro for superoxide scavenging activity in vivo in the carrageenan-induced rat paw edema assay and in the reverse passive Arthus reaction. The hydroxy-substituted compounds were found to be effective as in vitro scavengers of superoxide but were not effective as in vivo anti-inflammatory agents²⁵.

Peet and Sunder have synthesized several 5H-tetrazolo[1,5-d][1,4]benzodiazapin-6(7H)-ones **6a-b** and evaluated them for different pharmacological activities²⁶.



6a-b

	R	R ¹
a	OH	CH ₃
b	OCH ₃	CH ₃

In addition to these reports, there are many more evidences to point out biological importance of condensed triazole and tetrazole derivatives. However, detailed discussion about the research carried out in this field is not within the scope of this thesis.

It is clear from the above discussion that condensation of triazole and tetrazole moieties with various heterocyclic systems will result in new compounds with various biological and pharmacological properties. Encouraged by these reports, guided by the observation that many a times the condensation of one or more heterocyclic nuclei in a molecule enhances the biological profile many-folds and in continuation of our search for biologically potent naphthofuran derivatives, we report in this chapter, the synthesis and pharmacological investigation of 2-substituted-s-triazolo[4,3-c]pyrimido[4,5-b]naphtho[2,1-b]furans and tetrazolo[1,5-c]pyrimido[4,5-b]naphtho[2,1-b]furans.

Present work

One of the methods for the fusion of triazole/tetrazole moiety on pyrimidine ring involves the intermediacy of corresponding hydrazino compound with -NHNH_2 group in appropriate position. Hence, 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines **7a-b** synthesized during the present investigations (**Chapter-6**) were thought of as potential starting materials for the synthesis of title compounds. Thus 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines **7a-b** were treated with hydrazine hydrate solution (80%) in presence of ethanol. The nucleophilic substitution reaction occurred spontaneously and resulted in the formation of 2-substituted-4-hydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines **8a-b**.

The hydrazine compounds were confirmed on the basis of spectral data. The IR spectrum of **8a** (**Figure-7.1**) exhibited an absorption peak at 2918 cm^{-1} due to -NH_2 group.

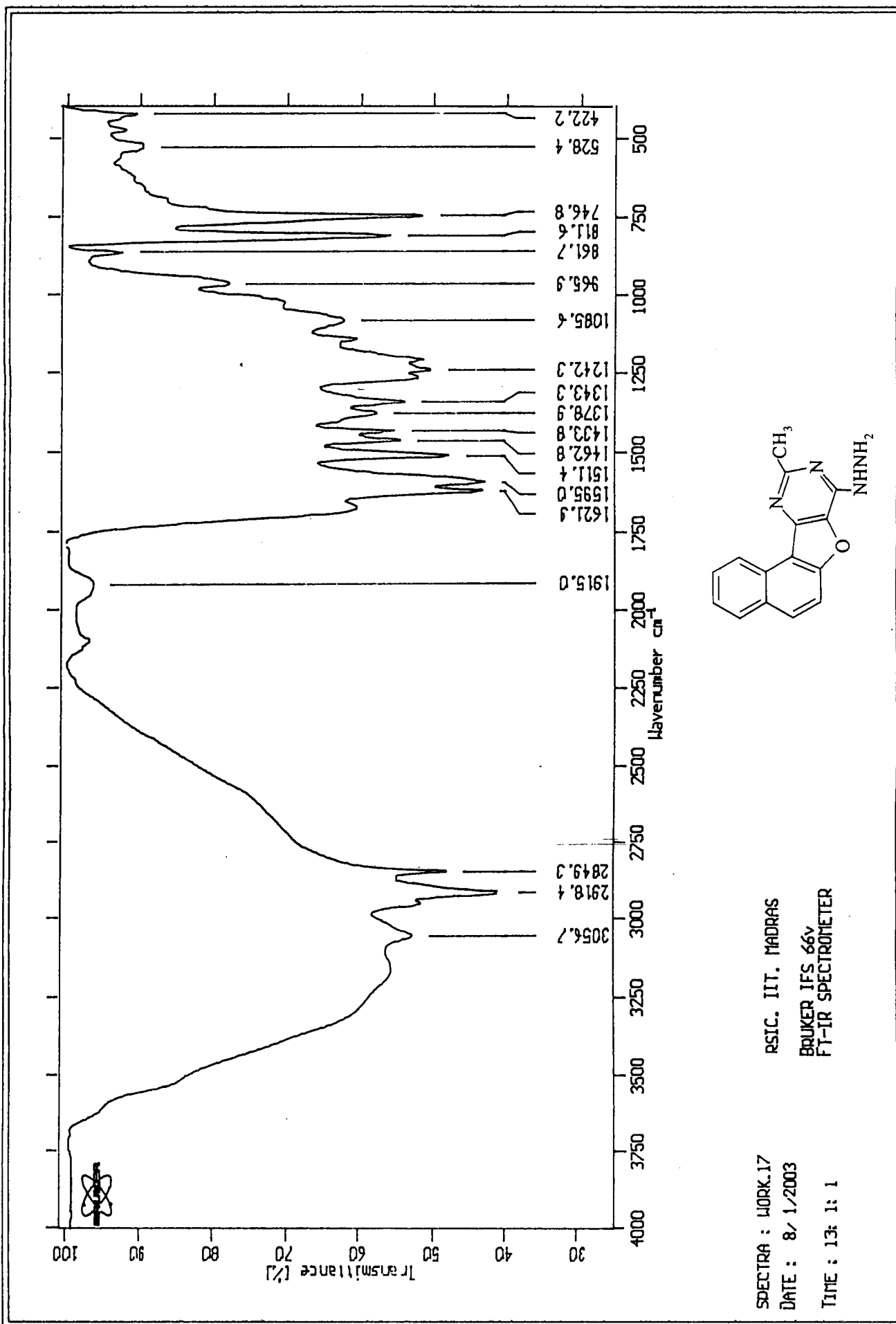
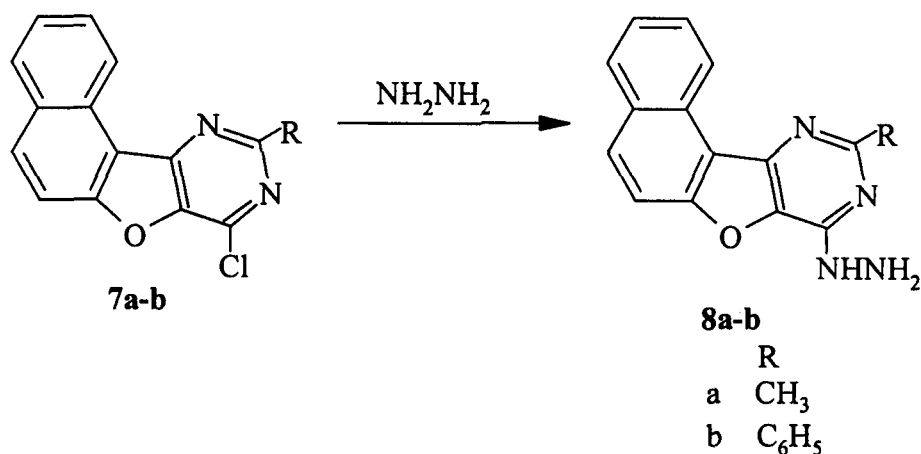
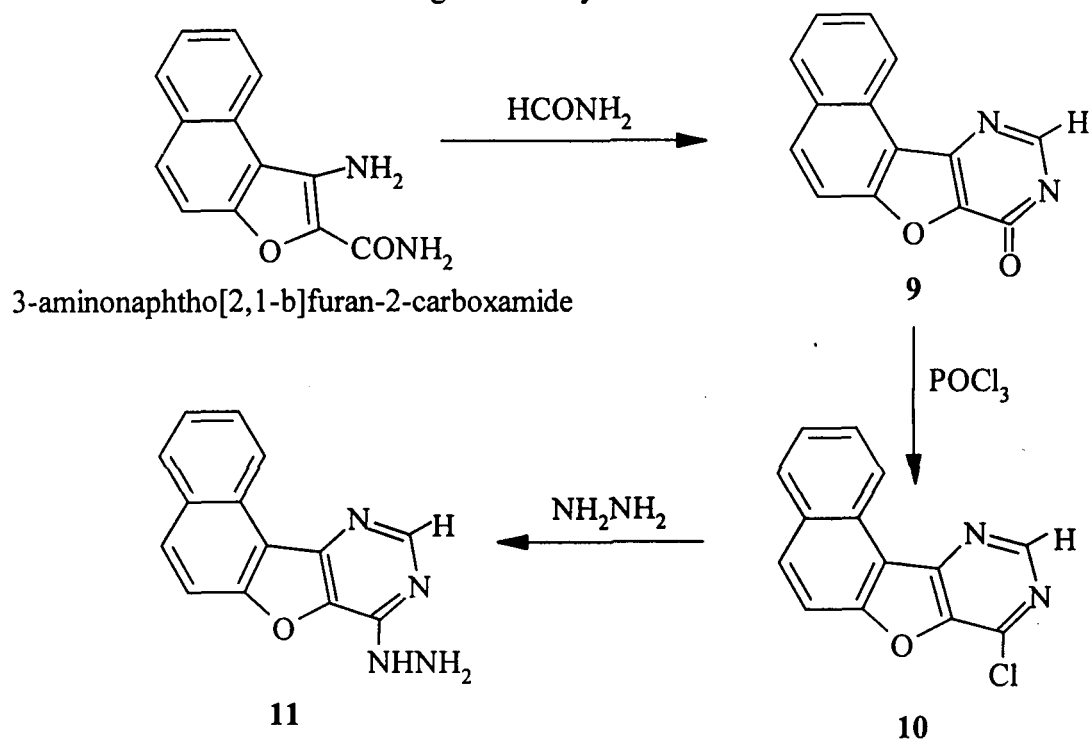


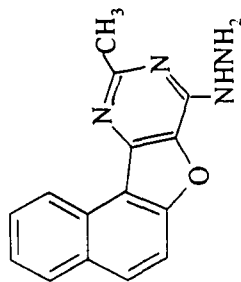
FIGURE-7.1



^1H NMR spectrum of **8a** (Figure-7.2) was recorded, which exhibited a singlet at δ 2.6 due to $-\text{CH}_3$ protons, a broad singlet at δ 6.0 (D_2O exchangeable) due to $-\text{NH}_2$ protons and a multiplet in the region δ 7.2-8.2 for six aromatic protons and a proton of $-\text{NH}$.

It was also envisaged to synthesize 4-hydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines **11** with no substitution at position-2 of pyrimidine ring, in order to compare the effect of substituents on biological activity.





Sample 6

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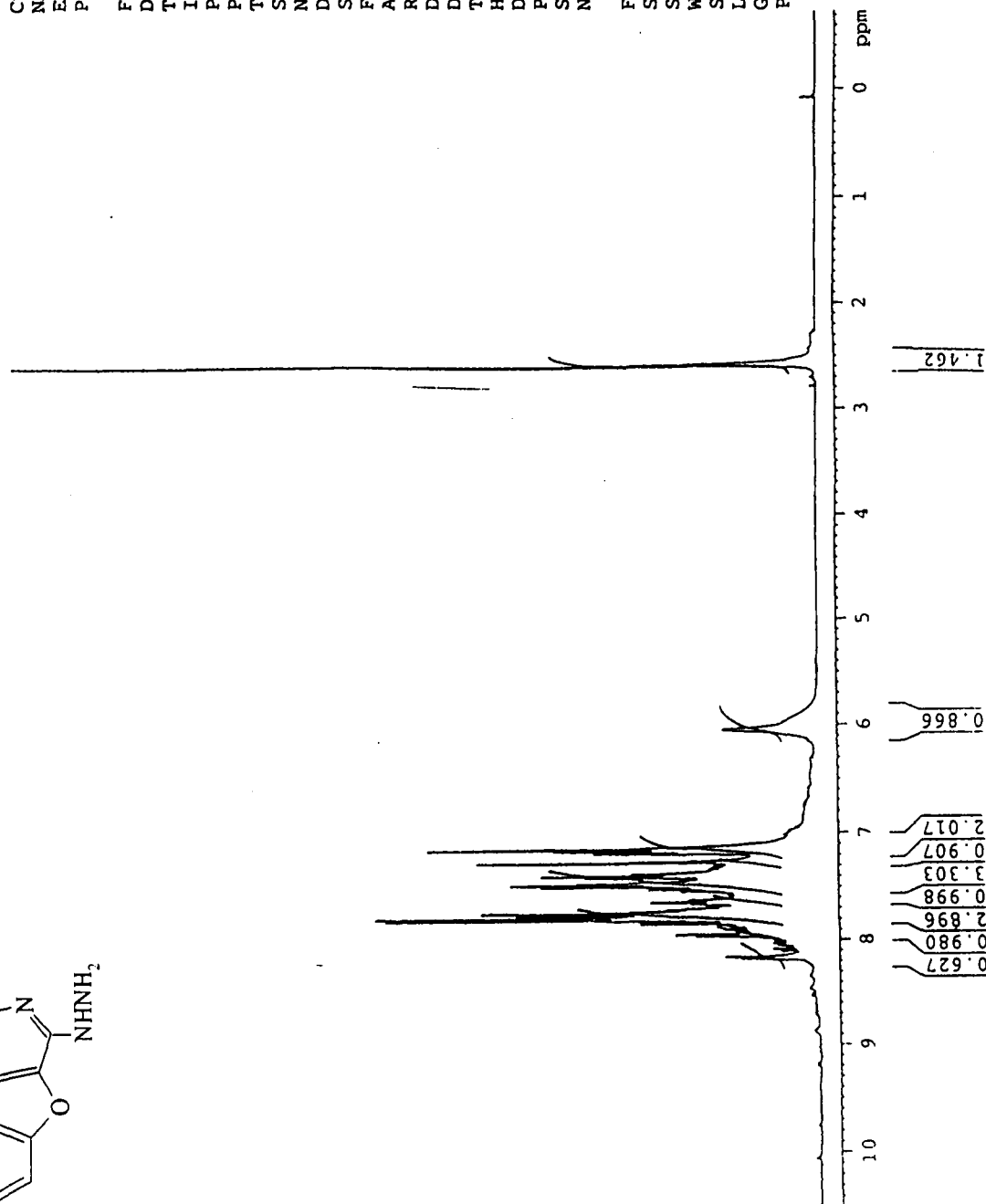
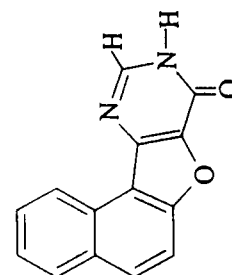


FIGURE-7.2

The synthesis of compound **11** was accomplished by using 3-aminonaphtho[2,1-b]furan-2-carboxamide as starting material. The 3-aminonaphtho[2,1-b]furan-2-carboxamide was refluxed with formamide in ethanol. The resulting compound was identified as 4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine **9** on the basis of its spectral data. The IR spectrum of compound **9** (Figure-7.3) exhibited absorption band at 1727 cm^{-1} due to carbonyl stretching frequency. ^1H NMR spectrum (Figure-7.4) of this compound showed a multiplet at δ 7.2- δ 8.2 integrating for six aromatic protons. As expected, the proton at C-2 position, which is flanked by two nitrogen atoms appeared as singlet at downfield of δ 10.2 whereas, the NH proton appeared at δ 9.1 as a singlet which was exchangeable with D_2O . 4-Oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine **9** was converted into 4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidine **10** by heating it with freshly distilled phosphorous oxychloride. The formation of 4-chloro compound **10** was evident by the absence of carbonyl peak in its IR spectrum (Figure-7.5). Nucleophilic substitution of chloro compound was carried out by reacting it with hydrazine hydrate in ethanol to obtain corresponding 4-hydrazino compound **11**. The ^1H NMR spectrum (Figure-7.6) of hydrazino compound was recorded to confirm the structure assigned to compound **11**. It showed a broad singlet at δ 6.0 (D_2O exchangeable) attributed to NH_2 protons. Other protons appeared as multiplet at δ 7.2 - δ 8.8.



RSIC, IIT, MADRAS
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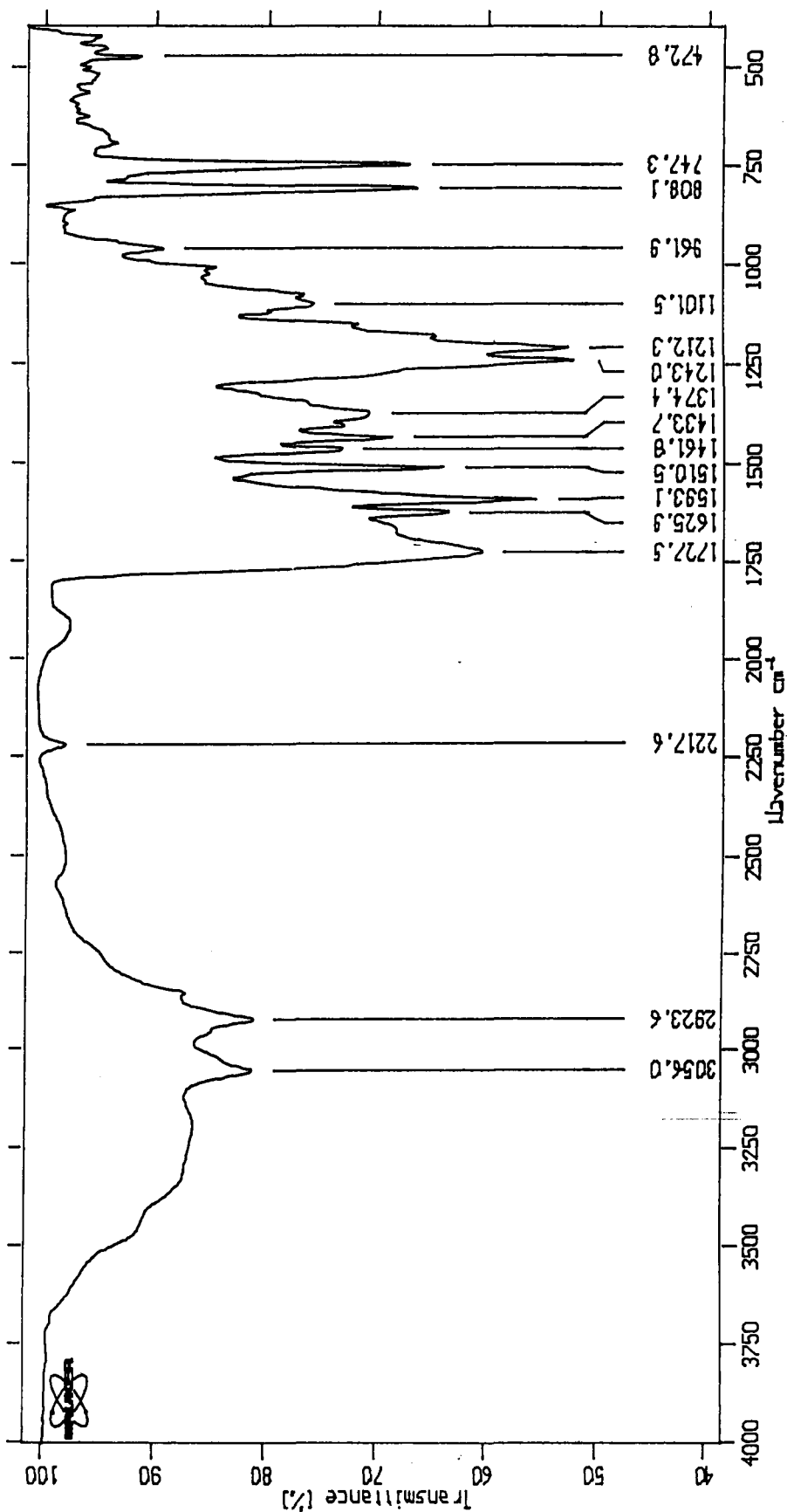
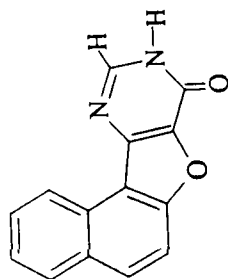


FIGURE-7.3



sample 3

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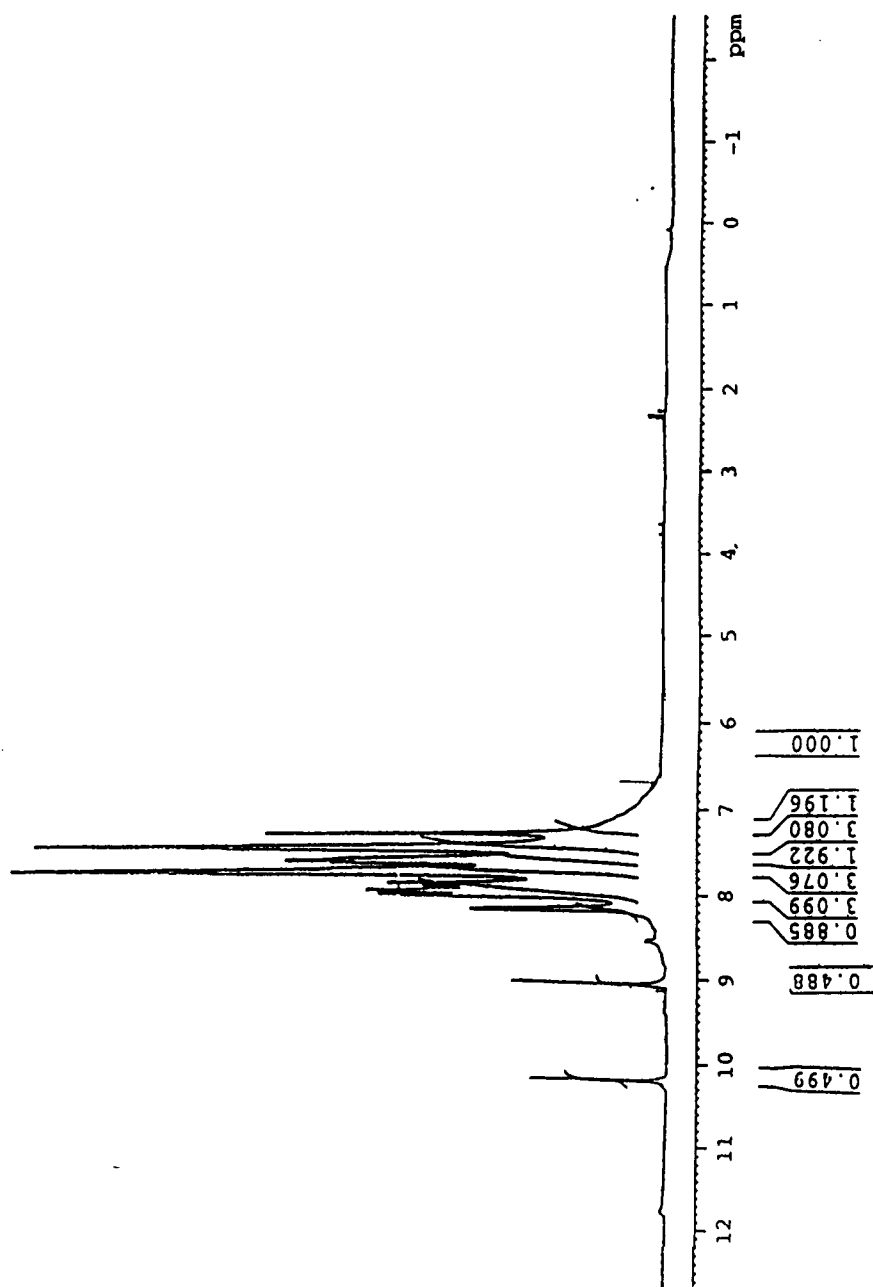


FIGURE-7.4

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RSIC. IIT. MADRAS
 BRUKER IFS 66V
 FT-IR SPECTROMETER

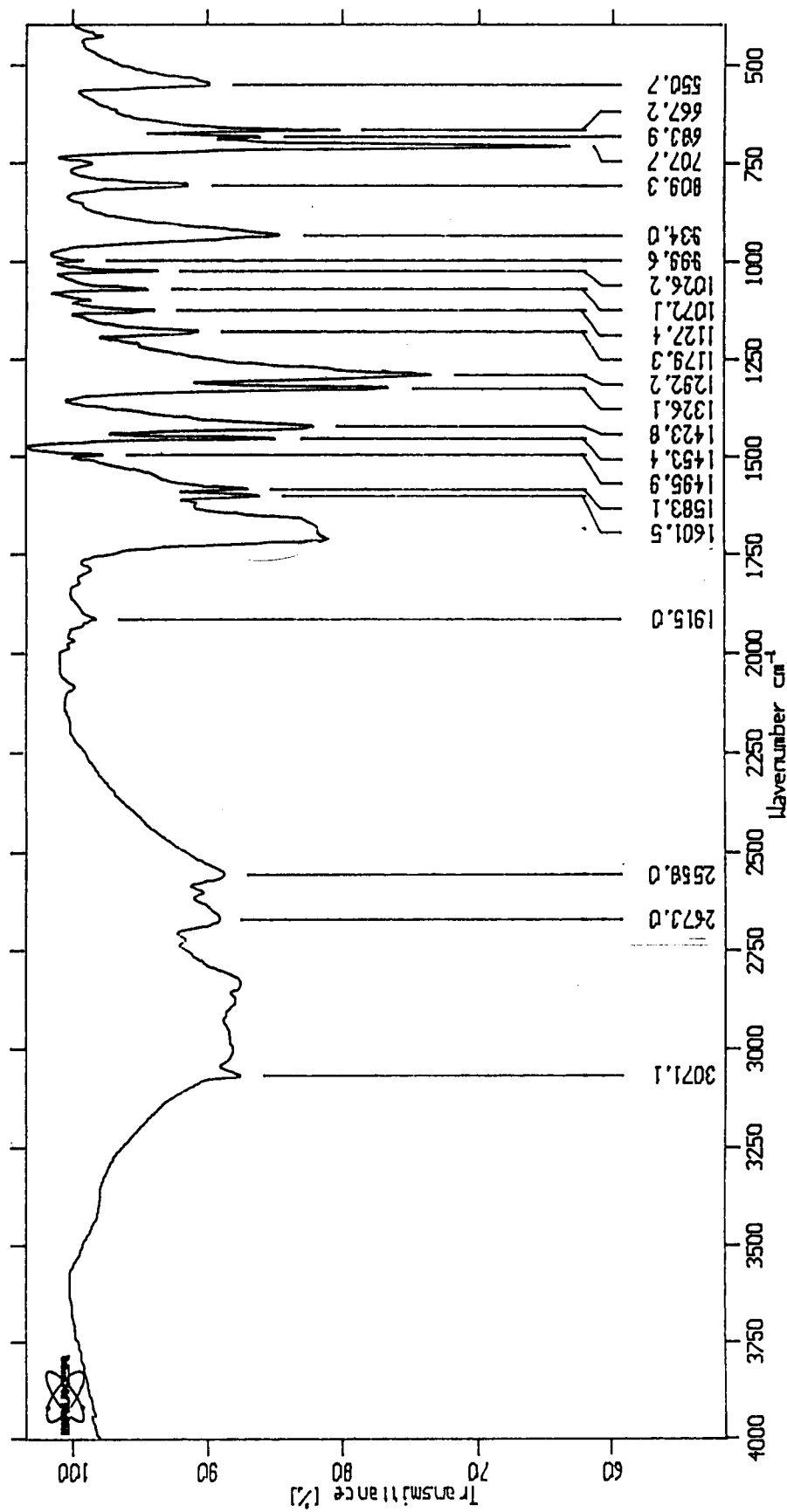
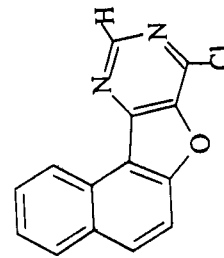
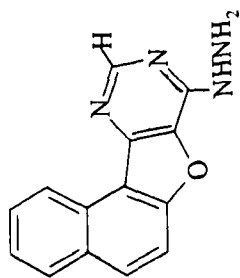


FIGURE-7.5



Sample 5

Current Data Parameters
NAME mahadev2405-1h
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters

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Time 14.45
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PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT cdcl3
NS 32
DS 0
SWH 6097.561 Hz
FIDRES 0.186083 Hz
AQ 2.6870260 sec
RG 512
DW 82.000 usec
DE 102.50 usec
TE 300.0 K
HL1 1 dB
D1 1.00000000 sec
P1 6.82 usec
SFO1 400.1364282 MHz
NUCLEUS 1H

F2 - Processing parameters

SI 32768
SF 400.1343949 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

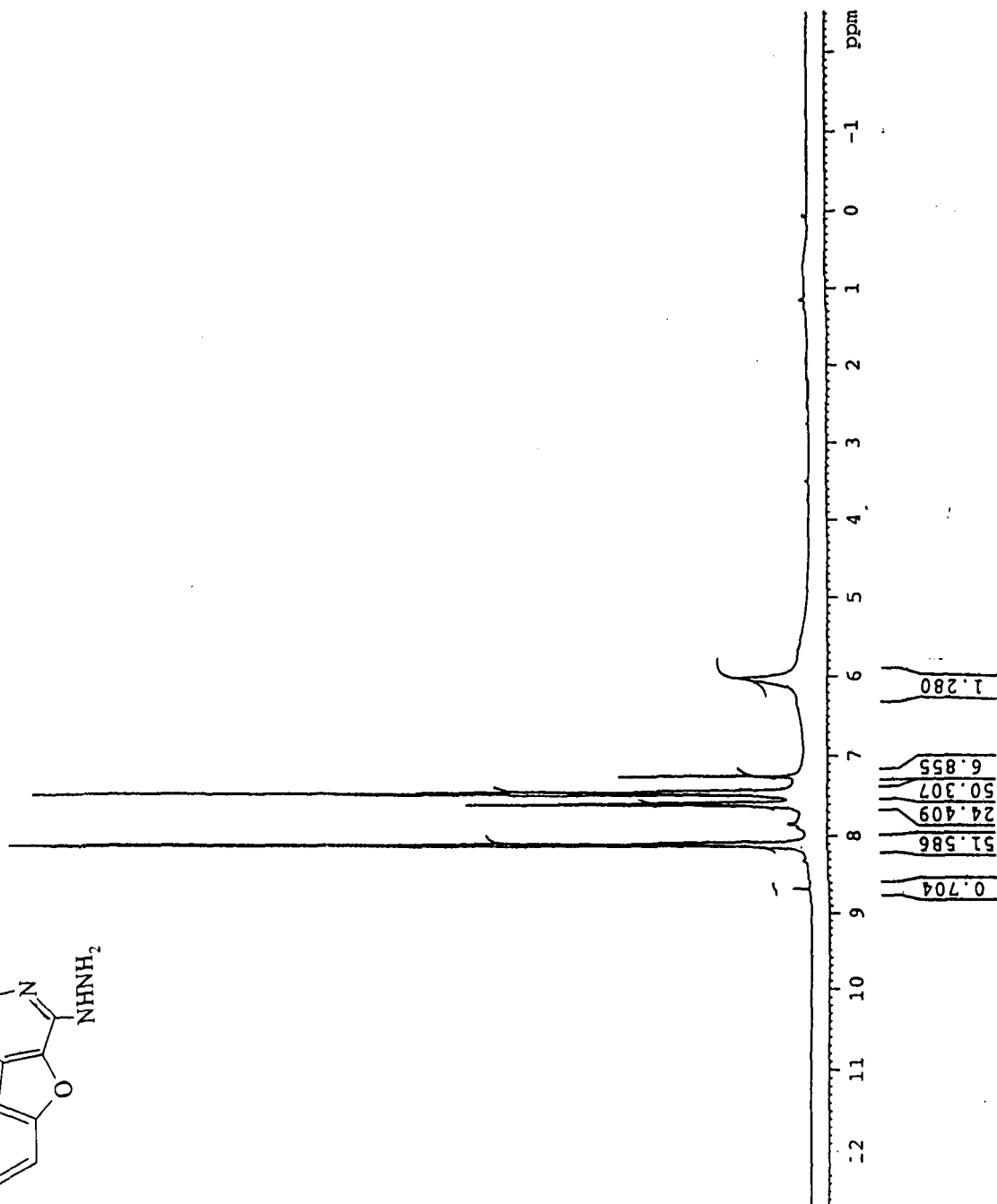
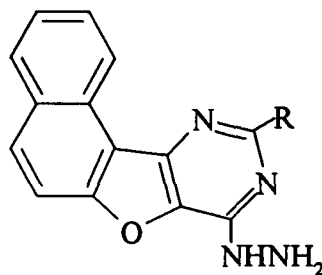


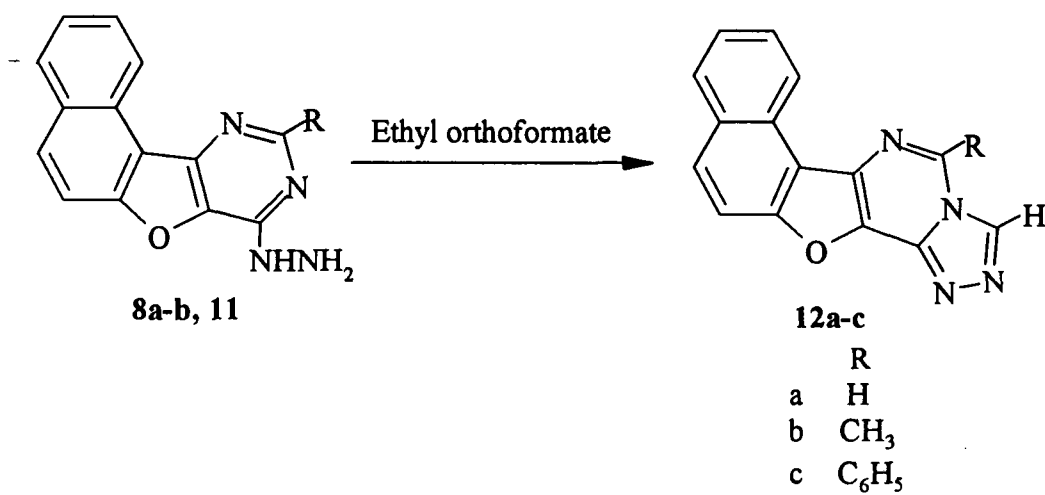
FIGURE-7.6

Table-7.1 IR spectral data of 2-substituted-4-hydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines**8a-b, 11**

R
 8a CH₃
 8b C₆H₅
 11 H

Compound	R	IR (KBr) cm ⁻¹	
		C=N	NH
8a	CH ₃	1582	2918
8b	C ₆ H ₅	1568	2910
11	H	1578	2912

The hydrazine compounds on refluxing with ethyl orthoformate afforded anticipated triazolo compounds i.e. 2-alkyl/aryl-s-triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furan **12a-c**.



The structure assigned to these compounds was established by recording the spectral data. The IR spectrum (**Figure-7.7**) was in accordance with its structure. ^1H NMR spectrum of **12a** (**Figure-7.8**) which exhibited a multiplet at δ 7.1- δ 8.3 integrating for six aromatic protons whereas the protons of pyrimidine ring and triazole ring appeared as singlet at δ 9.2 integrating for two protons. The IR spectrum (**Figure-7.9**) of **12b** confirmed the structure assigned. The ^1H NMR spectrum (**Figure-7.10**) exhibited a multiplet at δ 7.1- δ 8.3 integrating for six aromatic protons, a singlet at δ 2.6 integrating for three protons of methyl group, whereas the proton of triazole ring appeared as singlet at δ 9.4 integrating for one proton.

The other two methods for the construction of triazole moiety available in the literature were attempted. Thus hydrazine compounds when refluxed with acetyl chloride produced corresponding 2-methyl compounds **13a-c** and similar reaction with benzoyl chloride yielded corresponding 2-phenyl derivatives **13d-f**.

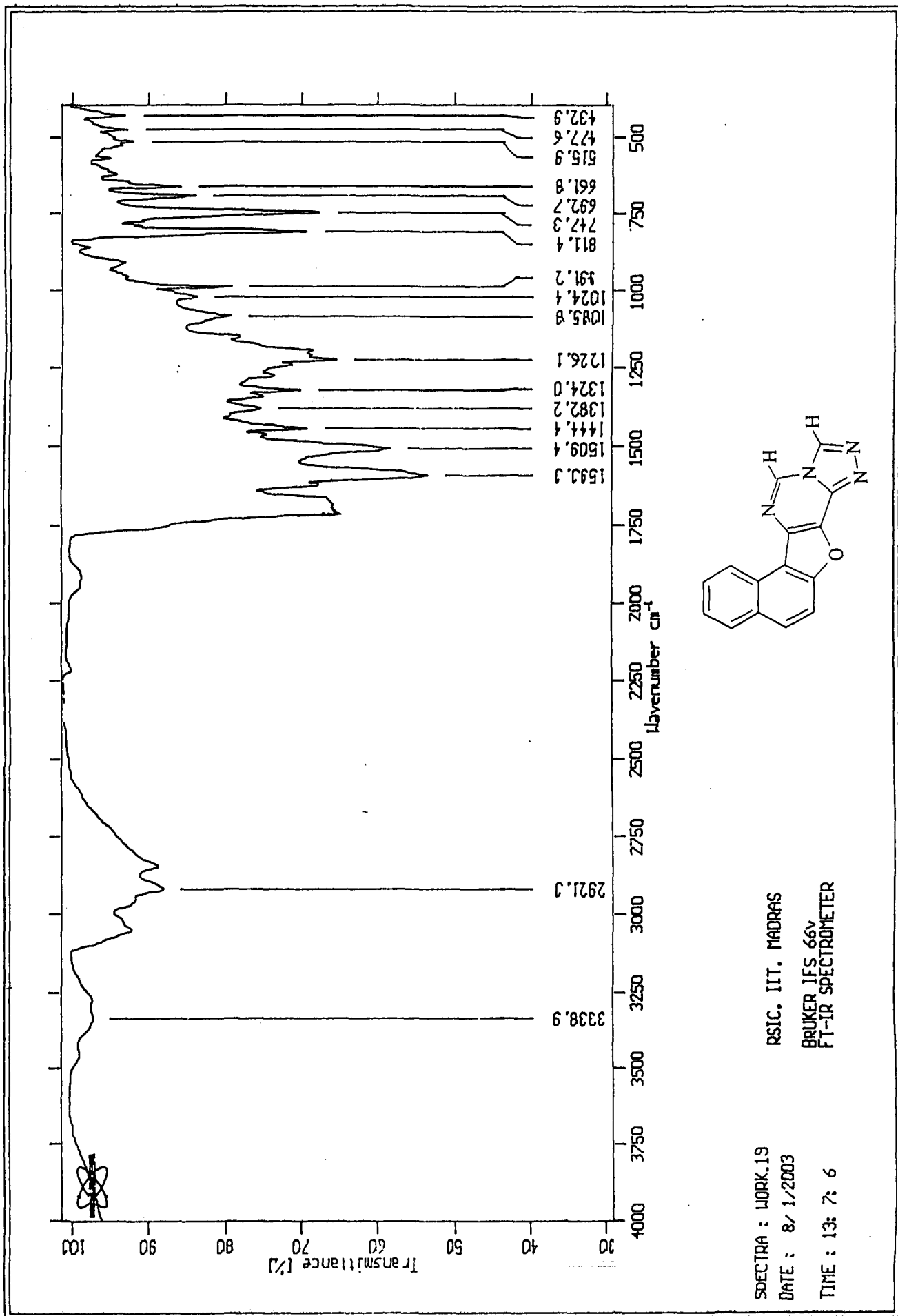
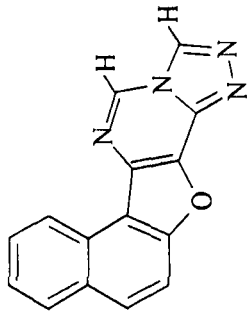


FIGURE-7.7



Sample 9

Current Data Parameters
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 EXPNO 13
 PROCNO 1

F2 - Acquisition Parameters
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 Time 16.07
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 PROBHD 5 mm Multinu
 PULPROG zg
 TD 32768
 SOLVENT cdcl3
 NS 32
 DS 0
 SWH 6097.561 Hz
 FIDRES 0.186083 Hz
 AQ 2.6870260 sec
 RG 512
 DW 82.000 usec
 DE 102.50 usec
 TE 300.0 K
 HLI 1 dB
 D1 1.00000000 sec
 P1 6.82 usec
 SFO1 400.1364282 MHz
 NUCLEUS 1H

F2 - Processing parameters
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 WDW EM
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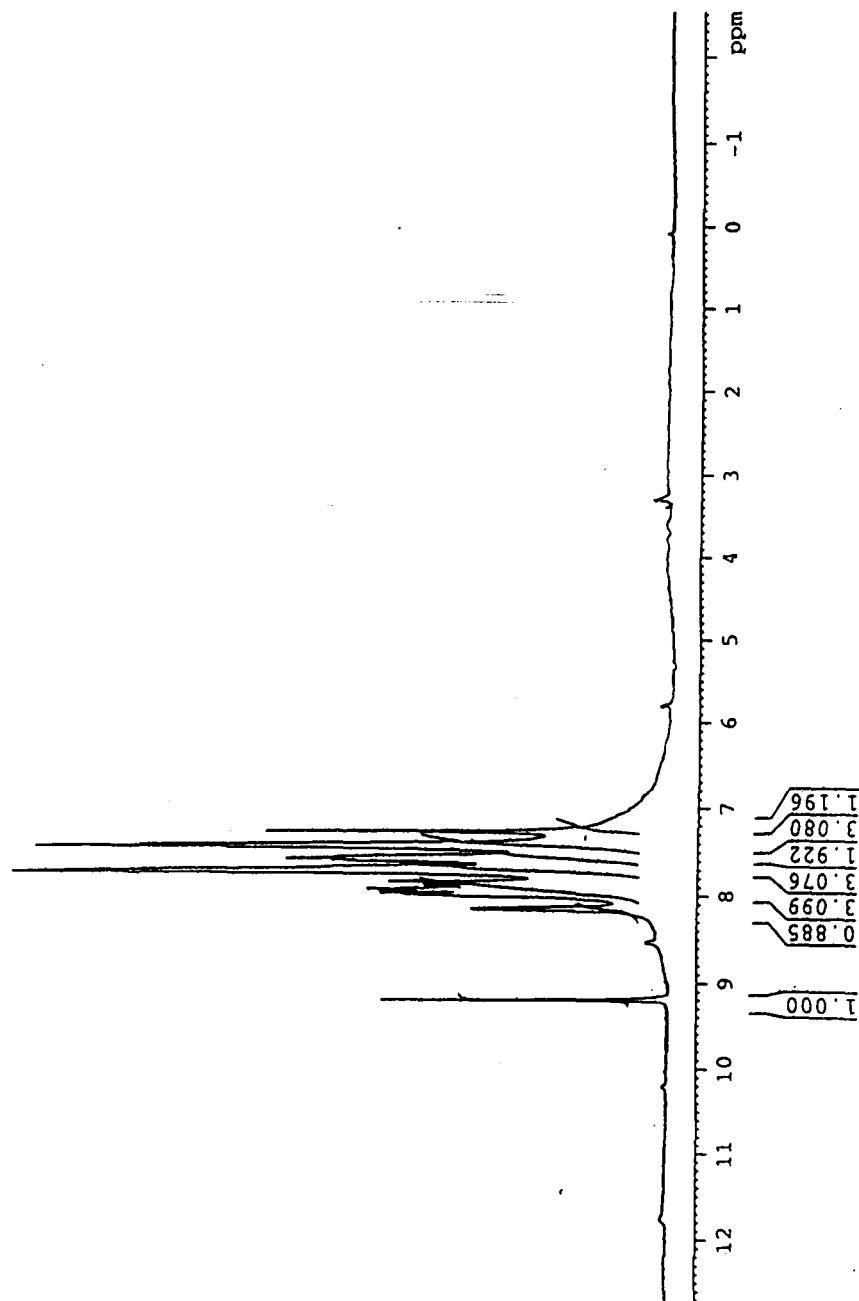
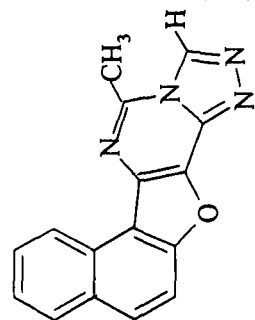
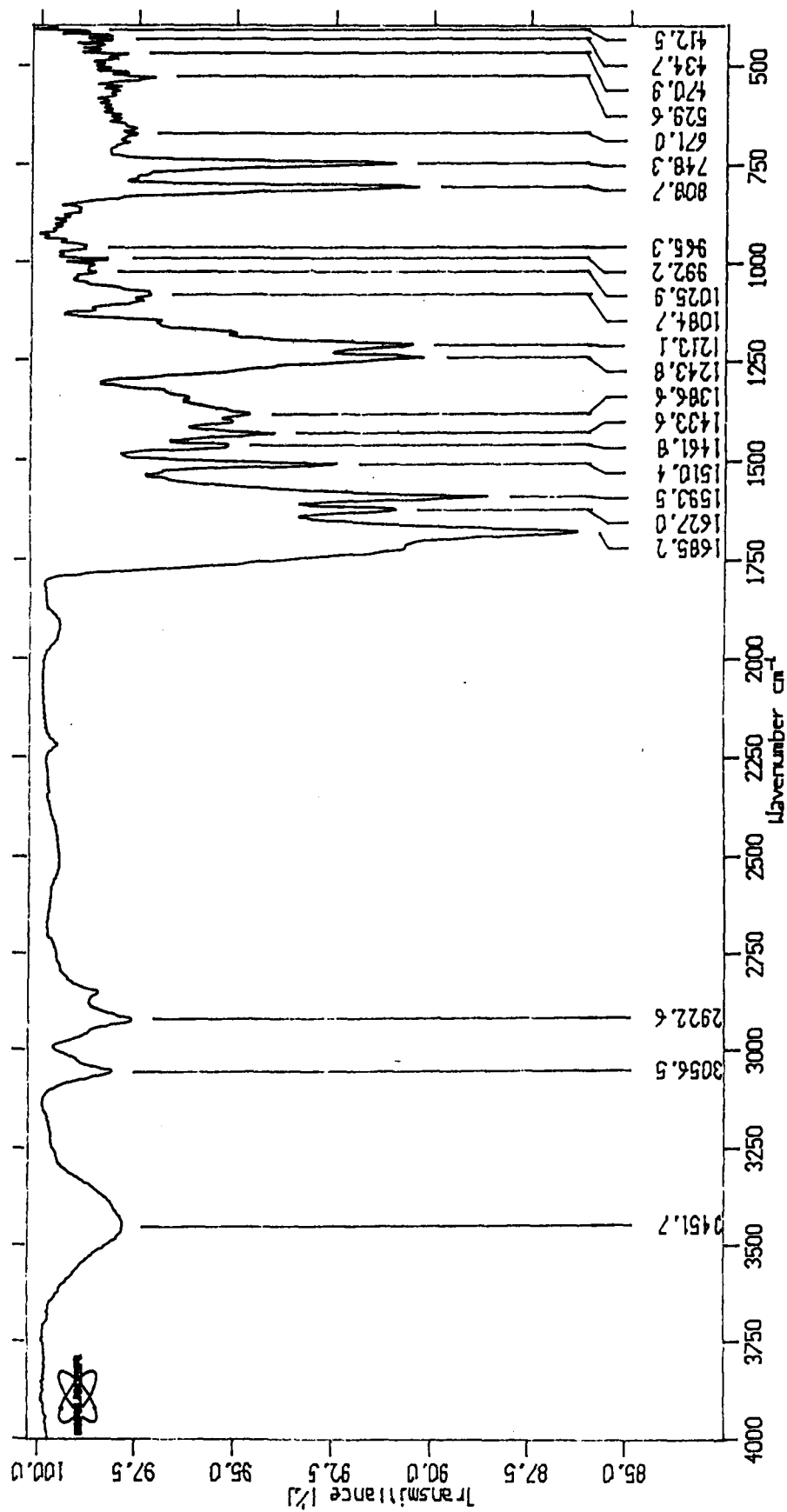
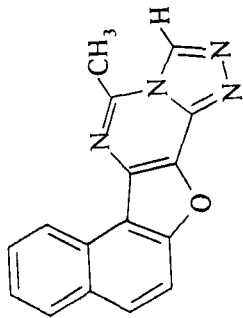


FIGURE-7.8



SPECTRA : WORK-21
 DATE : 8/1/2003
 TIME : 13: 9: 8
 RSIC, III, MADRAS
 BRUKER IFS 66V
 FT-IR SPECTROMETER

FIGURE-7.9



Sample 10:

Current Data Parameters
 NAME mahadev2405-1h
 EXPNO 14
 PROCNO 1

F2 - Acquisition Parameters

Date_ 20020524
 Time 16.11
 INSTRUM amx400
 PROBHD 5 mm Multinu
 PULPROG zg
 TD 32768
 SOLVENT cdcl3
 NS 32
 DS 0
 SWH 6097.561 Hz
 FIDRES 0.186083 Hz
 AQ 2.6870260 sec
 RG 512
 DW 82.000 usec
 DE 102.50 usec
 TE 300.0 K
 HL1 1 dB
 D1 1.00000000 sec
 P1 6.82 usec
 SFO1 400.1364282 MHz
 NUCLEUS 1H

F2 - Processing parameters

SI 32768
 SF 400.1343949 MHz
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 PC 2.00

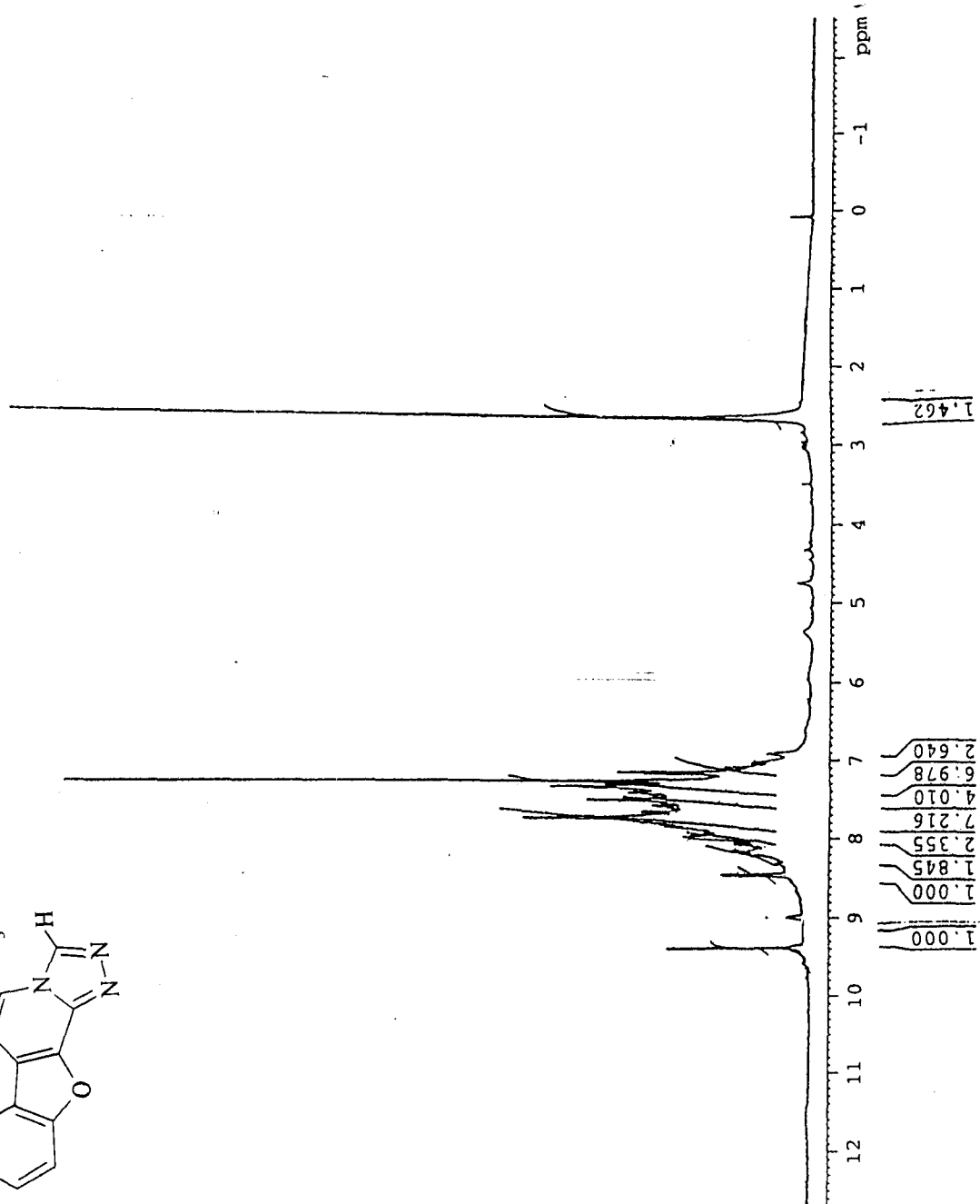
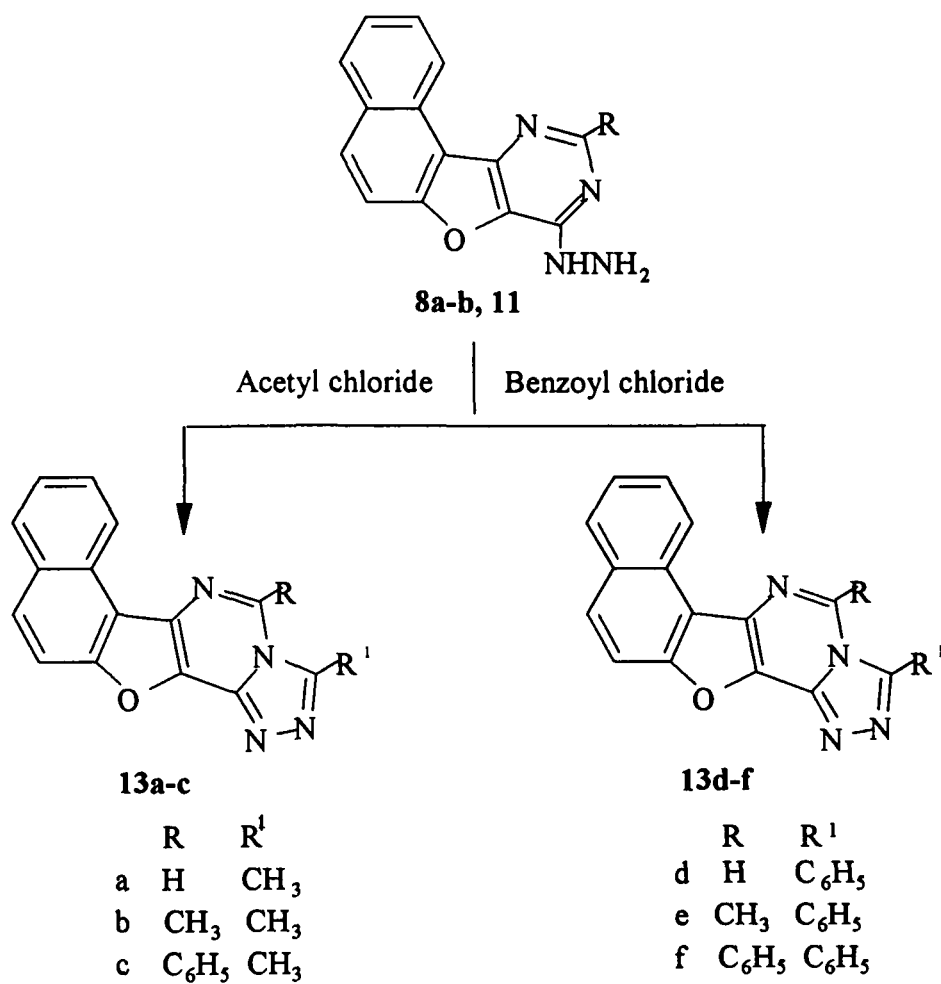
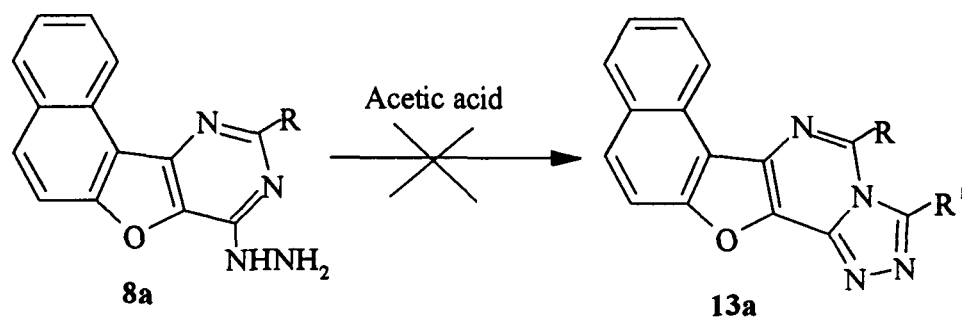


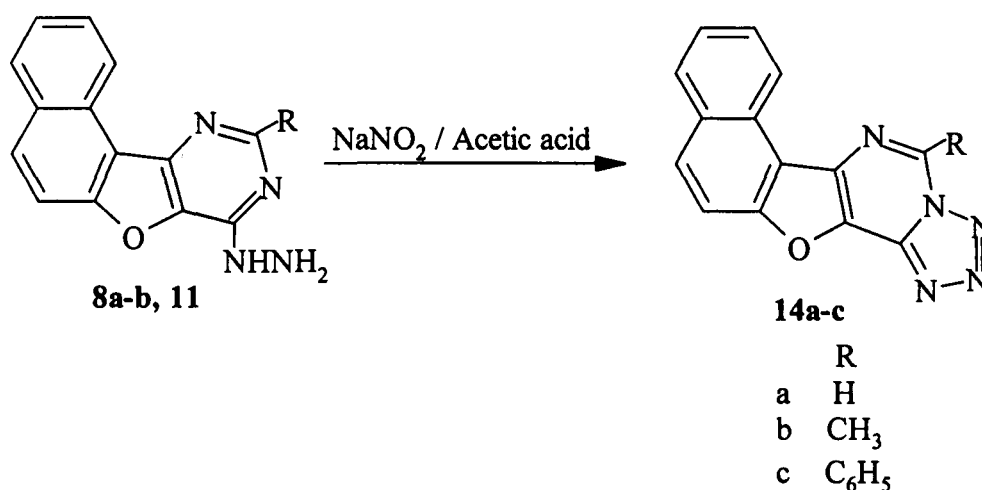
FIGURE-7.10



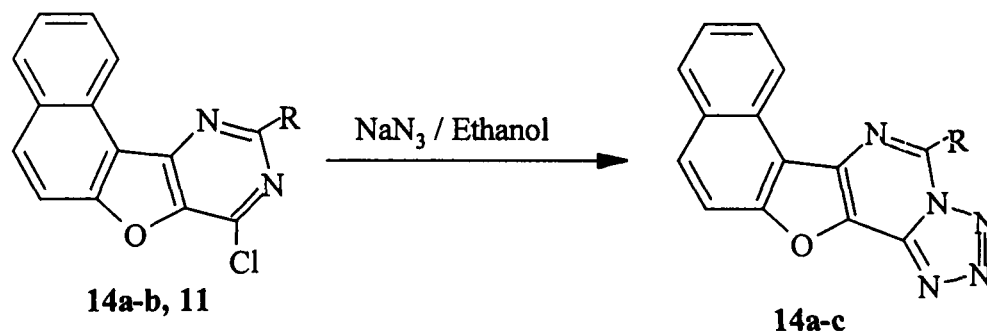
However, the method of refluxing hydrazine compound with formic acid to obtain triazolo compounds was not successful even after changing the reaction conditions.

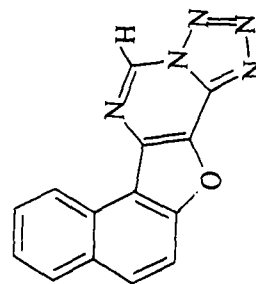
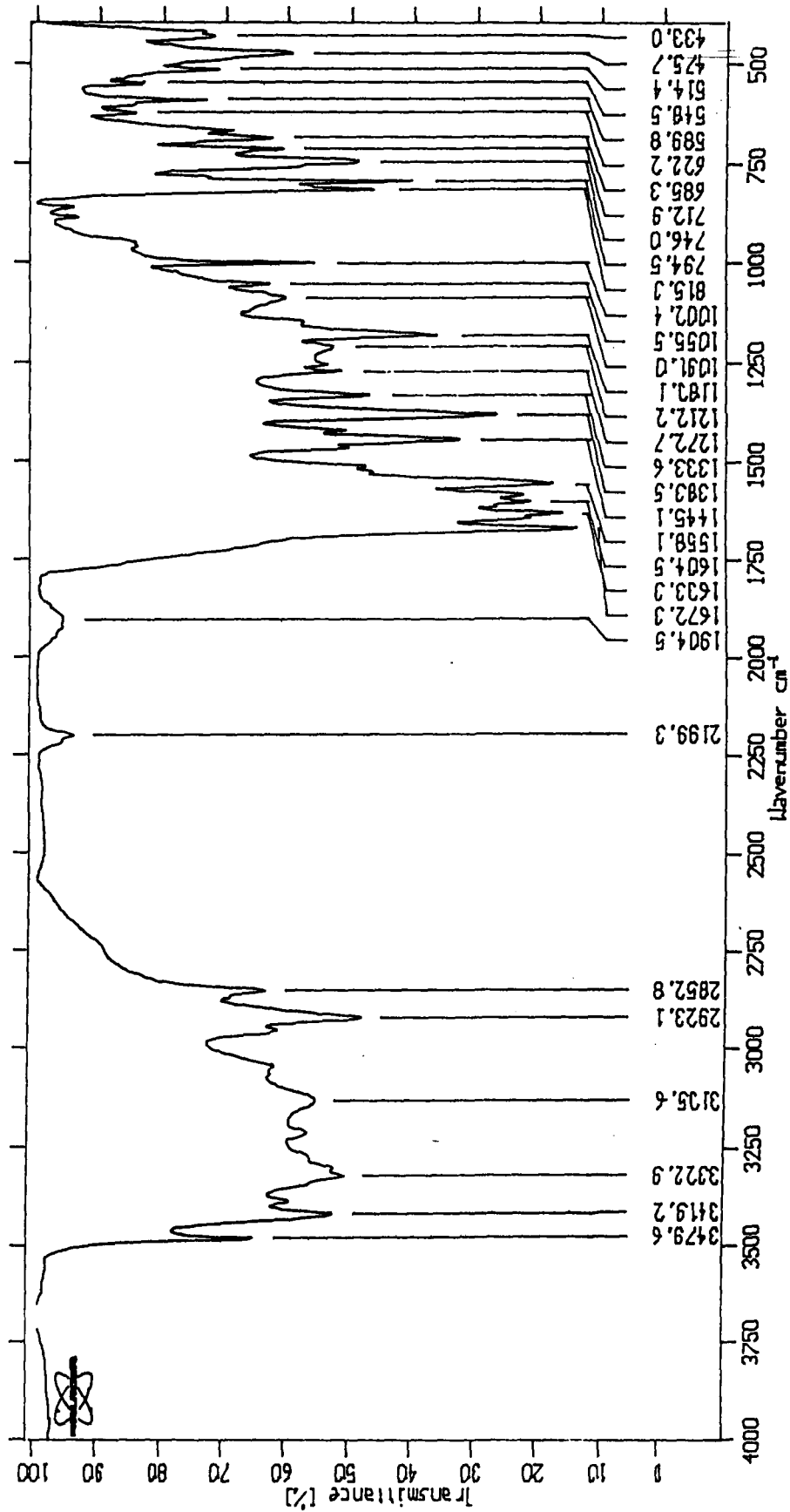


In view of the growing interest in newer heterocycles, it was contemplated to synthesize yet another pentacyclic heterocycle encompassing tetrazole moiety. The synthesis was achieved by subjecting hydrazine compound to diazotisation by using sodium nitrite in acetic acid. The structure of various tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans **14a-c** thus obtained were characterized by spectral studies. The structure assigned to **14a** was in well agreement with its IR spectrum (**Figure-7.11**). ^1H NMR spectrum (**Figure-7.12**), which exhibited only a multiplet at δ 7.2- δ 8.5 due to six aromatic protons including a proton of pyrimidine ring.



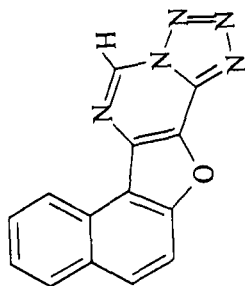
These new pentacyclic heterocyclic compounds were synthesized also by an alternative route, in which the respective chloro compounds **14a-b** and **11** were treated with sodium azide in ethanol.





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 DATE : 8/ 1/2003
 TIME : 12:52:52
 RSIC, IIT, MADRAS
 BRUKER IFS 66V
 FT-IR SPECTROMETER

FIGURE-7.11



sample 14

Current Data Parameters
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EXPNO 7
PROCNO 1

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PULPROG zg
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SOLVENT cdcl3
NS 96
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SWH 6097.561 Hz
FIDRES 0.186083 Hz
AQ 2.6870260 sec
RG 512
DW 82.000 usec
DE 102.50 usec
TE 300.0 K
HL1 1 CB
D1 1.00000000 sec
P1 6.82 usec
SFO1 400.1364282 MHz
NUCLEUS 1H

F2 - Processing parameters
SI 32768
SF 400.1343949 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

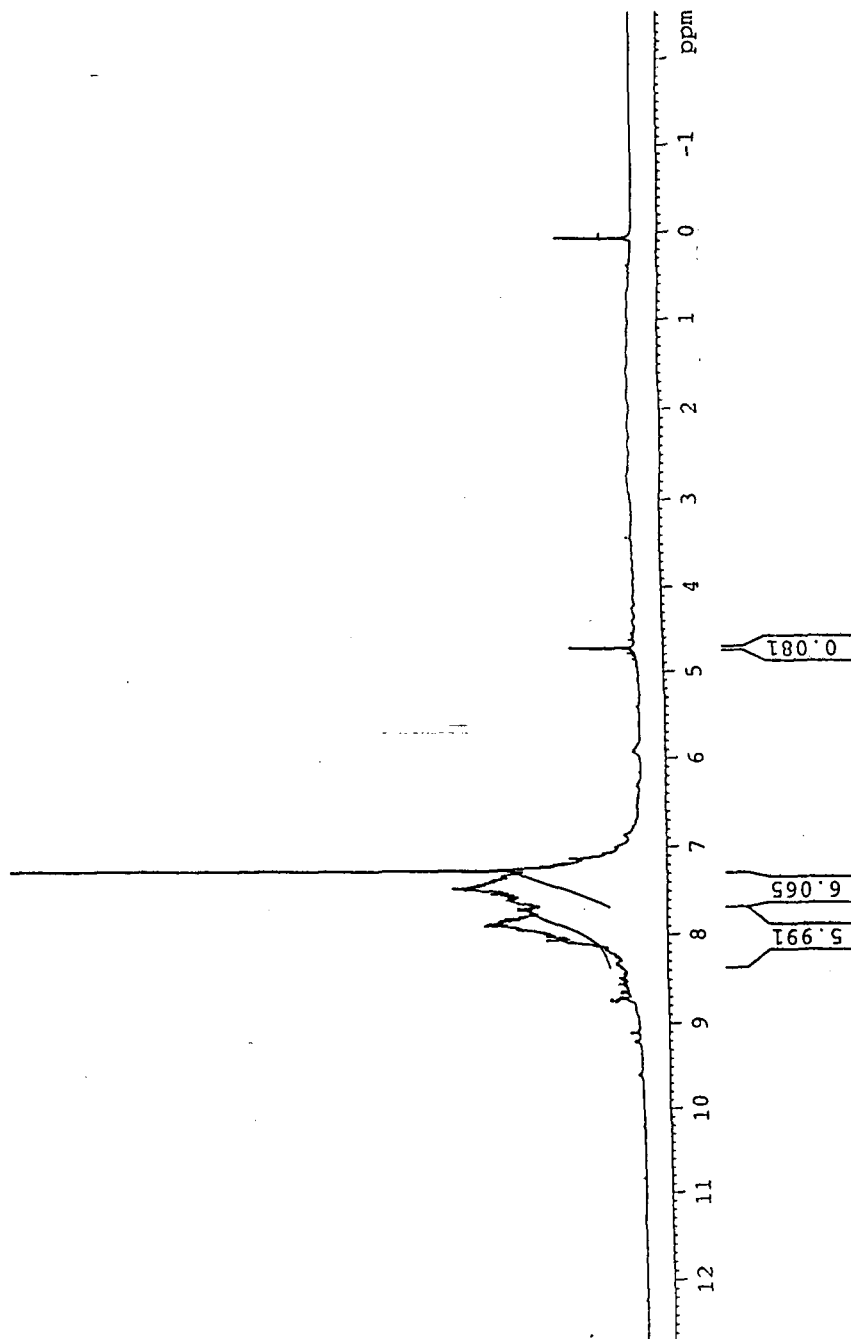
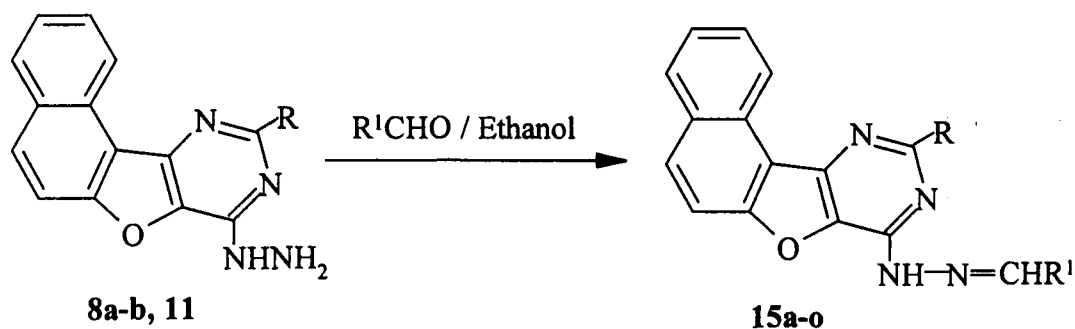


FIGURE-7.12

These compounds were found to be identical with the compounds obtained by earlier method as indicated by the superimposable IR and ^1H NMR spectra and also by the determination of mixed melting points.

It has been well established that $-\text{NH}-\text{N}=\text{CH}-$ group is necessary for antibacterial and antiparasitic activities of nitrofurans derivatives²⁷⁻²⁹. Further efforts are made to reduce the toxicity by using different heterocycles as carrier molecules³⁰. Receiving impetus from these reports, it was planned to utilize hydrazine compounds for the synthesis of various arylidene derivatives by condensation with different aldehydes in ethanol. The resulting compounds were identified as 4-arylidenehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines **9a-o** on the basis of spectral studies. The various aldehydes used for this reaction are listed below.

- Benzaldehyde
- 2-Hydroxybenzaldehyde
- 3-Nitrobenzaldehyde
- 4-Aminobenzaldehyde
- Furfural



	R	R ¹		R	R ¹
a	H	C ₆ H ₅	h	CH ₃	C ₆ H ₄ -3-NO ₂
b	H	C ₆ H ₄ -2-OH	i	CH ₃	C ₆ H ₄ -4-NH ₂
c	H	C ₆ H ₄ -3-NO ₂	j	CH ₃	2-Furyl
d	H	C ₆ H ₄ -4-NH ₂	k	C ₆ H ₅	C ₆ H ₅
e	H	2-Furyl	l	C ₆ H ₅	C ₆ H ₄ -2-OH
f	CH ₃	C ₆ H ₅	m	C ₆ H ₅	C ₆ H ₄ -3-NO ₂
g	CH ₃	C ₆ H ₄ -2-OH	n	C ₆ H ₅	C ₆ H ₄ -4-NH ₂
			o	C ₆ H ₅	2-Furyl

The structures assigned to compounds were authenticated by recording the IR and ¹H NMR spectra of the representative compounds. IR spectrum of **15a** (Figure-7.13) was in well agreement with the structure assigned. ¹H NMR (Figure-7.14) exhibited a singlet at a downfield of δ 9.3 assignable to methine protons and a multiplet in between δ 7.2 - δ 8.2 integrating for 12 protons, which was attributed to 11 aromatic and one -NH proton.

Similarly, ¹H NMR of **15k** (Figure-7.15) exhibited a singlet at δ 9.3 assignable to methine protons and a multiplet in between δ 7.2 - δ 8.2 integrating for 12 protons, which was attributed to 11 aromatic and one -NH proton. It also exhibited a singlet at δ 2.6 due to methyl protons integrating for three protons. IR spectral data of some representative 4-Arylidinehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines is given in Table-7.2.

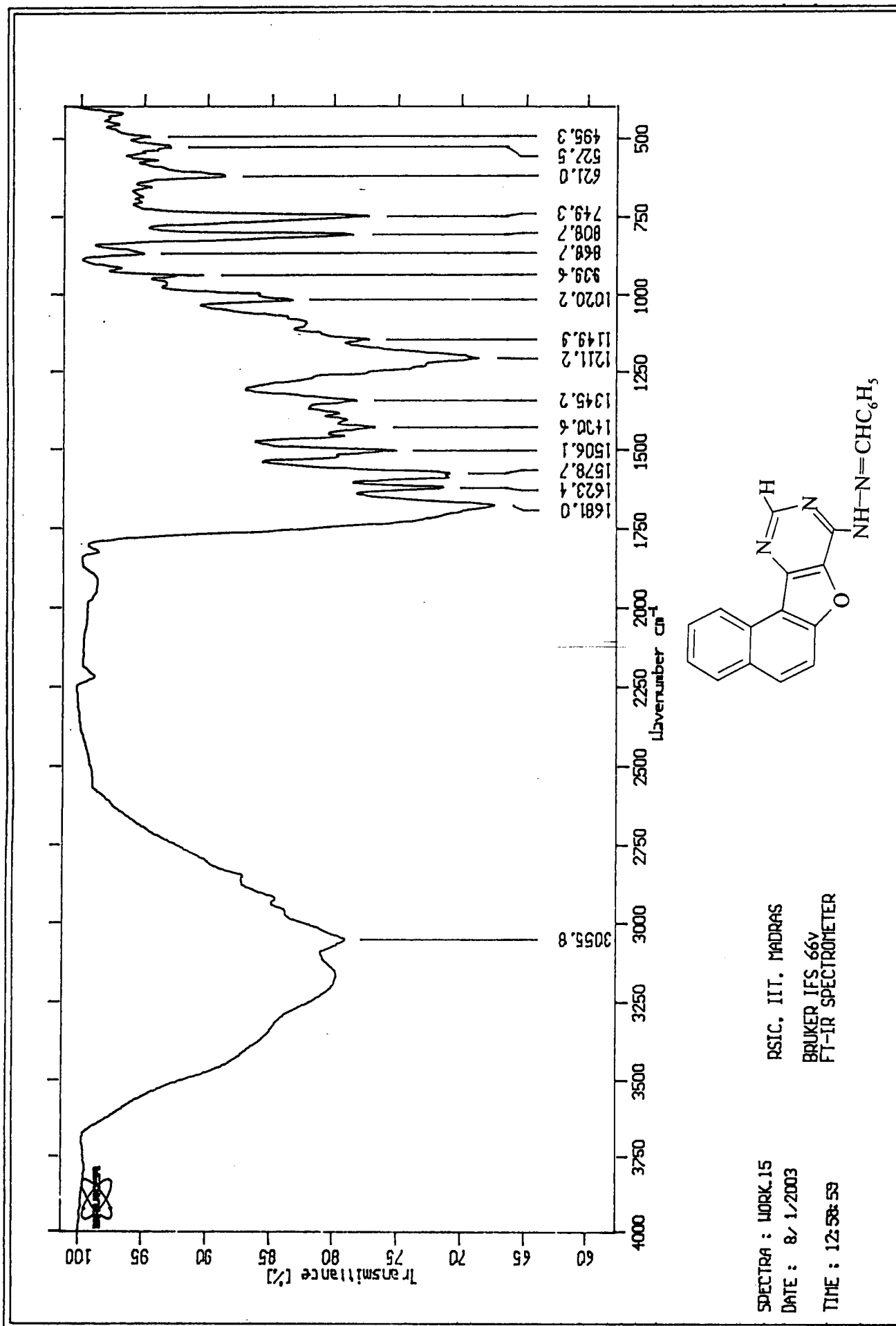
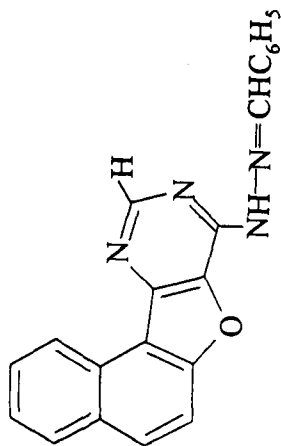


FIGURE-7.13



sample 7

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EXPNO 2
PROCNO 1

F2 - Acquisition Parameters

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PULPROG zg
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SOLVENT cdcl3
NS 32
DS 0
SWH 6024.096 Hz
FIDRES 0.183841 Hz
AQ 2.7197940 sec
RG 512
DM 83.000 usec
DE 103.75 usec
TE 300.0 K
HL1 1 dB
D1 1.00000000 sec
P1 12.25 usec
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F2 - Processing parameters

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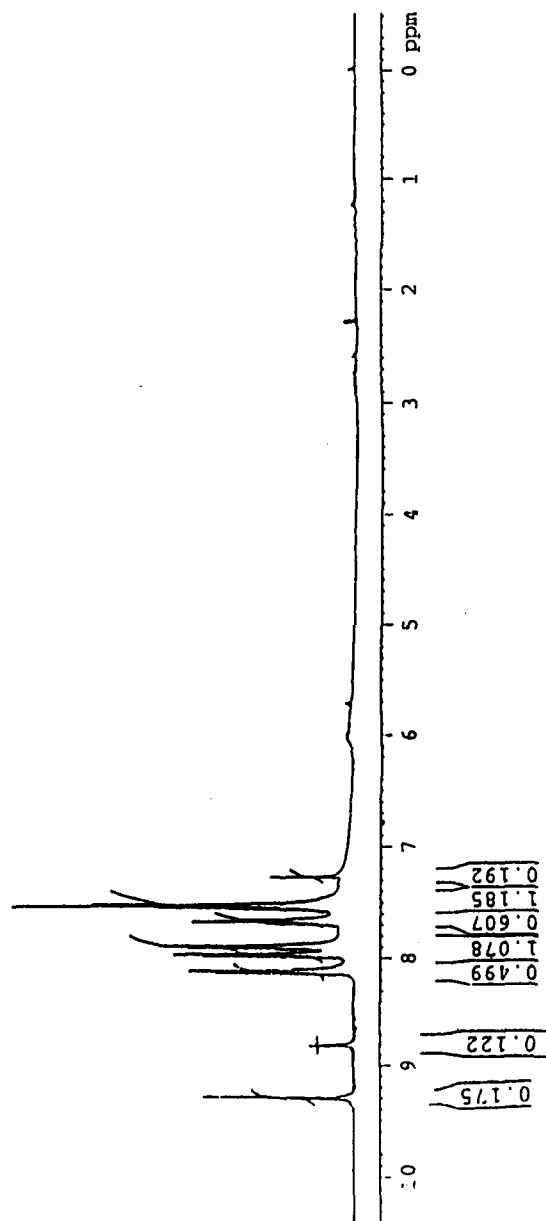
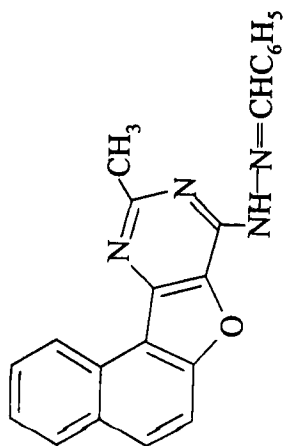


FIGURE-7.14



Sample 8

Current Data Parameters
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 EXPNO 14
 PROCNO 1

F2 - Acquisition Parameters

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 TD 32768
 SOLVENT cdcl3
 NS 32
 DS 0
 SWH 6097.561 Hz
 FIDRES 0.186083 Hz
 AQ 2.6870260 sec
 RG 512
 DW 82.000 usec
 DE 102.50 usec
 TE 300.0 K
 HL1 1 dB
 D1 1.00000000 sec
 P1 6.82 usec
 SFO1 400.1364282 MHz
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F2 - Processing parameters

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 PC 2.00

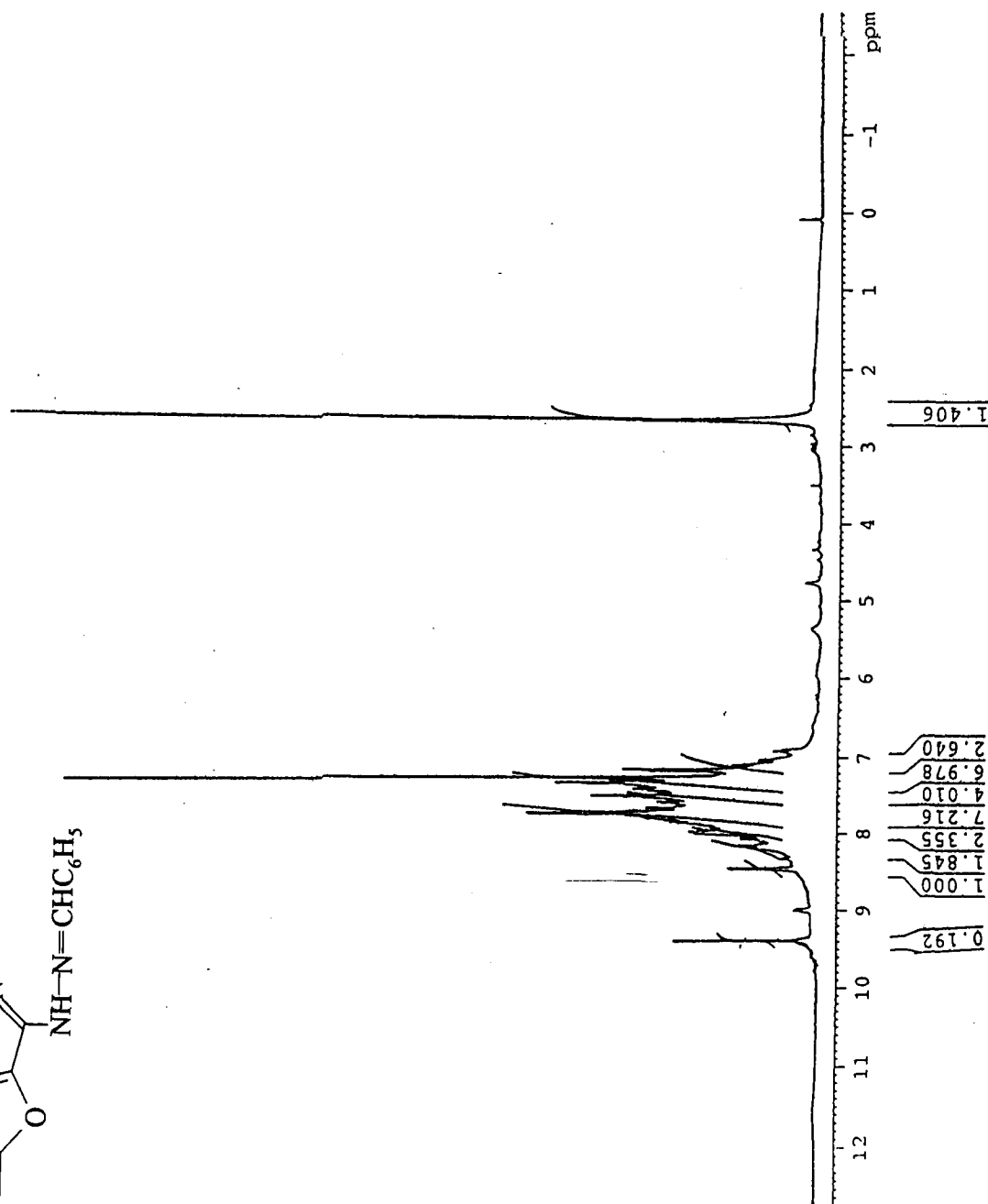
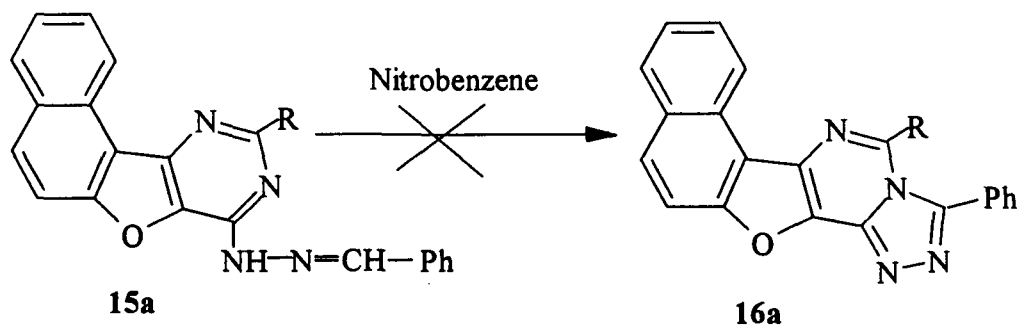


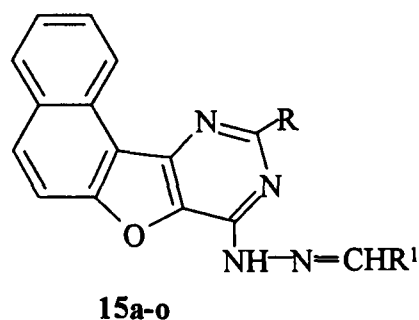
FIGURE-7.15

The attempted oxidative cyclization of **15a** to **16a** by using nitrobenzene under reflux condition was futile.



In view of the different biological and pharmacological activities exhibited by triazoles, tetrazoles and arylidine derivatives, some of the selected compounds synthesized during the present investigations have been evaluated for antimicrobial, anthelmintic, anticonvulsant and antipyretic activities. The method of screening, results and discussions are presented in **Chapter-8**.

Table-7.2 IR spectrum of 4-Arylidinehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines



Compound	R	R ¹	IR (KBr) cm ⁻¹	
			C=N	NH
15a	CH ₃	C ₆ H ₅	1582	3400
15b	C ₆ H ₅	C ₆ H ₅	1568	3360
15c	H	C ₆ H ₅	1578	3382

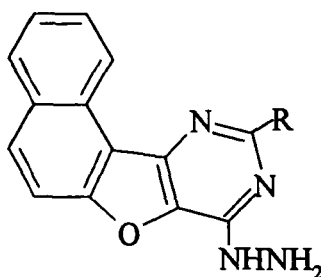
Experimental

2-Substituted-4-hydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines 8a-b, 11

To a solution of 7a (4 g) in methanol (80 ml) was added, 80% hydrazine hydrate (10 ml) and the mixture was heated under reflux on a water-bath for 4 hr. The yellow colored hydrazino compound 8a, which separated on cooling, was collected and crystallized from aqueous ethanol.

Similarly 8b and 11 were prepared. The physical and analytical data of the compounds synthesized is presented in Table-7.3.

Table-7.3



8a-b, 11

Compound	R	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
					C	H	N
8a	CH ₃	C ₁₅ H ₁₂ N ₄ O	68	144	64.28 (64.20)	4.28 (4.16)	20.00 (19.98)
8b	C ₆ H ₅	C ₂₀ H ₁₄ N ₄ O	67	168	73.62 (73.51)	4.29 (4.10)	17.17 (17.12)
11	H	C ₁₄ H ₁₀ N ₄ O	76	164	67.20 (67.11)	4.00 (4.02)	22.40 (22.42)

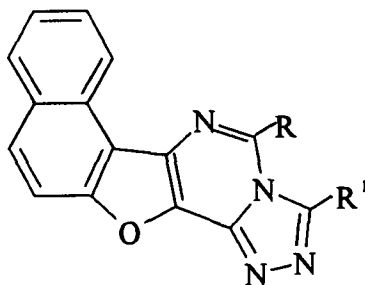
Triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans 12a-c

The compounds **8a** (0.002 mol) was heated in ethyl orthoformate (3 ml) at 80⁰c for 20 min. The reaction occurred within a few minutes and a solid cake **12a** was formed in each case. After cooling, it was triturated with petroleum ether and recrystallized from suitable solvent. The compounds **12b-c** were prepared similarly from **8b** and **11**. The physical and analytical data of the compounds synthesized is presented in **Table-7.4**.

The compounds **8a** (0.002 mol) was heated in acetyl chloride (4 ml) at 80⁰c for 20 min. The reaction occurred within a few minutes and a solid cake **13a** was formed in each case. After cooling, it was triturated with petroleum ether and recrystallized suitable solvent. The compounds **13b-c** were prepared similarly from **8b** and **11**. The physical and analytical data of the compounds synthesized is presented in **Table-7.4**.

The compounds **8a** (0.002 mol) was heated in acetic acid (4 ml) at 80⁰c for 20 min. The reaction occurred within a few minutes and a solid cake **13d** was formed in each case. After cooling, it was triturated with petroleum ether and recrystallized suitable solvent. The compounds **13e-f** were prepared similarly from **8b** and **11**. The physical and analytical data of the compounds synthesized is presented in **Table-7.4**.

Table-7.4



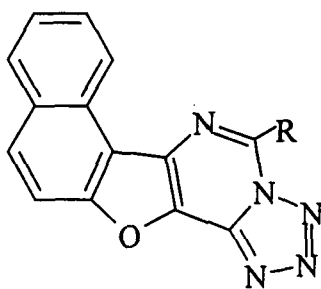
12a-c, 13a-f

Compound	R	R ¹	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
						C	H	N
12a	H	H	C ₁₅ H ₈ N ₄ O	67	254	69.23 (69.11)	3.07 (3.00)	21.53 (21.48)
12b	CH ₃	H	C ₁₆ H ₁₀ N ₄ O	69	189	70.07 (70.00)	3.65 (3.57)	20.43 (20.38)
12c	C ₆ H ₅	H	C ₂₁ H ₁₂ N ₄ O	68	201	75.0 (75.04)	3.57 (3.50)	16.66 (16.60)
13a	H	CH ₃	C ₁₆ H ₁₀ N ₄ O	82	252	70.07 (69.91)	3.65 (3.53)	20.43 (20.40)
13b	CH ₃	CH ₃	C ₁₇ H ₁₂ N ₄ O	74	264	70.83 (70.71)	4.16 (4.10)	19.44 (19.36)
13c	C ₆ H ₅	CH ₃	C ₂₂ H ₁₄ N ₄ O	68	198	75.42 (75.37)	4.00 (3.86)	16.00 (15.96)
13d	H	C ₆ H ₅	C ₂₁ H ₁₂ N ₄ O	70	194	75.00 (69.93)	3.57 (3.58)	16.66 (16.68)
13e	CH ₃	C ₆ H ₅	C ₂₂ H ₁₄ N ₄ O	75	279	75.42 (75.39)	4.00 (3.97)	16.00 (15.94)
13f	C ₆ H ₅	C ₆ H ₅	C ₂₇ H ₁₆ N ₄ O	65	233	78.64 (78.60)	3.88 (3.80)	13.59 (13.52)

Tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans 14a-c

Method A :- A suspension of **8a** (0.002 mol) in acetic acid (1N, 5 ml) was treated with sodium nitrite (0.2 g) at 45°. The reaction mixture was heated at this temperature for 2 hr. After cooling, the solid **14a**, which separated, was collected and recrystallized from ethanol. Similarly **14b** and **14c** were prepared from **8b** and **11**.

Method B.:- A mixture of **7a** (0.0015 mol) and sodium azide (0.3 g) in ethanol (10 ml) was heated under reflux on a water-bath for 4 hr. The resulting solid **14a**, that separated, after cooling the reaction mixture, was collected and recrystallized from ethanol. Compounds **14b** and **14c** were prepared in similar manner from the respective chloro compounds. The compounds were identical with those obtained in method A. The physical and analytical data of the compounds synthesized is presented in **Table-7.5**.

Table-7.5**14a-c**

Compound	R	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
					C	H	N
14a	H	C ₁₄ H ₇ N ₅ O	69	204	64.36 (64.28)	2.68 (2.60)	26.61 (26.52)
14b	CH ₃	C ₁₅ H ₉ N ₅ O	65	2174	65.45 (65.13)	3.27 (3.21)	25.45 (25.38)
14c	C ₆ H ₅	C ₂₀ H ₁₁ N ₅ O	69	198	71.21 (71.16)	3.26 (3.20)	20.77 (20.72)

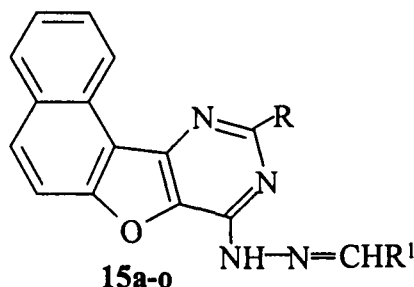
4-Arylidinehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines **15a-o**

To a solution of compounds **8a** (0.0025 mol) in methanol (50 ml), freshly distilled appropriate aldehyde (0.0025 mol) was added separately and the mixture stirred for about 2 hr. at room temperature and left overnight. The solid of **15a-e**, which separated, was collected and recrystallized from suitable solvent. Similarly, the compounds **15f-j** were prepared from **8b** and the compounds **15k-o** were prepared from **11** by using appropriate

aldehydes. The physical and analytical data of the compounds synthesized is presented in

Table-7.6.

Table-7.6



Comp	R	R¹	Molecular formula	Yield %	m.p. °C	Found (Calculated) %		
						C	H	N
15a	H	C ₆ H ₅	C ₂₁ H ₁₄ N ₄ O	75	122	74.55 (74.49)	4.14 (4.10)	16.56 (16.49)
15b	H	C ₆ H ₄ -2-OH	C ₂₁ H ₁₄ N ₄ O ₂	73	139	71.18 (71.11)	3.95 (3.89)	15.81 (15.76)
15c	H	C ₆ H ₄ -3-NO ₂	C ₂₁ H ₁₃ N ₅ O ₃	78	143	65.79 (65.72)	3.39 (3.43)	18.27 (18.22)
15d	H	C ₆ H ₄ -4-NH ₂	C ₂₁ H ₁₅ N ₅ O	81	150	71.38 (71.34)	4.25 (4.20)	19.83 (19.80)
15e	H	2-Furyl	C ₁₉ H ₁₂ N ₄ O ₂	69	187	69.51 (69.46)	3.65 (3.55)	17.07 (17.02)
15f	CH ₃	C ₆ H ₅	C ₂₂ H ₁₆ N ₄ O	69	210	75.00 (74.92)	4.54 (4.56)	15.90 (15.89)
15g	CH ₃	C ₆ H ₄ -2-OH	C ₂₂ H ₁₆ N ₄ O ₂	74	180	71.74 (71.65)	4.34 (3.50)	15.21 (15.19)
15h	CH ₃	C ₆ H ₄ -3-NO ₂	C ₂₂ H ₁₅ N ₅ O ₃	75	178	66.49 (66.41)	3.78 (3.76)	17.63 (17.60)
15i	CH ₃	C ₆ H ₄ -4-NH ₂	C ₂₂ H ₁₇ N ₅ O	67	197	71.93 (71.88)	4.63 (4.60)	19.07 (19.00)
15j	CH ₃	2-Furyl	C ₂₀ H ₁₄ N ₄ O ₂	70	245	70.17 (70.10)	4.09 (3.86)	16.37 (16.30)
15k	C ₆ H ₅	C ₆ H ₅	C ₂₇ H ₁₈ N ₄ O	82	187	78.26 (78.23)	4.34 (4.25)	13.52 (13.47)
15l	C ₆ H ₅	C ₆ H ₄ -2-OH	C ₂₂ H ₁₈ N ₄ O ₂	66	243	75.34 (75.21)	4.18 (4.01)	13.02 (12.98)
15m	C ₆ H ₅	C ₆ H ₄ -3-NO ₂	C ₂₇ H ₁₇ N ₅ O ₃	72	287	70.58 (70.51)	3.70 (3.66)	15.25 (15.21)
15m	C ₆ H ₅	C ₆ H ₄ -4-NH ₂	C ₂₇ H ₁₉ N ₅ O	74	263	75.52 (75.55)	4.42 (4.37)	16.31 (16.27)
15o	C ₆ H ₅	2-Furyl	C ₂₅ H ₁₆ N ₄ O ₂	71	234	74.25 (74.20)	3.96 (3.87)	13.866 (13.80)

References

1. A. Monge Vega, I. Aldana and E. Fernandez-Alvarez, *Eur. J. Med. Chem.*, **13**, 573 (1978).
2. S.P. Hiremath, A. Ullagaddi, S. Kalchar and M.G. Purohit, *Indian J. Heterocycl. Chem.*, **3**, 145 (1994).
3. M.S. Talwar, U.V. Laddi, Y.S. Somannanavar, R.S. Bennur and S.C. Bennur, *Indian J. Heterocycl. Chem.*, **4**, 297 (1995).
4. Vishnu J. Ram and Monika Verma, *Indian J. Chem.*, **31B**, 195 (1992).
5. Omodei-Sale Amedeo, Consonni Pietro, G. Galliani and L. Lerner, *Gen. Offen.*, **2**, 819 (1978).
6. Omodei-Sale Amedeo, Consonni Pietro, and L. Lerner, *Gen. Offen.*, **2**, 707 (1978).
7. U.S. Pathak, I.S. Rathod, K.S. Jain, N.S. Laddha and K.S. Kolhe, *Indian J. Chem.*, **36B**, 566 (1997).
8. J.E. Francis, W.E. cash, S. Psychoyos, G. Ghai, R. Wenk, R.C. Friedmann, C. Atkins, V. Wareen, P. Furness, H.L. Hyun, G.S. Sone, M.Desai and M. Wiliams, *J. Med. Chem.*, **31**, 1014 (1988).
9. J.J. Wade, *US Pat.* 4,528,288 ; *Chem. Abstr.*, 104, 5859 (1986).
10. K. Kottke, H. Kuesmstedt, V. Haegn, H. Renner and S. Schnitzler, *Ger(E) Pat.* 139 715, (1983) ; *Chem. Abstr.*, **99**, 70757 (1983).
11. H. Shailaja Rani, K. Mogilaiah and B. Sreenivasulu, *Indian J. Chem.*, **35B**, 106 (1996).
12. R.S. Robert Jean-Michel, Odile Rideau, Sylvie Riobert Piessard, Murcil Duflos, and Baut Guillaumele, *Arzneim. Forsch.*, **47(5)**, 635 (1997).
13. Heightmann Tom d, Monica Locatelli, Vasella Andrea, *Helv. Chim. Acta.*, **79(8)**, 2190 (1996).
14. A.I. Markosyan, R.A Kuroyan, M.O. Oganisyan, I.A. Dzhagatspanyan, A.B. Asryan and S.G. Zigilyan, *Pharm. Chem. J.*, **30(8)**, 498 (1996).
15. J.H. Wadsworth, M.S. Jenkins, S.B. Orlek, Frederick Canids and S.G. Michael Clark, *J. Med. Chem.*, **35(7)**, 1280 (1992).

16. -V. darias, S. Abdala, D. Martin-Herrera and S. Vega, *Arzeneimi. Helforschng.*, **50(4)**, 323 (2000).
17. F.G. Kathawala, U.S. Patent 3,850,932 (1974) ; *Chem. Abstr.*, 75, 140175d (1975).
18. G.E. Hardtmann, F.G. Kathawala, U.S. Patent 4,053,600 (1977) ; *Chem. Abstr.*, 88,22970k (1978).
19. E.S. Hand and D.C. Baker, *Can. J. Chem.*, **62**, 2570 (1984).
20. K. Noda, A. Nakagawa, Y. Nakajima and H. Ide, Japanese Patent 85,194 (1977) ; *Chem. Abstr.*, 88,50908q (1978).
21. G. Franco, L. Maria and P. Giueseppe, *J. Heterocyclic Chem.*, **26**, 613 (1989).
22. J.A. Montgomery, A.G. Laseter and K. Hewson, *J Heterocyclic Chem.*, **11**, 211 (1974).
23. A. Dlugosz, *Pharmazie.*, **50(3)**, 180 (1995).
24. E.B. Moawad, M.Y. Yousif and M.A. Metwally, *Pharmazie.*, **44(12)**, 820 (1989).
25. J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson and V.I. Stenberg, *J. Med. Chem.*, **27(12)**, 1565 (1984).
26. Norton P. Peet and Shyam Sunder, *J. Heterocyclic Chem.*, **14**, 561 (1977).
27. S.B. mahajan, S.S. Sangapure and Y.S. Agasimundin, *Indian J. Chem.*, **19(B)**, 596 ((1980).
28. Basavaraj Padmashali, V.P. Vaidya and M.L. Vijaya Kumar, *Indian J. Heterocyclic Chem.*, **10**, 253 (2001).
29. M.C. Dodd, D.L. Cramer and W.C. Ward, *J. Am. Pharm. Assoc.*, **39**, 313 (1950).
30. T. Novinson, B. Bhoshan, T. Okabe, G.R. Revankar, R.K. Robins, K. Senga, ar.d H.R. Wilson, *J. Med. Chem.*, **19**, 512 (1976).

CHAPTER-8

Pharmacological Evaluation of Synthetic Compounds

Section-A: Antifertility activity in female rats

Section-B: Antifertility activity in male rats

Section-C: Antimicrobial activity

Section-D: Anthelmintic activity

Section-E: Anti-inflammatory activity

Section-F: Anticonvulsant activity

Section-G: Antipyretic activity

INTRODUCTION

One of the major causes for the progress of chemistry of benzofurans and naphthofurans is the association of these moieties with various biological activities. There are numerous examples of plant extracts, containing these nuclei, being used for the treatment of various diseases. This fact stimulated several researchers to take up the work of isolation, identification and biological evaluation of many benzofuran compounds and few naphthofuran compounds. This has resulted in development of some benzofuran compounds as clinically useful drugs.

The major objective of the present investigation is to explore biologically more potent synthetic naphthofuran derivatives. Hence, a number of naphthofuran compounds with possible biological activities are designed, synthesized and selected compounds have been evaluated for

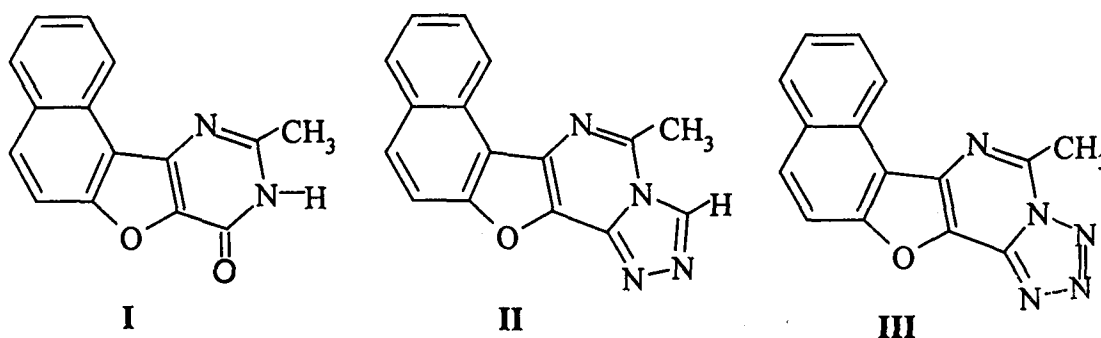
- A. Antifertility activity in female albino rats
- B. Antifertility activity in male albino rats
- C. Antimicrobial
- D. Anthelmintic
- E. Anti-inflammatory
- F. Anticonvulsant
- G. Antipyretic

activities, which are described in the following pages.

Section-A Antifertility activity in female rats

Antifertility effect of synthetic naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives in female rats was studied as described in **Chapter-4**. The observations made are presented in **Table-8.1** to **Table-8.3**.

Table-8.1 Abortifacient activity of some selected naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives.



Comp.	Dose mg/kg body weight	Group	No. of rats with implantation sites on day 10	Mean No. of implants on day 10	Mean No. of litters delivered	Percentage of abortifacient activity
Control	-	1	6	12.00	12.00	-
I	60 mg	2	6	11.60	5.82	49.82
II	60 mg	3	6	11.24	6.04	46.21
III	60 mg	4	6	10.36	6.20	40.15

As the compounds exhibited considerable abortifacient activity, it was contemplated to investigate them for possible estrogenic/antiestrogenic activity. The synthetic compounds were tested at the dose of 60 mg/kg body weight. The results are tabulated in **Table-8.2** and **Table-8.3**.

Table-8.2 Estrogenic/antiestrogenic activity of some synthetic naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives.

Group	Treatment (Dose/day)	Uterine weight in mg/100mg	Vaginal cornification
1	Control	70.62 ± 1.06	nucleated cells
2	Ethinyl estradiol (1 µg/rat)	322.43 ± 2.28	cornified cells
3	I (60 mg/kg)	187.13 ± 4.35*	nucleated and cornified
4	II (60 mg/kg)	164.54 ± 6.04*	nucleated and cornified
5	III (60 mg/kg)	140.17 ± 2.41*	nucleated cells

*p < 0.001 vs control

Table-8.3 Histological changes in the uterus.

Group	Treatment (Dose/day)	Diameter of the uterus (µm) ± SEM	Thickness of the endometrium (µm) ± SEM	Height of the endometrial epithelium (µm) ± SEM
1	Control	348.61 ± 12.72	48.06 ± 1.16	26.49 ± 5.43
2	Ethinyl estradiol (1 µg/rat)	832.14 ± 9.63	236.72 ± 8.20*	44.21 ± 6.64
3	I (60 mg/kg)	462.34 ± 6.82*	56.73 ± 1.44*	26.15 ± 1.25**
4	II (60 mg/kg)	420.32 ± 2.11*	54.08 ± 2.06*	24.54 ± 5.01*
5	III (60 mg/kg)	422.32 ± 2.35*	53.12 ± 5.72*	26.21 ± 3.24**

* p < 0.1 when compared with control, ** p < 0.01 when compared with control

Results and discussion

Some naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives were evaluated for post coital antifertility activity at the dose of 60 mg/kg body weight. No toxic effects were observed either by gross visual examination or in the weight of rats. After discontinuation of the treatment, all the rats were mated.

Since the number of corpora lutea and number of litters born were equal, it clearly indicates that naphthofuran derivatives do not possess antiimplantation activity. This observation revealed that all the implants have been resorbed by the uterus.

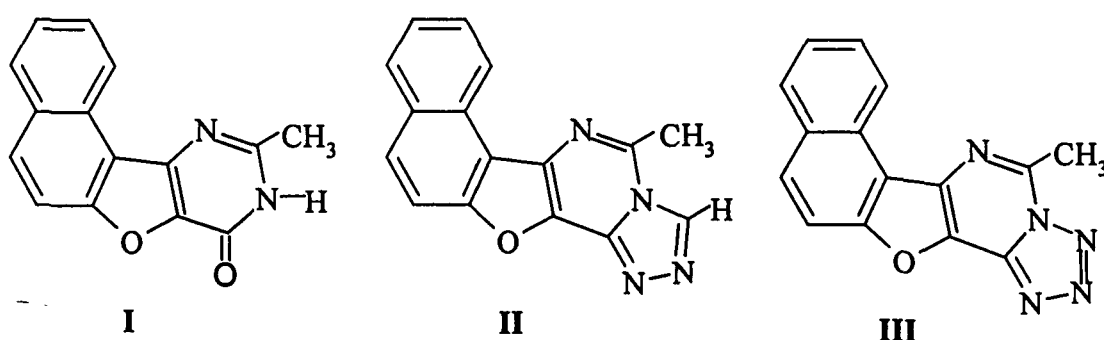
Oral administration of naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives caused significant increase in uterine weight. This suggested the mild estrogenic activity of the compounds. This fact was well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium and height of endometrial epithelium. Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

Amongst the tested compounds, 2-methyl-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidine exhibited comparatively more abortifacient activity. The construction of triazole moiety resulted in the decrease in abortifacient activity to some extent and building up of tetrazole moiety decreased the activity to a considerable extent. Surprisingly, this observation was contradictory to the anticipation of enhancement of activity when two or more heterocyclic systems are condensed with each other. Hence, findings of antifertility activity revealed that detailed investigation is required to assign any particular reason for the reverse trend in activity.

Section-B Antifertility activity in male rats

Naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives were also investigated for possible androgenic/antiandrogenic activity in male albino rats by following the procedure as described in **Chapter-4**. The synthetic compounds were tested at the dose of 60 mg/kg body weight. The results are tabulated in **Table-8.4** and **Table-8.5**.

Table-8.4 Androgenic/antiandrogenic activity of some synthetic naphtho[2,1-b]furo [3,2-d]pyrimidine derivatives.



Group	Treatment dose (mg/kg)	Testes ± SEM	Seminal vesicle ± SEM	Ventral prostrate ± SEM	Vas deferens ± SEM	Epididymes ± SEM
1	Control	638.44 ± 4.81	42.68 ± 15.30	49.54 ± 12.10	28.12 ± 23.51	76.65 ± 10.84
2	Testosterone (1 mg/kg)	679.80 ± 16.73*	71.32 ± 1.03*	82.62 ± 0.86*	40.28 ± 2.89*	84.38 ± 7.94*
3	I (60 mg/kg)	627.61 ± 11.21**	39.93 ± 2.09*	42.17 ± 9.62*	26.37 ± 9.72*	72.29 ± 0.27**
4	II (60 mg/kg)	664.52 ± 3.02*	63.04 ± 0.08**	70.16 ± 4.01*	32.66 ± 2.28*	80.41 ± 4.04*
5	III (60 mg/kg)	664.52 ± 3.02*	63.04 ± 0.08**	70.16 ± 4.01*	32.66 ± 2.28*	80.41 ± 4.04*

* $p < 0.1$ when compared with control, ** $p < 0.01$ when compared with control

Table-8.5 Changes in the testes.

Group	Treatment (Dose)	Diameter of the testes (μm) $\pm$ SEM	Diameter of the seminiferous tubules (μm) $\pm$ SEM
1	Control	39.58 $\pm$ 0.86	130.2 $\pm$ 4.1
2	Testosterone (1 mg/kg)	43.19 $\pm$ 2.03**	146.78 $\pm$ 2.9*
3	I (60 mg/kg)	40.13 $\pm$ 4.72*	140.02 $\pm$ 4.2**
4	II (60 mg/kg)	40.28 $\pm$ 3.01*	142.82 $\pm$ 1.6*
5	III (60 mg/kg)	40.47 $\pm$ 2.51*	138.52 $\pm$ 6.0**

* $p < 0.1$ when compared with control, ** $p < 0.01$ when compared with control

Results and discussion

The administration of the compounds to prepubertal male rats for seven days has increased the weights of testes, epididymes, seminal vesicle and ventral prostate when compared with the rats of control and testosterone group. The observations presented in Table-8.4 clearly indicate that all these compounds screened for androgenic activity are less active than the testosterone. However, in this case it was observed that annulation of naphtho[2,1-b]furo[3,2-d]pyrimidines with triazole and tetrazole ring system increased the androgenic activity, in accordance with the general observation.

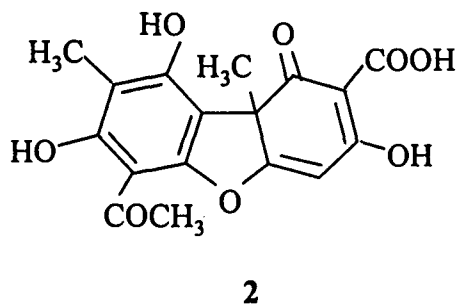
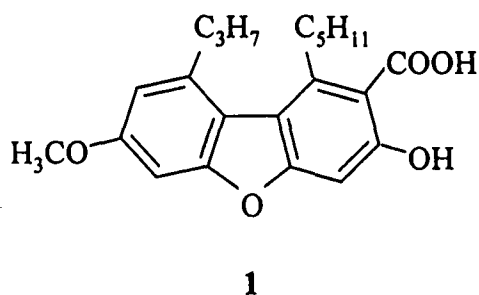
Section-C: Antimicrobial activity

Some selected compounds synthesized during the present research work have been screened for both antibacterial and antifungal activities.

C.1 Antibacterial activity

Introduction

One of the initial reports of antibacterial activity by furan compounds was due to Dodd and Stillman¹. Dibenzofuran derivatives such as didymic acid **1** and other hydroxylated dibenzofurans are known since long time, to possess some activity against *S. aureus* and *A. tubercle*². Usnic acid **2** has been found to inhibit many Gram-positive organisms³. The most interesting activity of this compound is its inhibition effect on *Mycobacterium tuberculosis* against which it is potent in conjunction with small quantities of streptomycin⁴.



The development of resistance among the various pathogenic organisms towards antibiotics stimulated the invention of newer antimicrobial agents⁵. Among the furan derivatives, nitrofurans were the first to be developed as antibacterial agents. This was followed by the use of alternative functional groups such as trifluoromethyl⁶, substituted phenyl⁷, alkylsulphonyl⁸, isostearic and isoelectronic groups like sulpho, sulphamoyl, carboxyl, methoxy carbonyl and cyano groups⁹ in place of nitro group to get compounds with improved chemotherapeutic indices.

In contrast to substituted furans, the benzofuran ring system has received little attention. The investigation of benzofuran derivatives as antimicrobial agents was initiated only in recent years. Within this short period of 2-3 decades, several benzofuran derivatives have been found to possess such an activity¹⁰⁻¹⁵. 2-Furylbenzofurans¹⁶, benzofuran analogues of chalcones¹⁷⁻²⁰ have been reported to possess antibacterial properties. *In vitro* testing of series of benzofuran, benzofuro[3,2-d]pyrimidine, 4H-benzofuro[3,2-d]-m-oxazin-4-one derivatives prepared are found to be significantly active against *S. aureus* and *E. coli*²¹.

Synthesis and antimicrobial screening of aminomethyl derivatives of naphtho [1,2-b]furan was carried out by Mukhanova²² *et al.*, they observed that all the synthesized compounds exhibited weak antimicrobial activity.

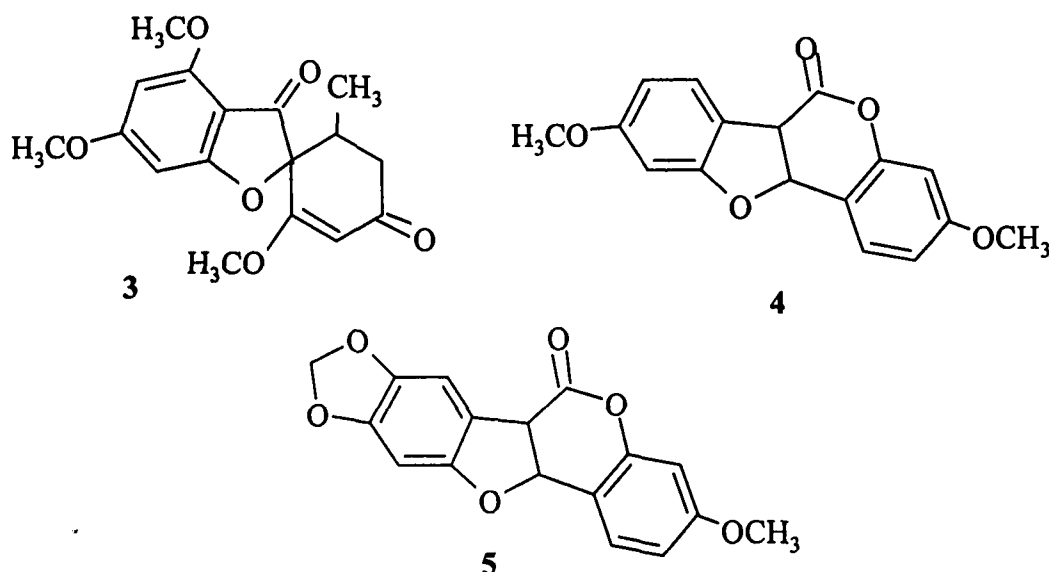
It is evident from the above facts, that several compounds possessing furan moiety are capable of exhibiting antibacterial activity. When this ring system is fused with other heterocycle or coupled with other heterocycle directly or through bridges, the resulting compounds would exhibit enhanced antibacterial property. Hence, selected naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives prepared in the course of present investigation were screened for antibacterial activity. In the present investigation, the bacteria used were *S. aureus* and *K. pneumoniae* and the fungi used were *A. niger* and *C. albicans*. The antibacterial activity of naphthofuran derivatives was studied in comparison with that of standard drug ciproflaxacin by cup plate method²⁸ as described in **Chapter-4**.

The results of antimicrobial activity of standard and selected compounds is given along with antifungal activity in **Table-8.6** to **Table-8.7**.

C.2 Antifungal activity

Introduction

β -Coumaranones are well known for their antifungal activity. Among these, griseofulvin is of great importance. It is quite active and harmless to the host. For this reason griseofulvin²³ 3 is used against several pathogenic fungi and ringworms. Chakraborty and co-workers have studied antifungal activity of furocoumarins and reported that psoralen was the most active antifungal agent²⁴. Many other naturally occurring benzofuran derivatives like homopterocarpin²⁵ 4, pterocarpin²⁶ 5 and pterocarpan phaseolidin²⁷ are reported to serve as antifungal agents in plants.



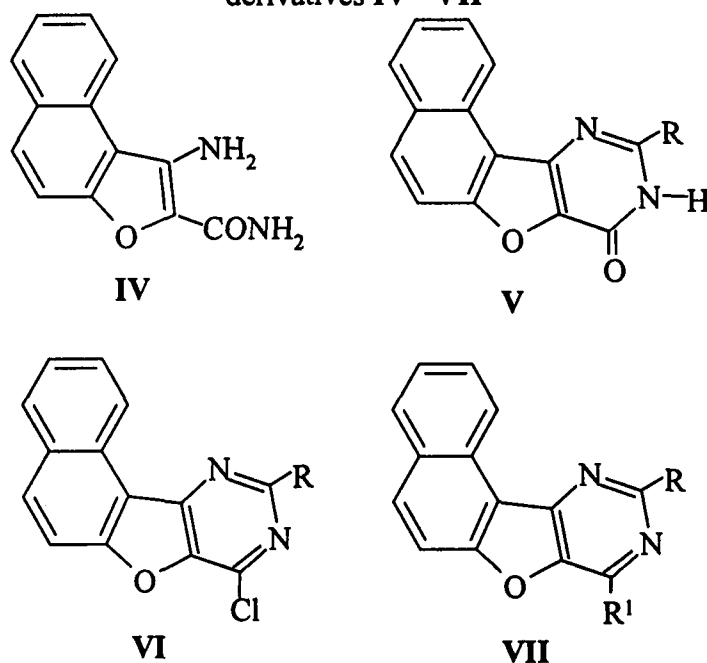
Not many synthetic benzofurans are investigated for antifungal activity. A few benzofurans derivatives with imidazole nucleus have been found to be useful as fungicides.

In view of these observations, some selected naphthofuran derivatives, now synthesized, have been subjected to antifungal screening against *Aspergillus niger* and *Candida albicans*.

Evaluation of antifungal activity

The antifungal activity of naphthofuran derivatives was studied in comparison with that of standard antifungal drug ciclopirox olamine, by cup-plate method²⁸ as described in Chapter-4.

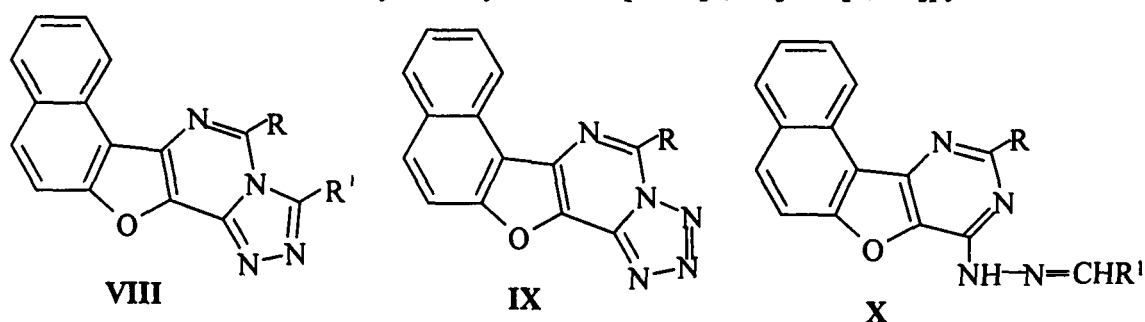
Table-8.6 Antimicrobial activity of some selected naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives IV - VII



Comp.	R	R ¹	Zone of inhibition in mm.			
			Antibacterial		Antifungal	
			<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. niger</i>	<i>C. albicans</i>
IV	-	-	04	12	14	10
Va	CH ₃	-	06	14	10	14
Vb	C ₆ H ₅	-	04	12	16	15
VIa	CH ₃	-	08	16	17	17
VIb	C ₆ H ₅	-	10	18	15	12
VIIa	CH ₃	OCH ₃	04	08	12	14
VIIb	CH ₃	OC ₆ H ₅	05	08	10	11
VIIc	CH ₃	NHC ₂ H ₅	04	06	09	10
VIIId	CH ₃	NHC ₆ H ₅	08	09	09	08
VIIe	CH ₃	NHC ₆ H ₄ -4-OCH ₃	08	06	10	06
VIIIf	CH ₃	NHC ₆ H ₄ -3-NO ₂	04	10	17	09
VIIg	C ₆ H ₅	OCH ₃	04	17	14	11
VIIh	C ₆ H ₅	OC ₆ H ₅	04	14	12	10

VIIIi	C ₆ H ₅	NHC ₂ H ₅	06	13	14	13
VIIj	C ₆ H ₅	NHC ₆ H ₅	05	11	15	12
VIIk	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃	06	14	14	10
VIII	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂	16	16	12	09
Ciprofloxacin			12	26	-	-
Ciclopirox olamine			-	-	24	22
DMF			Nil	Nil	Nil	Nil
Water (Distilled)			Nil	Nil	Nil	Nil

Table-8.7 The antimicrobial activity of some selected triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans **VIII**, tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans **IX** and 4-Arylidinehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines **X**.



Comp.	R	R ¹	Zone of inhibition in mm.			
			Antibacterial		Antifungal	
			<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
VIIIa	H	H	09	10	06	04
VIIIb	H	CH ₃	07	11	08	02
VIIIc	H	C ₆ H ₅	08	09	06	02
IXa	H	-	06	12	08	02
IXb	CH ₃	-	04	10	03	02
IXc	C ₆ H ₅	-	04	09	04	04
Xa	H	C ₆ H ₅	13	13	12	05
Xb	CH ₃	C ₆ H ₄ -2-OH	14	15	12	06
Xc	C ₆ H ₅	C ₆ H ₄ -3-NO ₂	14	14	13	06
Ciprofloxacin			19	25	-	-
Chlotrimazole			-	-	22	10
DMF			Nil	Nil	Nil	Nil
Water (Distilled)			Nil	Nil	Nil	Nil

Results and Discussion

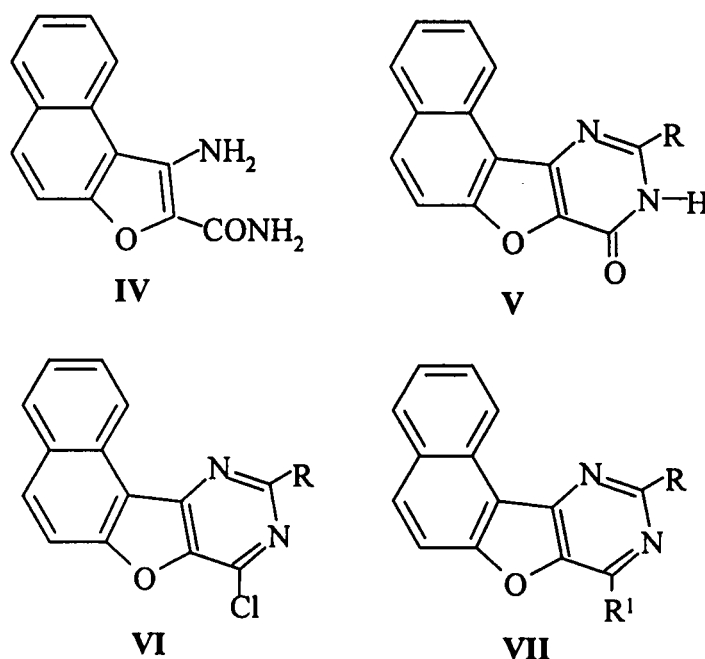
On the basis of the results obtained during the screening of various naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives for antimicrobial activity, the following conclusion could be drawn.

1. The compounds **VIa**, **VIb**, **VIIg**, **VIIIi**, **VIIIk**, **Xa**, **Xb** and **Xc** exhibited considerable antibacterial activity against *S. aureus* and *K. pneumoniae* and antifungal activity against *A. niger* and *C. albicans*.
2. The compounds **Xa**, **Xb** and **Xc** were found to exhibit moderate activity when compared with the standard drug.
3. Introduction of electron withdrawing group in the side chain resulted in the enhancement of antimicrobial activity.
4. On the other hand, introduction of electron donating group in different position of the side chain has no significant impact on the antimicrobial activity.
5. The remaining compounds were found to be less active or inactive against all the four microorganisms when compared with the standard drug.

Section-D Anthelmintic activity

The anthelmintic activity of some selected naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives and some angularly fused pentacyclic heterocycles was done as described in Chapter-4. The results are shown in Table-8.8 and Table-8.9.

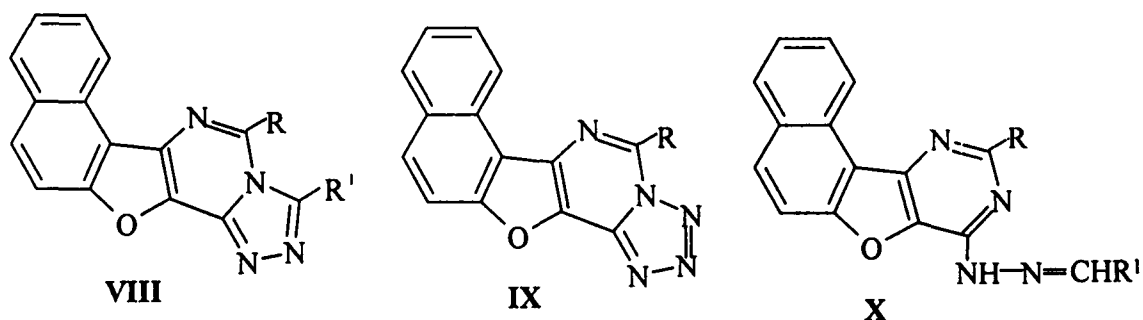
Table-8.8 The anthelmintic activity of some selected naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives IV - VII



Comp.	R	R ¹	No. of earth worms tested	Conc. (Wt./Vol., mg)	Mean paralysis time (in min.)	Mean death time (in min.)
Control	-	-	8	-	-	-
Standard	-	-	8	100	100	120
IV	-	-	8	100	162	185
Va	CH ₃	-	8	100	130	169
Vb	C ₆ H ₅	-	8	100	142	160
VIa	CH ₃	-	8	100	122	120
VIb	C ₆ H ₅	-	8	100	116	122
VIIa	CH ₃	OCH ₃	8	100	133	160
VIIb	CH ₃	OC ₆ H ₅	8	100	140	190
VIIc	CH ₃	NHC ₂ H ₅	8	100	134	166
VIIId	CH ₃	NHC ₆ H ₅	8	100	120	168
VIIe	CH ₃	NHC ₆ H ₄ -4-OCH ₃	8	100	112	160

VII f	CH ₃	NHC ₆ H ₄ -3-NO ₂	8	100	132	166
VII g	C ₆ H ₅	OCH ₃	8	100	147	170
VII h	C ₆ H ₅	OC ₆ H ₅	8	100	133	180
VII i	C ₆ H ₅	NHC ₂ H ₅	8	100	160	209
VII j	C ₆ H ₅	NHC ₆ H ₅	8	100	146	172
VII k	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃	8	100	122	160
VIII	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂	8	100	135	164

Table-8.9 The anthelmintic activity of some selected triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans VIII, tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans IX and 4-Arylidinehydrazinonaphtho[2,1-b]furo[3,2-d]pyrimidines X.



Comp.	R	R ¹	No. of earth worms tested	Conc. (Wt./Vol., mg)	Mean paralysis time (in min.)	Mean death time (in min.)
Control			8	-	-	-
Standard			8	100	100	120
VIIIa	H	H	8	100	146	172
VIIIb	H	CH ₃	8	100	164	186
VIIIc	H	C ₆ H ₅	8	100	120	168
IXa	H	-	8	100	112	160
IXb	CH ₃	-	8	100	132	170
IXc	C ₆ H ₅	-	8	100	146	172
Xa	H	C ₆ H ₅	8	100	133	180
Xb	CH ₃	C ₆ H ₄ -2-OH	8	100	160	209
Xc	C ₆ H ₅	C ₆ H ₄ -3-NO ₂	8	100	122	170

Results and discussion

The following conclusions could be drawn from the observations presented in **Table-8.10** and **Table-8.11**.

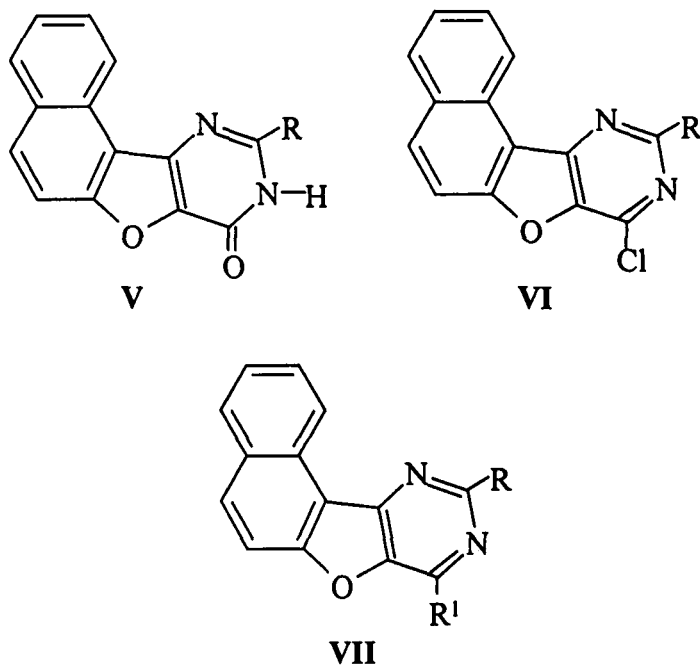
1. All the synthesized compounds screened for anthelmintic response, were less active than the standard drug as regards both vermifuge and vermucidal activities.
2. Amongst the tested compounds **VIIIa** and **VIIIb** exhibited almost equipotent vermucidal activity when compared with the standard piperazine citrate.
3. The presence of electron withdrawing groups decreased the anthelmintic activity.
4. Introduction of electron donating groups had no marked effect on the activity even after changing the position of such groups in the side chain.

Section-E Anti-inflammatory activity

In recent years, in a search for potent anti-inflammatory agents, several benzofuran compounds have been explored. Mooradin²⁹, prepared a series of benzofuro[2,3-b]pyridine derivatives and found them to possess anti-inflammatory activity. N-(O-carboxyphenyl)-3-methyl-benzofuran-2-carboxamide³⁰ and some 2,3-dihydro-2-oxo-benzofuran-3-carboxanilides³¹ have been reported to possess anti-inflammatory activity almost equal to that of aspirin. Since then a number of reports revealing the potential anti-inflammatory properties of various benzofuran compounds have appeared in literature³²⁻³⁸.

Hence, it was contemplated that some of the naphtho[2,1-b]furo[3,2-d]pyrimidine derivatives synthesized in the course of present investigation may exhibit interesting anti-inflammatory property. Thus, these compounds are now subjected for evaluation of anti-inflammatory activity. The materials and method are discussed in **Chapter-4**. The results are shown in **Table-8.10**.

Table-8.10 Anti-inflammatory activity of some selected naphtho[2,1-b]furo[3,2-d]Pyrimidines V - VII



Compound	R	R ¹	Paw volume±SEM after 3 hr.	Inhibition (%) of edema after 3 hr.
Control	-	-	1.24±0.0204	-
Standard	-	-	0.28±0.0600	77.50
Va	CH ₃	-	0.41±0.0126	66.93
Vb	C ₆ H ₅	-	0.70±0.0190	43.54
VIa	CH ₃	-	0.32±0.0135	74.19
VIb	C ₆ H ₅	-	0.68±0.0089	45.16
VIIa	CH ₃	OCH ₃	0.36±0.0152	70.96
VIIb	C ₆ H ₅	OCH ₃	0.48±0.0170	61.21
VIIc	CH ₃	NHC ₆ H ₅	0.37±0.012	69.23
VIIId	C ₆ H ₅	NHC ₆ H ₅	0.46±0.011	64.17

Results and Discussion

The only selected compounds were screened for anti-inflammatory activity by carrageenan induced rat paw edema method using Ibuprofen as the standard drug.

On the basis of results during the evaluation of anti-inflammatory activity, the following conclusions could be drawn.

1. All the compounds tested for anti-inflammatory activity were found to be less active than the standard drug.
2. Anti-inflammatory activity of the compounds **Va**, **VIa**, **VIIa** and **VIIc** was almost equal to the activity of Ibuprofen.
3. The presence of electron donating $-\text{CH}_3$ group on pyrimidine moiety relatively increased the activity.
4. The introduction of electron withdrawing $-\text{C}_6\text{H}_5$ group pyrimidine ring relatively decreased the activity.

Section-F Anticonvulsant activity

Introduction

Different types of epilepsies i.e. grand mal, petit mal or psychomotor type, can be studied in laboratory animals. The maximal electroshock (MES) induced convulsions in animals represent grand mal type of epilepsy. Similarly, chemo-convulsions due to pentylenetetrazole, which produce clonic type of convulsions resemble petit mal type of convulsions in human beings. These are the two procedures used to study convulsions and to test anticonvulsant drugs in laboratory animals. In the present study, the MES induced convulsion method was adopted to carryout anticonvulsant activity in rats^{17, 39}.

In MES convulsions, electroshock is applied through corneal electrodes. Corneal excitation is produced through optic stimulation. The MES convulsions are divided into five phages such as

- a) tonic flexion
- b) tonic extensor
- c) clonic convulsions
- d) stupor and
- e) recovery or death

A substance is known to possess anticonvulsant activity if it reduces or abolishes the extensor phase of the MES convulsions. Some of the selected angularly fused pentacyclic heterocycles have been tested for anticonvulsant activity.

Materials and method

Albino rats

Electroconvulsometer

Corneal electrodes

Stopwatch

Preparation of test solutions of the extracts

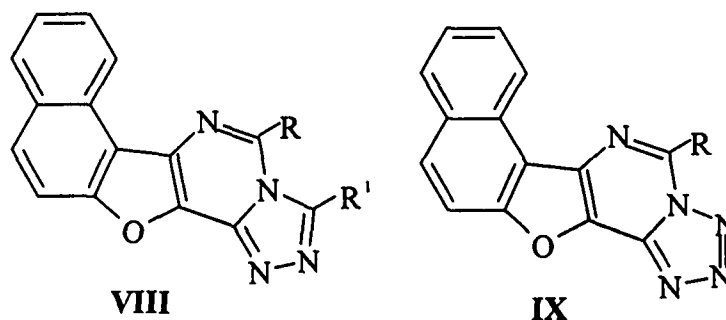
The suspension of compound (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.

Index for anticonvulsant activity in rats

Method	:	MES induced convulsion method
Animals	:	Albino rats (Wistar strain weighing 80-100 mg)
Standard	:	Diazepam (25 mg/kg body weight)
No. of animals in each group	:	06
Route of administration	:	Oral

The albino rats were weighed, numbered and were divided into eight groups of six animals each. The first group received vehicle Tween-80 (1%) and served as control and second group received the Diazepam at the dose of 25 mg/kg body weight and served as standard. The groups 3 to 8 received the compounds at the dose of 80 mg/kg body weight respectively. After 45 minutes, the animal was held properly and the corneal electrodes were placed on both the ears. Then 150 mA of current was applied for 0.2 seconds and time spent by the rat in different stages of convulsion were noted. The experiment was repeated for all the animals of the groups. The results are recorded in the **Table-8.11**.

Table-8.11 The anticonvulsant activity of some selected triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans **VIII**, tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans **IX**



Comp.	Group	R	R ¹	Time in various phases of convulsion (Mean in sec.)			
				Flexion	Extensor	Clonus	Stupor
Control	1	-	-	3.3	10.4	2.4	120
Standard	2	-	-	3.2	0	4.8	123
VIIIa	3	H	H	4.4	6.4	5.6	126
VIIIb	4	H	CH ₃	5.4	7.5	3.2	120
VIIIc	5	H	C ₆ H ₅	4.7	6.8	6.6	128
IXa	6	H	-	4.8	8.4	7.4	136
IXb	7	CH ₃	-	5.4	8.6	6.7	128
IXc	8	C ₆ H ₅	-	6.4	8.2	6.4	130

Results and discussion

Anticonvulsant activity was determined by comparing the time spent in extensor i.e. the second phase of convulsion. The following conclusions could be drawn from the observations tabulated in the **Table-8.13**.

1. All the pentacyclic heterocycles screened for anticonvulsant activity, were found to possess less activity when compared with the standard.
2. Relative anticonvulsant activity of these compounds was observed to be more when compared to the control group.
3. Amongst the compounds screened for this activity, the parent heterocycle i.e. triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furan **VIIIa** was found to possess maximum anticonvulsant activity.
4. Introduction of electron donating group leads to reduction of activity to more extent whereas electron withdrawing group leads to reduction of activity to lesser extent.
5. All the three tetrazole compounds **IXa-c** were found to be less active when compared with their triazole counterparts.

Section-G Antipyretic activity

Introduction

The human body works almost like a thermostat. It always maintains the constant body temperature. In feverish condition, this thermostat is set at high temperature. The drugs that bring down the body temperature are called antipyretics. Some of the selected angularly fused pentacyclic heterocycles have been tested for anticonvulsant activity.

Materials and method

Albino rats

Digital Tele-thermometer

Preparation of test solutions of the extracts

The suspension of compound (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.

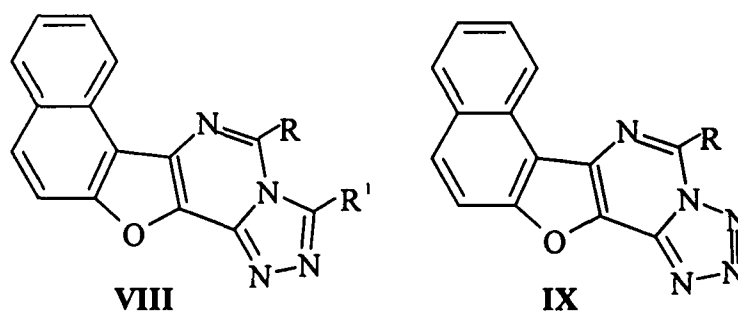
Antipyretic activity in rats

Method	:	Yeast induced hyperpyrexia
Animals	:	Albino rats (Wistar strain weighing 80-100 mg)
Standard	:	Acetyl salicylic acid (10 mg/kg body weight)
No. of animals in each group	:	06
Route of administration	:	Oral

The antipyretic activity was carried out on Wistar strain albino rats by adopting yeast induced hyperpyrexia method^{18, 40}. The rats weighing between 80-120 gm were divided into 8 groups of 6 each. The normal rectal temperature and its hourly variation were recorded at the beginning of the experiment using a digital thermometer. The

animals were fasted for 24 hrs and kept at a constant temperature of 24-25°C before giving the drugs but were freely permitted. The hyperpyrexia was induced by subcutaneous injection of 2.0 ml of 15% yeast suspension in saline solution. The rats developing 0.5°C or more rise in the rectal temperature were noted 18 hrs after the administration of the suspension. Then drugs were administered to each test group animals orally. The group 1 received Tween-80 solution (1%) and served as control, group 2 received acetyl salicylic acid and served as standard and the groups 3 to 8 received test compounds **VIIIa-c** and **IXa-c** respectively at the dose of 80 mg/kg body weight. The rectal temperature was noted after 1, 3, 6 hrs interval and compared with control group values. All values are expressed as mean $\pm$ SEM. The results are recorded in **Table-8.12**.

Table-8.12 The antipyretic activity of some selected triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans **VIII** and tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans **IX**



Comp.	Group	R	R ¹	Mean rectal temperature after hours shown $\pm$ SEM			
				0 hr	1 hr	3 hr	6 hr
Control		-	-	38.00 $\pm$ 1.42	38.3 $\pm$ 0.58	38.4 $\pm$ 0.06	38.3 $\pm$ 0.45
Standard		-	-	38.17 $\pm$ 0.12	37.82 $\pm$ 0.63	37.62 $\pm$ 1.04	37.4 $\pm$ 1.26
VIIIa		H	H	38.96 $\pm$ 0.23	38.2 $\pm$ 0.62	38.1 $\pm$ 1.00	37.8 $\pm$ 0.35
VIIIb		H	CH ₃	38.16 $\pm$ 0.83	37.84 $\pm$ 1.26	37.64 $\pm$ 0.64	37.6 $\pm$ 1.41
VIIIc		H	C ₆ H ₅	39.08 $\pm$ 0.64	38.4 $\pm$ 0.16	38.3 $\pm$ 0.23	38.2 $\pm$ 0.58
IXa		H	-	39.16 $\pm$ 0.76	38.4 $\pm$ 1.02	38.3 $\pm$ 1.34	38.2 $\pm$ 0.24
IXb		CH ₃	-	39.42 $\pm$ 0.17	39.6 $\pm$ 0.23	39.3 $\pm$ 1.22	38.1 $\pm$ 0.26
IXc		C ₆ H ₅	-	39.10 $\pm$ 0.62	38.84 $\pm$ 0.16	38.3 $\pm$ 0.28	38.2 $\pm$ 0.48

Results and discussion

The antipyretic activity of the pentacyclic heterocycles under investigation, was determined by comparing the temperature recorded after 6 hr. The following conclusions could be drawn from the observations recorded in **Table-8.14**.

1. The compounds **VIIIa** and **VIIIb** were found to exhibit equipotent antipyretic activity when compared with the standard drug.
2. Introduction of electron donating group in the triazole moiety decreases the activity to certain extent.
3. Introduction of electron withdrawing group in the triazole moiety decreases the activity to more extent.
4. All the tetrazole compounds **IXa-c** were found to possess less antipyretic activity.
5. Substitution by either electron donating or electron withdrawing groups in pyrimidine ring of tetrazoles **IXa-c** had no marked effect on antipyretic activity.

References

1. H.C. Dodd and W.B. Stillman, *J. Pharmacol. Exp. Ther.*, **82**, 11 (1944).
2. S. Shibata, Y. Miura, H. Sugimura and Y. Tozoizumi, *J. Pharma. Soc.*, **68**, 300 (1948).
3. J.B. Stark, E.D. Walter and H.S. Owans, *J. Am. Chem. Soc.*, **72**, 1819 (1950).
4. A. Stoll, A. Brack and J. Renz, *Schewarz, Zpathol*, **13**, 729 (1950).
5. K. Miura and H.K. Reckindort, *Progr. Med. Chem.*, **5**, 320 (1967).
6. R.E. Bambury, H.K. Yaktin and K.K. Wycoff, *Heterocyclic Chem.*, 595 (1968).
7. S. Somashekara, G.K. Suthar, N.V. Upadhyaya and S.L. Mukherjee, *Curr. Sci.*, **21**, 614 (1968).
8. S. Toyoshima, K. Shimada, K. Kawabe and T. Kanzawa, *Yakugaku Zasshi*, **89**, 779 (1969).
9. W. Hoyle and G.P. Roberts, *J. Med. Chem.*, **16**, 709 (1973).
10. L.J. Powers and M.P. Metes, *J. Med. Chem.*, **13**, 1102 (1970).
11. R. Royer, L. Rene, P. Demerseman, R. Cavier and J. Cenac, *Chem. Ther.*, **6**, 79 (1971).
12. R. Cavier, J. Cenac, R. Royer and L. Rane, *Chem. Ther.*, **7**, 361 (1972).
13. A. Moradian, U.S.3, 558, 667 (1971); *Chem. Abstr.*, **75**, 20176 (1971).
14. M. Steinman, *Ger. Offen.*, 2,522,669 (1973); *Chem. Abstr.*, **84**, 135460 (1973).
15. J.P. Buisson, R. Cavier, J.P. Lemonie, L. Rene and R. Royer, *Chem. Ther.*, **1C**, 43 (1975).
16. J. Guillaumel, R. Royer, M. Lecorre, A. Hercourt and R. Cavier, *Fur. Med. Chem. Ther.*, **18**, 431 (1983).
17. N.L. Doss, A. Gamil, S.F. Selim and M.S. Nossier, *J. Chem.*, **20(3)**, 315 (1979).
18. J.N. Chatherjea, A.K. Sinha, C. Bhakta and S.N. Mukharjee, *Indian J. Chem.*, **17B**, 329 (1980).
19. J. Tulecki, S. Sobiak and W. Drwewiecki, *Pol. J. Chem.*, **54**, 1563 (1980).

20. K.A. Thakur and R.C. Joshi, *J. Indian Chem. Soc.*, **57**, 1106 (1980).
21. S.S. Sangapure, *Ph. D. Thesis*, Karn. Univ., (1978).
22. T.I. Mukhanova, O.A. Lykova, L.M. Alekseeva and V.G. Granik, *Chem. Heterocyclic Compd.*, **34(6)**, 651 (1998).
23. "Indian Pharmacopoeia", Controller of Publications, Delhi, India, **I**, 353 (1996).
24. Chakraborty, D. Gupta and P.K. Bose, *Ann. Biochem. Expe. Med.*, **17**, 59 (1957); *Chem. Abstr.*, **52**, 1352 (1958).
25. McGookin, A. Robertson and W.S. Whalley, *J. Chem. Soc.*, 787 (1940).
26. P. Cazeneuve, *Chem. Ber.*, **7**, 1798 (1974).
27. D.R. Perrin and C.E. Whittle, *Tetrahedron Lett.*, 1673 (1971).
28. "Indian Pharmacopoeia", Controller of Publications, Delhi, India, **II**, A-9.1, 100 (1996).
29. Mooradin Aram, U.S. 3,452,033, (1969); *Chem. Abstr.*, **71**, 11284 (1969).
30. S.K. Saksena, A.V. Radhakrishna, C.C. Kartha and S.V. Gokhale, *Indian J. Med. Res.*, **59**, 1283 (1971).
31. S.B. Kadin, *J. Med. Chem.*, **15**, 551 (1972).
32. F. Cleude, E. Jeamnine, B. Ghy, R. Guj and P. Bernard, *Ger. Offen.*, 2,405,278 (1973); *Chem. Abstr.*, **81**, 151980 (1974).
33. K.F. Swingle, R.A. Scherrer and T.J. Grant, *Arch. Int. Pharmacodyn. Ther.*, **214**, 2040 (1975); *Chem. Abstr.*, **83**, 90835 (1975).
34. H. Noriyasu, K. Shizuo, Kyoshinor and T. Shoji, *J. Med. Chem.*, **19**, 303 (1975).
35. A. Alex, M. Jean, V. Francis, H. Christian, M. Descamps, B. Joseph, M. Colot, J.P. Heyndrickx, Van. D. Marcel and C. Robert, *Eur. J. Med. Chem. Chim. Ther.*, **11**, 57 (1976); *Chem. Abstr.*, **85**, 46281 (1976).
36. S. Schiapparelli, C. Farenceutici and S.P.A. Ruinitis, *Fr. Demande*, 2,274,296 (1976); *Chem. Abstr.*, **85**, 123769 (1976).
37. K. Mieczyslaw, J. Pomorski and K. Lidia, *Arch. Immunol. Ther. Expl.*, **24**, 603 (1976); *Chem. Abstr.*, **85**, 17166 (1976).

38. H.K. Hsieh., L.T. Tsao, J.P. Wang and C.N. Lin, *J. Pharm. Pharmacol*, **52(2)**, 163 (2000).
39. A.C. Glasser, L. Diamond and G. Combs, *J. Pharm. Sci.* **60**, 127 (1971).
40. S.C. Pal and A. Nandy, *Indian Drugs*, **36(2)**, 106 (1999).

OVERALL CONCLUSIONS

From the results arrived at the investigations on *Balanites Roxburghii* and some derivatives of naphtho[2,1-b]furo[3,2-d]pyrimidine, regarding antifertility and other biological activities, the following conclusions can be drawn,

1. The fruits of the plant *Balanites Roxburghii* have considerable abortifacient activity.
2. It has no toxic and side effects and the abortifacient activity is reversible.
3. Hence, fruits of this plant can serve as potential abortifacient agent.
4. This plant is commonly grown in many places throughout Karnataka. The fruits can be preserved several days without any deterioration.
5. The fruits can be used, very safely by pregnant woman, even without the consultation of the physician. and thus physiological and social inhibition of woman for abortion can be avoided.
6. The active component present in the crude ethanol extract, which could be responsible for abortifacient activity is found to be a flavonone. Generally flavonoids are responsible for antifertility activity.
7. In the present investigation, it was not possible to isolate any other non-steroidal antifertility agent from the fruits of *Balanites Roxburghii*.
8. The various extracts of the fruits of this plant are found to be associated with the following pharmacological activities,
 - Anthelmintic activity
 - Anti-inflammatory activity
 - Analgesic activity
 - Diuretic activity.

Hence, in addition to potent antifertility agent, the fruits of this plant can also be used safely for the treatment of helmenthiasis and rheumatic arithritis. It can also be used as a pain reliever and in urinary disorders.

9. Amongst the heterocyclic compounds synthesized during the present investigation,
 - 4-Chloronaphtho[2,1-b]furo[3,2-d]pyrimidines were found to exhibit antibacterial and antifungal activities.
 - 4-Alkyl/arylaminonaphtho[2,1-b]furo[3,2-d]pyrimidines were found to possess anthelmintic activity.
 - 4-Arylidinonaphtho[2,1-b]furo[3,2-d]pyrimidines were found to show antibacterial activity.
 - Angularly fused pentacyclic heterocycles viz., triazolo[4,3-c]pyrimido[5,4-b]naphtho[2,1-b]furans and tetrazolo[1,5-c]pyrimido[5,4-b]naphtho[2,1-b]furans were found to possess significant anti-inflammatory and anticonvulsant activity.
 - s-Triazolo[4,3-c]pyrimido[5,4-b] naphtho[2,1-b]furan was found to exhibit considerable antipyretic activity.
10. Further systematic investigation of the newly synthesized pharmacologically potent compounds would lead to the discovery of new agents possessing relatively wide spectrum of activities.

LIST OF PUBLICATIONS

1. "Analgesic and anthelmintic activity of crude extracts of the plant *Balanites Roxburghii*".
Basavaraj Padmashali and V.P. Vaidya*
Kuvempu Univ. Sci. J., **1(1)**, 56 (2001).
2. "Synthesis and pharmacological evaluation of some naphtho[2,1-b]furo[3,2-d]pyrimidines".
Basavaraj Padmashali, V.P. Vaidya* and M.L. Vijaya Kumar
Indian J. Heterocycl. Chem., **11**, 15 (2001).
3. "Synthesis of novel angularly fused pentacyclic heterocycles of pharmacological interest".
Basavaraj Padmashali, Vaidya, K.M. Mahadevan and H.M. Vagdevi,
(Accepted for "The Proceedings of International Symposium on Drug Discovery and Process Research" in Elsevier journal).
4. "Antifertility efficacy of the plant *Balanites Roxburghii* (Simurubeaceae)".
Basavaraj Padmashali, V.P. Vaidya*, N.D. Satyanarayan and C.S. Shreedhara
(Communicated to Journal of Ethnopharmacology).

PAPERS PRESENTED AT CONFERENCES

1. "Analgesic and anthelmintic activity of crude extracts of the plant *Balanites Roxburghii*".
Basavaraj Padmashali, K.M Mahadevan and V.P. Vaidya,
20th Annual conference of Indian Council of Chemists, 23 - 25th of December 2001, Mysore University, Mysore (Karnataka).
2. "Synthesis and pharmacological evaluation of some naphtho[2,1-b]furo[3,2-d]pyrimidines".
Basavaraj Padmashali and V.P. Vaidya,
21st Annual conference of Indian Council of Chemists, 24 – 26th of October 2002, Rani Durgavathi University, Jabalpur (Madhya Pradesh).
3. "Synthesis of novel angularly fused pentacyclic heterocycles of pharmacological interest".
Basavaraj Padmashali, K.M. Mahadevan and V.P. Vaidya,
"International Symposium on Drug Discovery and Process Research", 23-25th of January 2003, Shivaji University, Kolhapur (Maharashtra).

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NAPHTHO [2,1-*b*] FURO [3,2-*d*] PYRIMIDINES

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2-Hydroxy-1-naphthonitrile **1** has been condensed with chloroacetamide and the resulting 1-cyano-2-naphthoxyacetamide **2** on Thorpe-Ziegler cyclization furnished 3-aminonaphtho [2,1-*b*] furan-2-carboxamide **3** in good yield. This compound served as an intermediate for the synthesis of 2-substituted-4-oxo-naphtho [2,1-*b*] furo [3,2-*d*] pyrimidines **5a-b**. The compounds **5a-b** have been converted into 2-substituted-4-chloronaphtho [2,1-*b*] furo [3,2-*d*] pyrimidines **6a-b** which on nucleophilic substitution reactions with alcohols, phenols, aliphatic and aromatic amines afforded 2-substituted-4-alkoxy/4-aryloxy/4-alkylamino and 4-arylaminonaphtho [2,1-*b*] furo [3,2-*d*] pyrimidines **7a-p** and **8a-p**. All the newly synthesized compounds have been characterized by elemental analysis and spectroscopic data and have been screened for antimicrobial, anthelmintic and antiinflammatory activities.

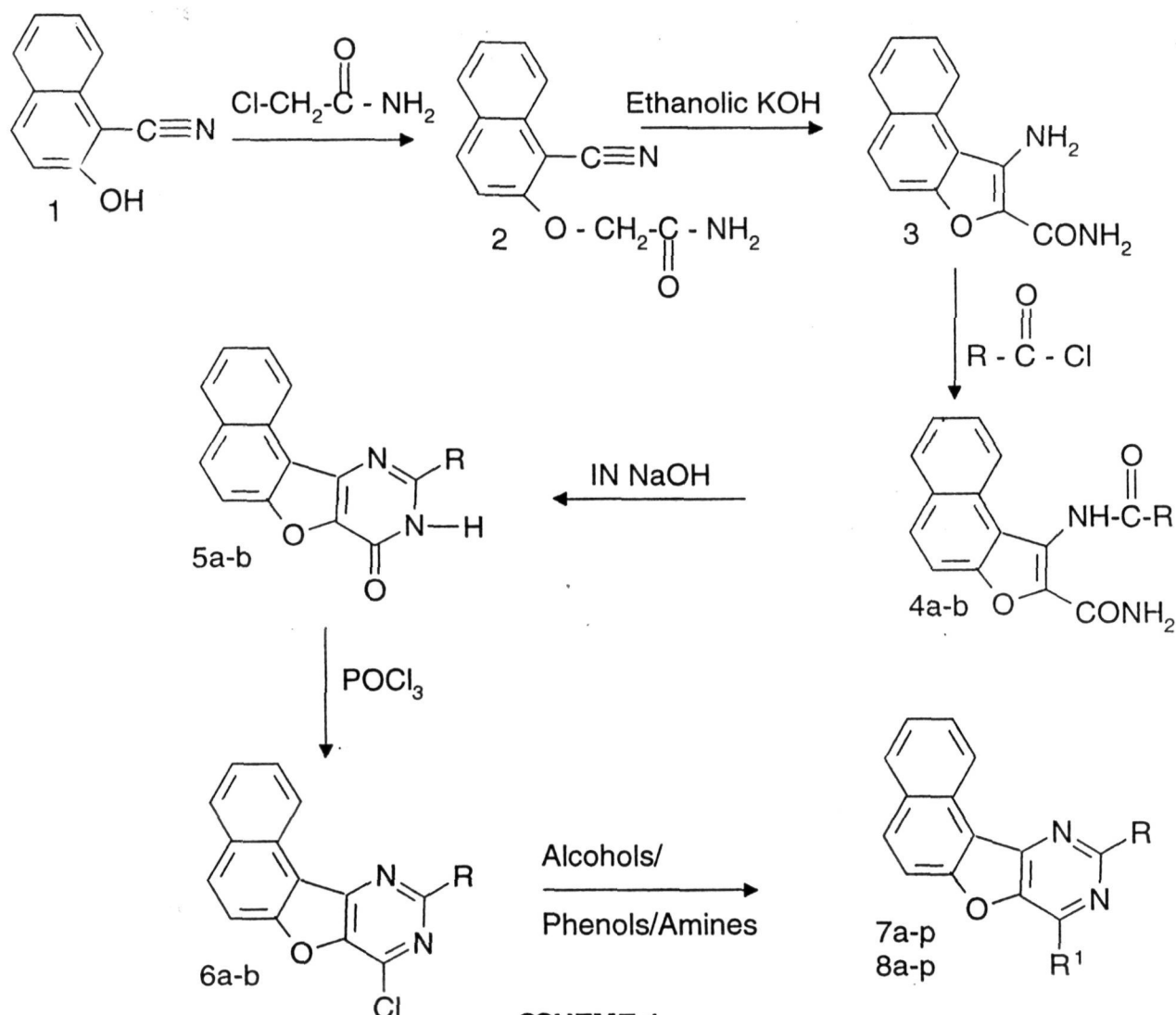
Pyrimidine is an important ring system found in nucleic acids, antibiotics, antimalarials, anticancer and antiinflammatory drugs and is associated with diverse biodynamic properties¹⁻³. Many of the pyrimidine derivatives are reported to assess potential analgesic, CNS depressant and antipyretic properties^{4,5}. Numerous compounds containing such a ring system fused with nitrogen heterocycles have been extensively investigated^{6,7}. However there are only few reports concerning pyrimidines condensed with oxygen heterocycles^{8,9}. Several derivatives of naphtho [2,1-*b*] furan have been synthesized in our laboratory¹⁰⁻¹² and are found to possess significant biological activities. Hence it was thought of interest to condense pyrimidine nucleus with naphtho [2,1-*b*] furan ring and investigate the biological activities of the resulting angularly fused tetracyclic heterocycles.

One of the methods for the construction of pyrimidine nucleus requires *o*-amino carboxamide system. For building pyrimiding moiety on naphtho [2,1-*b*] furan moiety, such a functionality in **2** and **3** positions was necessary. To accomplish this, easily accessible 2-hydroxy-1-naphthonitrile¹⁰ **1** was condensed with chloroacetamide in presence of hydrous potassium carbonate and dry acetone. The condensed product 1-cyano-2-naphthoxyacetamide

2 underwent Thorpe-Ziegler cyclization on refluxing with ethanolic potassium hydroxide and produced the desired 3-aminonaphtho [2,1-*b*] furan-2-carboxamide **3** in good yield (Scheme-1).

The IR spectrum of **2** exhibited absorption bands at 3453 cm⁻¹, 2280 and 1678 due to -NH₂, -C≡N and C=O stretching frequencies. Appearance of peaks at 4.6 δ as a singlet integrating for two protons of -CH₂ and at 5.8 at 7.0-9.0 as a multiplet integrating for 6 aromatic protons in the ¹H NMR spectrum of **2** established the structure assigned. The IR spectrum of **3** was conspicuous by the absence of peak at 2280 cm⁻¹ due to -C≡N. As expected the absorption band due to C=O group appeared at 1628. ¹H NMR spectrum of **3** exhibited peaks at 3.7 as a singlet and 6.0 as a broad singlet (D₂O exchangeable), and multiplet at 7.0-9.0 were attributed to -NH₂, -CONH₂ and 6 aromatic protons respectively.

The compound **3** was acylated using acetic anhydride and benzoyl chloride/sodium hydroxide to obtain corresponding acylated compounds **4a-b**. These compounds underwent smooth ring closure on treatment with sodium hydroxide and resulted in the formation of anticipated 2-substituted-4-oxo-naphtho [2,1-*b*] furo [3,2-*d*] pyrimidines **5a-b**. In order to synthesize various derivatives of **5a-b** it was decided to replace the carbonyl group by reactive chlorine atom. This was achieved by



SCHEME-1

refluxing **5a-b** with phosphorus oxychloride and 2-substituted-4-chloronaphtho [2,1-*b*] furo [3,2-*b*] pyrimidines **6a-b** were obtained in excellent yield. With a view to study structure activity relationships; it was contemplated to replace chlorine atom by various electron withdrawing and electron donating groups. The desired nucleophilic substitution reactions were brought about by the reaction of **6a-b** with various alcohols, phenols, aliphatic and aromatic amines under appropriate reaction conditions. The products **7a-p** and **8a-p** thus obtained were screened for antimicrobial, anthelmintic and antiinflammatory activities.

Antimicrobial activity

The antimicrobial activity of synthesized compounds was determined by cup plate method¹³⁻¹⁴

The *in vitro* antimicrobial activity was carried out against 24 hr old cultures of two bacteria and two fungi. The bacteria used were *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungi used were *Aspergillus niger* and *Candida albicans*. The compounds were tested at a concentration of 0.001 mol/ml in dimethylformamide against all organisms. Ciprofloxacin (0.001 mol/ml) and Clotrimazole (0.001 mol/ml) were used as standard for comparison of antibacterial and antifungal activities respectively. The plates were inoculated with 24 hr old culture of bacteria and fungi. The zone of inhibition was compared with the standard drug after 24 hr of incubation at 37° for antibacterial activity and 72 hr at 25° for antifungal activity. All the compounds tested showed poor antimicrobial activity.

Table-1
Characterisation data of compounds

Compd	R	R ¹	Mol formula	M.P. (°C)	Yield (%)	Nitrogen analysis %	
						Found	(Calcd)
2	-	-	C ₁₃ H ₁₀ N ₂ O ₂	107	82	12.26	(12.38)
3	-	-	C ₁₃ H ₁₀ N ₂ O ₂	96	86	12.24	(12.38)
4a	CH ₃	-	C ₁₅ H ₁₂ N ₂ O ₃	148	74	10.37	(10.44)
4b	C ₆ H ₅	-	C ₂₀ H ₁₄ N ₂ O ₃	152	70	08.40	(08.48)
5a	CH ₃	-	C ₁₅ H ₁₀ N ₂ O ₂	160	64	11.03	(11.20)
5b	C ₆ H ₅	-	C ₂₀ H ₁₂ N ₂ O ₂	144	61	08.78	(08.97)
6a	CH ₃	-	C ₁₅ H ₉ ClN ₂ O	168	76	10.28	(10.42)
6b	C ₆ H ₅	-	C ₂₀ H ₁₁ ClN ₂ O	122	72	08.34	(08.47)
7a	CH ₃	OCH ₃	C ₁₆ H ₁₂ N ₂ O ₂	139	65	10.30	(10.52)
7b	CH ₃	OC ₂ H ₅	C ₁₇ H ₁₄ N ₂ O ₂	143	64	09.82	(10.00)
7c	CH ₃	OC ₆ H ₅	C ₂₁ H ₁₄ N ₂ O ₂	150	58	08.51	(08.58)
7d	CH ₃	OC ₆ H ₄ -2-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	136	70	07.98	(08.23)
7e	CH ₃	OC ₆ H ₄ -3-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	153	64	07.96	(08.23)
7f	CH ₃	OC ₆ H ₄ -4-CH ₃	C ₂₂ H ₁₆ N ₂ O ₂	160	55	08.02	(08.02)
7g	CH ₃	NHC ₂ H ₅	C ₁₇ H ₁₅ N ₃ O	129	42	15.02	(15.16)
7h	CH ₃	NHC ₆ H ₅	C ₂₁ H ₁₅ N ₃ O	136	58	12.67	(12.92)
7i	CH ₃	NHC ₆ H ₄ -4-OCH ₃	C ₂₂ H ₁₇ N ₃ O ₂	142	64	11.80	(11.83)
7j	CH ₃	NHC ₆ H ₄ -4-Br	C ₂₁ H ₁₄ BrN ₃ O	153	62	10.19	(10.39)
7k	CH ₃	NHC ₆ H ₄ -3-CH ₃	C ₂₂ H ₁₇ N ₃ O	169	70	12.24	(12.38)
7l	CH ₃	NHC ₆ H ₄ -4-CH ₃	C ₂₂ H ₁₇ N ₃ O	142	72	12.19	(12.38)
7m	CH ₃	NHC ₆ H ₄ -4-COOH	C ₂₂ H ₁₅ N ₃ O ₃	156	60	11.32	(11.38)
7n	CH ₃	NHC ₆ H ₄ -3-NO ₂	C ₂₁ H ₁₄ N ₄ O ₃	164	78	15.02	(15.13)
7o	CH ₃	NHC ₆ H ₄ -4-NO ₂	C ₂₁ H ₁₄ N ₄ O ₃	168	76	15.10	(15.13)
7p	CH ₃	NHC ₆ H ₄ -4-OH	C ₂₁ H ₁₅ N ₃ O ₂	140	58	12.28	(12.31)
8a	C ₆ H ₅	OCH ₃	C ₂₁ H ₁₄ N ₂ O ₂	134	67	08.50	(08.58)
8b	C ₆ H ₅	OC ₂ H ₅	C ₂₂ H ₁₆ N ₂ O ₂	140	65	08.16	(08.21)
8c	C ₆ H ₅	OC ₆ H ₅	C ₂₆ H ₁₆ N ₂ O ₂	180	82	07.12	(07.19)
8d	C ₆ H ₅	OC ₆ H ₄ -2-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	210	80	06.83	(06.96)
8e	C ₆ H ₅	OC ₆ H ₄ -3-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	206	79	06.87	(06.96)
8f	C ₆ H ₅	OC ₆ H ₄ -4-CH ₃	C ₂₇ H ₁₈ N ₂ O ₂	218	86	06.90	(06.96)
8g	C ₆ H ₅	NHC ₂ H ₅	C ₂₂ H ₁₇ N ₃ O	195	70	12.34	(12.38)
8h	C ₆ H ₅	NHC ₆ H ₅	C ₂₆ H ₁₇ N ₃ O	240	80	10.76	(10.88)
8i	C ₆ H ₅	NHC ₆ H ₄ -4-OCH ₃	C ₂₇ H ₁₉ N ₃ O ₂	168	68	10.00	(10.07)
8j	C ₆ H ₅	NHC ₆ H ₄ -4-Br	C ₂₆ H ₁₆ BrN ₃ O	175	74	09.02	(09.01)
8k	C ₆ H ₅	NHC ₆ H ₄ -3-CH ₃	C ₂₇ H ₁₉ N ₃ O	186	86	10.34	(10.47)
8l	C ₆ H ₅	NHC ₆ H ₄ -4-CH ₃	C ₂₇ H ₁₉ N ₃ O	190	79	10.37	(10.47)
8m	C ₆ H ₅	NHC ₆ H ₄ -4-COOH	C ₂₇ H ₁₇ N ₃ O ₃	238	76	09.76	(09.74)
8n	C ₆ H ₅	NHC ₆ H ₄ -3-NO ₂	C ₂₆ H ₁₆ N ₄ O ₃	192	64	12.50	(12.66)
8o	C ₆ H ₅	NHC ₆ H ₄ -4-NO ₂	C ₂₆ H ₁₆ N ₄ O ₃	189	69	12.52	(12.66)
8p	C ₆ H ₅	NHC ₆ H ₄ -4-OH	C ₂₆ H ₁₇ N ₃ O ₂	166	72	10.43	(10.42)

Satisfactory C and H analysis were obtained for all the compounds.

Anthelmintic activity

The activity was evaluated on earthworms *Pheritima posthuma* by reported method¹⁵. The compounds were tested at a dose of 0.001 mol/ml suspension in 0.1% Tween-80 solution. Solution of 0.1% Tween-80 in 6% dextrose, 6% dextrose solution and Piperazine citrate suspension were poured into separate petridishes as a control, blank and standard respectively. The time taken by the worm to become motionless was noted as paralysis time. The time taken for the complete death of worms was recorded.

Study of anthelmintic activity showed that compound 8g possessed equipotent activity with that of standard drug. Compounds 7g, 8a and 8j were moderately active whereas remaining compounds were found to be less active.

Antiinflammatory activity

Ca.rageenan induced rat paw oedema method was employed for evaluating the antiinflammatory activity of some selected compounds¹⁶. The compounds were given at a dose of 80 mg/kg body weight in albino rats (Wistar strain) weighing between 150 and 200g. The oedema was produced by injecting 1% carrageenan solution at the left hind paw. Ibuprofen was used as standard drug. The percentage inhibition of oedema was calculated and compared with the standard.

Antiinflammatory response of the tested compounds was weak than the standard drug. However it was observed that the compounds containing electron-withdrawing groups exhibited more activity than the compounds containing electron-donating groups.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR spectra (in cm^{-1}) were recorded in KBr on FT-IR (Research spectrophotometer series) and Perkin-Elmer FT-IR (spectrum 1000) NMR spectra on Jeol GSX 270 FT NMR spectrophotometer using DMSO-d_6 or CDCl_3 as solvent and TMS as an internal standard [chemical shifts are given in δ (ppm) values]. Compounds were checked for their purity by TLC on silica gel G plates using carbon tetrachloride-methanol (v/v) of varying polarity and the spots were located by iodine vapours.

1-Cyano-2-naphthoxy acetamide 2

To a solution of 2-hydroxy-1-naphthonitrile (16 g, 0.07 mol) in dry acetone (175 ml), 2-chloroacetamide

(7 g, 0.074 mol) and anhyd potassium carbonate (30 g) were added and refluxed for 8 hr. The reaction mixture was filtered and the filtrate on evaporation of the solvent gave 2 as light yellow colored solid. Some amount of 2 was recovered from the potassium salts by suspending in large excess of water and collecting the insoluble material. The combined yield was 96%. It was recrystallized from ethanol.

3-Aminonaphtho [2,1-b] furan-2-carboxamide 3

The compound 2 (12g, 0.05 mol), and potassium hydroxide (8g, 0.05 mol) in ethanol (160 ml) was refluxed for 2 hr on a water bath. The reaction mixture was diluted with water and cooled in ice and neutralized. The brown colored solid thus separated was collected, dried and recrystallized from aq ethanol.

3-Acetylaminonaphtho [2,1-b] furan-2-carboxamide 4a

A mixture of 3 (5g, 0.01 mol) and acetic anhydride (25 ml) was warmed on water-bath for 15-20 min and then decomposed in ice water to get a crystalline solid. It was recrystallized from aq ethanol. IR: 1602 ($-\text{COCH}_3$), 1624 ($-\text{CONH}_2$). ^1H NMR : 2.4 (s, 3H, CH_3), 4.8 (s, 2H, NH_2), 10.4 (s, 1H, NH) and 7.0-9.0 (m, 6H, ArH).

3-Benzoylaminonaphtho [2,1-b] furan-2-carboxamide 4b

The compound 3 (5g, 0.01 mol) was warmed on water-bath for 20 min with benzoyl chloride (4 ml) in presence of aq sodium hydroxide (2N, 2 ml). The product 4b thus separated as solid was collected and recrystallized from aq ethanol.

2-Methyl-4-oxo-naphtho [2,1-b]-furo - [3,2-d] pyrimidine 5a

Method A

Acetyl compound 4a (5g, 0.02 mol) was suspended in aq sodium hydroxide (1N, 500 ml) and warmed on a water-bath for 20 min. The resulting solution when acidified with dil hydrochloric acid gave 5a, which was recrystallized from aq ethanol. IR : 1595 ($-\text{C}=\text{O}$), ^1H NMR : 2.5 (s, 3H, CH_3), 10.2 (s, 1H, NH) and 7.0-9.0 (m, 6H, ArH).

Method B

To a mixture of hydrogen peroxide (3%, 20 ml) and aq potassium hydroxide (10%, 15 ml), the acetyl compound 4a (1.5g, 0.006 mol) was added and the

mixture was heated at 70-75° for about 30 min. The resulting solution when acidified with gl acetic acid furnished 5a as light brown colored crystals, which was identical with the sample obtained in method (A).

2-Phenyl-4-oxo-naphtho [2,1-b] furo [3,2-d] pyrimidine 5b

The benzoyl derivative 4b (5g, 0.015 mol) was suspended in aq sodium hydroxide (1N, 500 ml) and warmed on a water-bath for 2 hr. The resulting solution when acidified with dil hydrochloric acid gave 5b as solid, which was recrystallized from aq ethanol. IR: 1590 (-C=O).

2-Substituted-4-chloronaphtho [2,1-b] furo [3,2-d] pyrimidines 6a-b

Compounds 5a (5g, 0.02 mol) and 5b (5g, 0.016 mol) in freshly distilled phosphorus oxychloride (50 ml) were heated separately under reflux until a clear solution was obtained. The resulting solution was cooled and poured into crushed ice with stirring. The chloro compounds thus separated were collected and crystallized from suitable solvents. 6a. ¹H NMR : 2.5 (s, 3H, CH₃), and 7.0-9.0 (m, 6H, ArH); 6b. ¹H NMR : 7.0-9.0 (m, 11H, ArH).

2-Substituted-4-methoxynaphtho [2,1-b] furo [3,2-d] pyrimidines 7a, 8a

To a solution of 6a (0.6 g, 0.0022 mol) in abs methanol (10 ml), a solution of sodium methoxide prepared from sodium metal (0.3 g) and abs methanol (10 ml) was added. Immediately sodium chloride separated. The reaction mixture was warmed on a water-bath for 30 min., cooled and diluted with water. 7a was collected and recrystallized from methanol. Similarly 8a was also prepared. 7a. ¹H NMR : 2.5 (s, 3H, CH₃), 3.4 (s, 3H, OCH₃) and 7.0-9.0 (m, 6H, ArH); 8a. ¹H NMR : 3.6 (s, 3H, OCH₃) and 7.0-9.0 (m, 11H, ArH).

2-Substituted-4-ethoxynaphtho [2,1-b] furo [3,2-d] pyrimidines 7b, 8b

To a solution of 6a (0.6g, 0.0022 mol) in abs ethanol (10 ml), a solution of sodium ethoxide prepared from sodium metal (0.3 g) and abs ethanol (10 ml) was added. Immediately sodium chloride was separated. The reaction mixture was warmed on a water-bath for 30 min, cooled and diluted with water. 7b was collected and recrystallized from methanol. Similarly 8b was also

prepared. 7b. ¹H NMR : 1.2 (t, 3H, CH₃), 4.0 (q, 2H, CH₂) and 7.0-9.0 (m, 11H, ArH).

2-Substituted-4-aryloxynaphtho [2,1-b] furo [3,2-d] pyrimidines 7c-f, 8a-f

A mixture of 6a (0.6g, 0.0022 mol), freshly distilled phenol (6g) and powdered ammonium carbonate (2g) was initially heated gently followed by heating on a waterbath for 40-45 min. Upon dil with appropriate amount of aq sodium hydroxide solution (10.6), the solid that separated was filtered, washed with water and recrystallized from suitable solvent. Compounds 8c-f were also prepared in the same way.

2-Substituted-4-alkyl and arylaminonaphtho [2,1-b] furo [3,2-d] pyrimidines 7g-p, 8g-p

A mixture of 6a (0.6g, 0.0022 mol) and appropriate amine (0.005 mol) in abs ethanol (12 ml) was heated on a waterbath for 4-6 hr. After dilution with water, it was allowed to stand overnight. The solid product was collected and crystallized from a suitable solvent. The compounds 8g-p were also prepared in the same manner. 7i. ¹H NMR 2.5 (s, 3H, CH₃), 3.6 (s, 3H, OCH₃), 10.2 (s, 1H, NH) and 7.0-9.0 (m, 10H, ArH); 8k. ¹H NMR : 2.4 (s, 3H, CH₃) and 7.0-9.0 (m, 15H, ArH).

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References

1. R.G. Menon and E. Purushothaman, *J. Indian Chem., Soc.*, **70** (1993), 553.
2. R.G. Memon and E. Purushothaman, *J. Indian Chem. Soc.*, **72** (1995), 731.
3. R.G. Menon and E. Purushothaman, *J. Indian Chem. Soc.*, **35** (1996), 1185.
4. C.J. Shishoo, M.B. Devani, G.V. Ullas, S. Anandan and V.S. Bhadti, *J. Heterocyclic Chem.*, **18** (1981), 43.
5. *Chem. Abstr.*, **88** (1978), 50908q.
6. Bharati Mishra and Nizamuddin, *Indian J. Chem.*, **28B** (1989), 243.

7. Seema Mishra, S.K. Srivastava and S.D. Srivastava, *Indian J. Chem.*, **36B** (1997), 826.
8. S.S. Sangapure and S. Agasimundin, *Indian J. Chem.*, **14B** (1976), 6686.
9. V.P. Vaidya and Y.S. Agasimundin, *Indian J. Chem.*, **20B** (1981), 114.
10. K.M. Mahadevan, Basvaraj Padmashali and V.P. Vaidya, *Indian J. Heterocyclic Chem.*, **11** (2001), 15.
11. H.M. Vagdevi, K.P. Latha, V.P. Vaidya, M.L. Vijaya Kumar and K.S.R. Pai, *Indian J. Pharm. Sci.*, **63(4)** (2001), 286.
12. K.M. Mahadevan and V.P. Vaidya, *J. Ind. Council Chem.*, **18** (2001), 78.
13. A.R. Saundane, K. Rudresh, N.D. Satyanarayan and S.P. Hiremath, *Indian J. Pharma. Sci.*, **60** (1998), 379.
14. H.M. Vagdevi and V.P. Vaidya, *Indian J. Heterocyclic Chem.*, **10** (2001), 253.
15. K.N. Giand, R.N. Dhar, B.M. Chopra and R.N. Kaul, *Indian J. Pharm.*, **27** (1965), 141.
16. V.N. Sonar and N. Sreenivasulu, *Indian J. Heterocyclic Chem.*, **4** (1995), 203.

1254/02

Anthelmintic activity of fruits of *Balanites roxburghii* (Balanitaceae)

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ABSTRACT

The anthelmintic efficacy of *Balanites roxburghii* (fruits) has been studied on earthworms *Pheritima posthuma*. The crude extracts have been obtained by Soxhlet extraction, of powdered fruit, with different solvents and further purification has been carried out by chromatographic separation. The methanol soluble fraction showed considerable activity. Piperazine citrate was used as a standard drug.

Key words : Anthelmintic activity, *Balanites roxburghii*, *Pheritima posthuma*, Piperazine citrate.

1. Introduction

Balanites roxburghii, Planch is an important medicinal plant (small evergreen tree) found in dry parts of India[1-2]. In Karnataka it grows in both black and red soils of Chitradurga and Bellary districts. Survey of the literature revealed that there are only few reports regarding the study of this plant. The bark, unripe fruit and leaves are reported to have anthelmintic property. The bark is given to cattle as an anthelmintic by the people of Western India. In addition, the fruits are associated with analgesic, purgative and antidysenteric properties[3-4].

Antifeedant active saponins have been isolated from the stem bark[5] Studies

regarding hyperglycemia induced testicular dysfunction after chronic administration of fruit pulp extract in dogs have been reported[6]. Chemical investigation and vegetative propagation of these plants have appeared in literature[7-8].

Similarly, studies have been made regarding phytochemistry and pharmacological activity of different parts of the plants of *Balanites* genus[9-12]. Encouraged by these reports it is contemplated to take up the investigation of anthelmintic activity of fruits of the plant.

2. Experimental

2.1 Plant material

The fruits were collected from Chitradurga

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in the month of June (fruiting season). The plant was authenticated by comparing herbarium (F.D.D-822) maintained at Department of Biotechnology, Kuvempu University, Shankaraghatta.

2.2 Extract Preparation

The fruits were shade dried, coarsely powdered and subjected to Soxhlet extraction for 6 hours using 2 liters each of solvents with increasing polarity viz., petroleum ether (60°-80°C), chloroform, ethanol and finally macerated with water for 7 days at room temperature. Solvents were removed from each extract by using rotary flash evaporator under reduced pressure and controlled temperature. The ethanolic extract showed considerable anthelmintic activity and hence was taken up for investigation in detail.

Ethanolic extract, which was obtained as dark brown coloured gummy mass (80 g.), was stirred with ethyl acetate (500 ml) to remove chlorophyll. The remaining residue was stirred with *n*-butanol (500 ml) and left over night. The supernatant liquid was collected and solvent was removed by using rotary flash evaporator, which yielded brown coloured pasty mass (8 g.).

The remaining residue was subjected to column chromatographic separation. The column (75 cm in length, 4 cm in diameter) was filled with Silica Gel (100-200 mesh) using chloroform. The gummy mass (20 g) obtained from ethanolic extract was mixed intimately with Silica Gel (20 g, 100-200 mesh), dried and loaded on the top of the column and eluted with solvent or mixture of solvents as shown in Table-2. The fractions showing similar spot on TLC were pooled together and solvent was

removed by using rotary flash evaporator. Elution with ethyl acetate and methanol (60:40) gave yellowish crystalline solid (2.5 g.) and was named as IBR-1. Similarly elution with ethyl acetate and methanol (30:70) yielded light brown coloured solid (2.5 g.), which was named as IBR-4. All these fractions i.e. IBR-1, IBR-2 and IBR-4 were screened for anthelmintic activity.

2.3 Phytochemical investigation of crude extracts

Phytochemical tests were carried out to find out the presence of alkaloids, carbohydrates and glycosides, flavonoids, proteins and free aminoacids and saponins by the methods described in the literature. Results of the phytochemical investigation of the crude extracts are reported in Table-1.

2.4 Animals

Earthworms *Pheritima posthuma* (Order-Annelida, class-Oligochaeta) measuring 8-10 cm in length were procured from local supplier at Shimoga and maintained at Sahyadri Science College, Shimoga.

2.5 Anthelmintic activity

The anthelmintic activity was carried out on earthworms *Pheritima posthuma* by reported method[13-14]. The worms were washed with saline (5%) to remove adhering material and sorted out for uniform size and length. They were kept in glucose solution (6%) and the worms with normal motility were selected for the test.

Petridishes of equal size taken and in the first set of petridishes dextrose solution (6%, 20 ml) was placed. In the second, third, fourth, fifth and sixth set of petridishes, Piperazine citrate solution (20

mg in 20 ml of 0.1% Tween-80) and test compounds IBR-1, IBR-2, IBR-3 and IBR-4 (20 mg in 20 ml of water) were placed. In each set of petridishes three worms were kept. The time taken for paralysis

and death was recorded. The experiment was repeated with different concentrations i.e., 30, 60, 100, 160 and 200 mg of Piperazine citrate and test compounds. The results are shown in Table-4.

Table-1 : Phytochemical investigation of crude extracts.

Sl. No.	Phytochemical Constituents	Petroleum ether	Chloroform extract extract	Ethanollic extract	Aqueous extract
1.	Alkaloids	-	-	+	+
2.	Carbohydrates and Glycosides	-	-	+	+
3.	Flavonoids	-	-	-	-
4.	Phytosterols	+	+	-	-
5.	Proteins and Free Amino acids	-	-	+	+
6.	Saponins	+	+	+	+

"+" Indicates the presence of phytochemical constituents.

"-" Indicates the absence of phytochemical constituents.

Table-2 : Chromatographic separation of ethanolic fraction

No. of Fractions	Solvent System*	Remarks
1-7	Petroleum ether	No residue
	<u>Petroleum ether:Chloroform</u>	
8-15	90:10	"
16-22	80:20	"
23-28	70:30	"
29-34	60:40	"
35-40	50:50	"
41-46	40:60	"
47-52	30:70	"
53-58	20:80	"
59-64	10:90	"
65-70	Chloroform	"
	<u>Chloroform:Ethyl acetate</u>	
41-76	90:10	"
77-82	80:20	"
83-88	70:30	"
89-94	60:40	"
95-100	50:50	"
101-106	40:60	"
107-112	30:70	"
113-118	20:80	"
119-124	10:90	"
125-130	Ethyl acetate	"
	<u>Ethyl acetate:Methanol</u>	
131-136	90:10	"
137-142	80:20	"
143-154	70:30	"
155-169	60:40	Yellowish white mass
170-185	50:50	(IBR-1)
185-210	40:60	
211-225	30:70	Light brown mass
226-242	20:80	(IBR-2)
243-265	10:90	
	Methanol	Dark brown mass
		(IBR-3)

*Solvents were distilled and dried before use.

Table-3 : Chromatographic separation of butanolic extract

No. of Fractions	Solvent System*	Remarks
100ml	Petroleum ether	No residue
	<u>Petroleum ether:Chloroform</u>	
1-7	90:10	"
8-15	80:20	"
16-21	70:30	"
22-27	60:40	"
28-33	50:50	"
34-40	40:60	"
41-47	30:70	"
48-51	20:80	"
52-57	10:90	"
58-63	Chloroform	"
	<u>Chloroform:Ethyl acetate</u>	
64-70	90:10	"
71-75	80:20	"
76-81	70:30	"
82-87	60:40	"
88-93	50:50	"
94-99	40:60	"
100-106	30:70	"
107-112	20:80	"
113-116	10:90	"
117-121	Ethyl acetate	"
	<u>Ethyl acetate:Methanol</u>	
122-136	90:10	"
128-142	80:20	"
141-154	70:30	"
150-169	60:40	"
157-185	50:50	Brown coloured mass
181-210	40:60	(IBR-4)
186-225	30:70	"
191-242	20:80	"
197-265	10:90	"
201-204	Methanol	

Solvents were distilled and dried before use.

Table-4 : Anthelmintic activity of IBR-1, IBR-2, IBR-3 and IBR-4

Compound	Dose in mg.	No. of Worms used	Time of Paralysis in min.	Time of death in min.
Control	–	03	–	–
Standard drug	20	03	140	160
	30	03	130	150
	60	03	125	140
	100	03	120	138
	160	03	106	130
	200	03	80	100
IBR-1	20	03	–	–
	30	03	–	–
	60	03	–	–
	100	03	–	–
	160	03	–	–
	200	03	–	–
IBR-2	20	03	180	–
	30	03	160	–
	60	03	130	–
	100	03	100	–
	160	03	70	–
	200	03	40	–
IBR-3	20	03	–	–
	30	03	–	–
	60	03	–	–
	100	03	–	–
	160	03	–	–
	200	03	–	–
IBR-4	20	03	150	–
	30	03	120	–
	60	03	95	–
	100	03	70	–
	160	03	50	–
	200	03	45	–

Standard drug used
Worms used

- Piperazine citrate
- *Pheritima posthuma*

3. Results and discussions

The compounds IBR-2 and IBR-4 showed considerable anthelmintic activity by paralyzing earthworms in a quicker way as compared with the standard drug. The compounds IBR-1 and IBR-3 showed no activity.

Earthworms have the ability to move by ciliary movement. Outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm. The drugs possessing anthelmintic property will paralyze the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis. Since worm is incapable of movement it will be expelled from the body. The anthelmintic activity of the extracts of *Balanites roxburghii* may be attributed to similar type of action.

4. Conclusion

Present study on the fruits of the *Balanites roxburghii* plant reveals that ethanolic fraction eluted by ethyl acetate:methanol (30:70) has more anthelmintic activity than the standard drug at higher concentration. On the other hand butanolic extract (obtained from ethanolic fraction) eluted by ethylacetate:methanol (50:50) has less activity, whereas other fractions failed to exhibit any activity.

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References

- [1] J.S. Cecil, "Flora of Karnataka", Oxford and IBH Publications, 2, 212-213, (1996).
- [2] R.N. Chopra, S.L. Nayar and I.C. Chopra, "Glossary of Indian Medicinal Plants", CSIR Publication, New Delhi, (1992).
- [3] G. Watt, "Dictionary of The Economic Products of India", Vol.1, Periodical Experts, New Delhi, (1972).
- [4] S.K. Bhattacharjee, "Handbook of Medicinal Plants", Pointer Publishers, Jaipur, (1998).
- [5] D.C. Jain, *Phytochemistry*, 26(8), 2223, (1987).
- [6] V.P. Dixit, S.K. Bhargava, and R.A. Gupta, *Indian J. Exp. Biol.*, 10, 918, (1981).
- [7] M.S.V. Khan, A.A. Siddiqui and J. Kalim, *Asian J. Chem.*, 9(2), 324, (1997).
- [8] V.A. Amalraj and K.A. Shankaranarayan, *Indian For.*, 118(4), 271, (1992).
- [9] M.S. Kamel, *Phytochemistry*, 48(4), 755, (1998).
- [10] M.S. Kamel, and A. Koskinen, *Phytochemistry*, 40(6), 1773, (1995).
- [11] M.S. Kamel, K. Ohtani, K. Tomonori and M. Assaf., *Chem. Pharm. Bull.*, 39(5), 1229, (1991).
- [12] Liu Hungweri and Nakanishi Koji, *Tetrahedron*, 38(4), 513, (1982).
- [13] K.N. Giand, R. N. Dar, B.M. Chopra and R.N. Kaul, *Indian J. Pharm. Sci.*, 27, 141, (1996).
- [14] H.M. Vagdevi, K.P. Latha, V.P. Vaidya, M.L. Vijayakumar and K.S.R. Pai, *Indian J. Pharm. Sci.*, 63(4), 279, (2001).

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ABSTRACTS

OP-95 : ANTHELMINTIC AND ANALGESIC ACTIVITY OF FRUITS OF *BALANITES ROXBURGHII* PLANCH (BALANITACEAE)

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Balanites Roxburghii Planch (Balanitaceae) is an important medicinal plant (small evergreen tree) found in drier parts of India. In Karnataka it grows in both black and red soils of Chitradurga and Bellary districts. The fruit has sharp bitter taste having anthelmintic, analgesic, purgative and antidysenteric properties. Hence it is contemplated to take up the investigation of anthelmintic and analgesic activities of fruits of this plant.

The fruits were collected from Chitradurga in the month of June (fruiting season), shade dried, coarsely powdered and subjected to Soxhlet extraction using solvents with increasing polarity viz. petroleum ether (60°-80°C), chloroform, ethanol and water. Solvents were removed by using rotary flash evaporator under reduced pressure and controlled temperature. Ethanolic extract, which showed considerable anthelmintic and analgesic activity, was subjected to column chromatographic separation. The four fractions collected were labelled as IBR-1, IBR-2, IBR-3 and IBR-4. These fractions were screened for anthelmintic and analgesic activity.

Anthelmintic activity was carried out on earthworms *Pheritima posthuma* by reported method. Piperazine citrate was used as a standard drug. Analgesic activity was performed on albino mice (German strain) weighing between 20g. and 30g. Acetic acid solution (0.6%) was used to induce writhings and acetyl salicylic acid as standard drug.

The fractions IBR-2 and IBR-4 showed significant anthelmintic and analgesic activities where as remaining two fractions exhibited negligible activity.

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ABSTRACTS

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OO-23: SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NAPHTHO[2,1-b]FURO[3,2- d]PYRIMIDINES

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Pyrimidine is an important ring system found in nucleic acid, antibiotics, antimalarials, anticancer and anti-inflammatory drugs and is associated with diverse biodynamic properties. Many of the pyrimidine derivatives are reported to possess potential analgesic, CNS depressant and antipyretic properties. Though numerous compounds containing such a ring system fused with nitrogen heterocycles have been extensively investigated, there are only few reports concerning pyrimidines condensed with oxygen heterocycles. With a view to explore pharmaceutical leads, the fusion of pyrimidine ring with naphtho[2,1-b]furan moiety and the synthesis of its various derivatives and biological evaluation of these compounds was undertaken.

2-Hydroxy-1-naphthonitrile 1 has been condensed with chloroacetamide and the resulting 1-cyano-2-naphthoxyacetamide 2 on Thorpe-Ziegler cyclization furnished 3-aminonaphtho[2,1-b]furan-2-carboxamide 3 in good yield. This compound served as an intermediate for the synthesis of 2-substituted-4-oxo-naphtho[2,1-b]furo[3,2-d]pyrimidines 5a-b. The compounds 5a-b have been converted into 2-substituted-4-chloronaphtho[2,1-b]furo[3,2-d]pyrimidines 6a-b which on nucleophilic substitution reactions with alcohols, phenols, aliphatic and aromatic amines afforded 2-substituted-4-alkoxy/4-aryloxy/4-alkylamino and 4-arylamino-naphtho[2,1-b]furo[3,2-d]pyrimidines 7a-p and 8a-p.

All the newly synthesized compounds have been characterized by elemental analysis and spectroscopic data and have been screened for antimicrobial, anthelmintic and anti-inflammatory activities.

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Basavaraj Padmashali

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SHIVAJI UNIVERSITY
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P-60

**SYNTHESIS OF NOVEL ANGULARLY FUSED PENTACYCLIC
HETEROCYCLES OF PHARMACOLOGICAL INTEREST**

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Historically, a wide range of biological activities have been attributed to fused triazoles and tetrazoles. A number of [1,2,4]triazole derivatives were reported to show broad spectrum biological activities. Some triazoloquinazolines and triazolo pyrimidines have been synthesized for their anxiolytic, antitumor, analgesic, CNS depressant and antipyretic activities.

Encouraged by these reports and guided by the observation that many a times the combination of one or more heterocyclic nuclei in a molecule enhances the biological profile many-folds, it was contemplated to take up the synthesis of new 2-substituted s-triazolo[4,3-c]pyrimido[4,5-b]naphtho[2,1-b]furans and tetrazolo [1,5-c] pyrimido[4,5-b]naphtho[2,1-b]furans.

The required starting materials 2-substituted-4-chloropyrimido[3,2-d]naphtho [2,1-b]furans 1a-c were synthesized from 2-hydroxy-1-naphthaldehyde by series of reactions already reported from our laboratory. Compounds 1a-c on reaction with hydrazine hydrate gave corresponding 4-hydrazino derivatives 2a-c, which on subsequent treatment with ethyl orthoformate, acetic acid and benzoyl chloride separately, yielded 2-substituted-s-triazolo[4,3-c]pyrimido[4,5-b]naphtho[2,1-b] furans 3a-i. On the other hand compounds 2a-c on diazotisation reaction produced 2-substituted-tetrazolo [1,5-c] pyrimido [4,5-b] naphtho[2,1-b]furans 4a-c.

The newly synthesized compounds have been characterized by spectral studies and have been evaluated for anticonvulsant activity by MES induced convulsion method and for antipyretic activity by yeast induced hyperpyrexia method on albino rats.