PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES ON LEAVES OF *Erythrina variegata* Linn., AND EVALUATION OF ITS EFFECT ON METABOLIC SYMPTOMS OF EXPERIMENTALLY INDUCED POLYCYSTIC OVARIAN DISEASE

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY CHENNAI – 32

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY IN PHARMACOGNOSY

Submitted by

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INTRODUCTION

The field of Pharmacognosy has well advanced in the past centuries. Natural products from the medicinal plant source are the main cast of modern drugs in clinical use[1].

Hippocrates says that[2]

" Life is short, and the art long; the occasion fleeting, experience fallacious; and judgment difficult".

Millions of people in the third world have used and will always use herbal medicines because they believe in them and regard them as their Medicine, in contrast to the **"Allopathic"** (conventional medicine) brought it form **"outside".**

In western countries, there is now an increased use of Herbal medicines, largely because of a belief that powerful synthetic agents used in western medicine can exert more unwanted side effects and are too often used indiscriminately and irrationally.

"Nature is the finest doctor, India is a medicinal meadow with over 7500 medicinal plants that cure naturally. Reviving this ancient, effective and rich medicinal heritage is the right way to improve the health status of the people".

It is estimated that 2% of landmass has 25% of biodiversity and 7000 species of herbs are available in different ecofloristic regions of India. Also 159 Pharmaceutical companies are utilizing about 300 species of herbs in their formulations and the production has recently increased from Rs 100 crore in 1991 to Rs 400 crore[3].

Plants have curative measures for all the ailments but man has to discover it out. Herbs act in almost magical and astonishing ways. However, the ultimate objective of this is that they should interest directly with our body chemistry. Their active constituents must be absorbed into the body for deriving the required benefits. Once they are absorbed in the blood stream, they circulate to influence our whole system. Herbs are considered food for natural medicine, vitamins, minerals and phytochemicals that have a remarkable history of curative effects[4].

Pharmacognostic science, considered the mother of all the sciences, owes its birth to the advent on earth of the earliest man, who had no choice but to search his surroundings for some product to relieve his pain and cure the diseases[5].

Today the world is moving from spiritual to digital. The digitalization of herbal medicines, once considered spiritual, is progressing at a rapid space. There is global resurgence in the use of traditional systems of medicine which are based on drugs of natural origin[6].

Herbal medicine

The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (WHO, 1991).

Why herbal medicine? [7]

Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a

part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body.

Back ground of Traditional Herbal Medicine [8]

Plants, animals and the human beings have intimate biological relationships since remote past and have evolved along parallel lines co-operating and depending upon each other for existence. The primitive men during the course of their struggle for existence in the forest must have encountered the miseries of pains and sickness, sustained injuries and lived under the circumstances of abject poverty disease and hunger. To liberate themselves from these sufferings they should have looked towards their natural fellow friends- the plants. And this inevitably led to the experimentation through trial and error and discovery of the healing properties of plants. It must have begun with the **"Miraculous healing effects"** of leaves of some plants with they covered their wounds to avoid flies and dust and with the soothing and stimulating effect of the leaves, root and stems of some plants which they chewed to satisfy their hunger. Once having been realized the significance of these "**Wonder Herbs**" they started communicating about them to their fellow friends through signs and symbols. Undoubtedly nature has all along with the diseases, has created their cure and for every disease that arises on this planet, plants have a cure. Nature keeps in her green bag, the secret of healthy life on this earth, perhaps in the luxuriant green cover, the bio-diversity.

History of Traditional medicine in the world [9]

Archaeological discoveries of 60,000 year old Neanderthal burial grounds in Iraq, point to the use of plants like marsh mallow, yarrow and ground sel. Sumerians (4000 BC) from their cusseiform writing on clay tablets are reported to have used opium, liquorice, thyme, mustard and sulphur. Egyptian's first medical text "The Ebers Papyrus" contains 800 recipes and 700 drugs. The Chinese pharmacopoeia, Pen- Sao keng Mu, is the oldest record of herbal medicine.

Theophrastus (300 BC) a botanist whose treatise 'Inquiry Into Plants' influenced both botany and medicine for centuries is called the 'Father of Pharmacognosy' (Farooqi & Sreeramu 2001). In De Materia Medica, Dioscorides (64-120 AD) discussed in detail the identification, collection, adulteration and therapeutic uses of several thousands of plants.

History of traditional medicine in India

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurveda, Siddha, Unani, Homeopathy have been in existence for several centuries.

The earliest mention of the medicinal use of plants is found in the Rig Veda (4500-1600 BC). In Ayurvedha, the properties of various drugs have been given in detail. Susruta Samhita (1000 BC) contains a comprehensive chapter on therapeutics and Charaka Samhita, gives a remarkable description of the Materia Medica. Later

during the Buddhist period, the medicinal plants were cultivated. Contacts with Greece and Rome, and later with Arabia and Persia, contributed to the enrichment of the Indian Materia Medica and a large number of vegetable and other products came into use for the treatment of diseases.

IMPORTANCE OF HERBAL MEDICINE [5]

Medicinal plants are used at the house hold level to improve the health of the family members.

These herbs are used in the various forms.

- Food for growth
- Food and herb as medicine

REASONS FOR THE USE OF NATURAL PRODUCTS [6]

They provide a number of extremely useful drugs that are difficult, if not impossible, to produce commercially by synthetic means. They include alkaloid of poppy, ergot and solanaceous.

Natural sources also supply basic compounds that may be modified slightly to render them more effective or less toxic. The numerous variations of the morphine molecule serve as example here.

Utility of natural products as prototypes or models for synthetic drug processing physiological activities similar to the original drug. Procaine and similar local anesthetics are commonly cited as representatives of this category.

Other important application is that the natural products contain compounds that demonstrate little or no activity themselves but which can be modified by chemical or biological methods to produce potent drugs not easily obtained by other methods.

APPLICATION OF HERBAL MEDICINE [7]

- Herbal medicines have long history of use and better patient tolerance as well as acceptance
- Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicines for the world growing population.
- Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity
- The cultivation and processing of medicinal herbs and herbal products is environmental friendly
- Cost-effectiveness-prescription drugs cost much more money than herbal medicines
- Lower side effects herbal medicines are generally a far heal their solution than prescription drugs due to potential harmful side effects caused by unpredicted body chemistry interactions.
- Obesity is the cause of many of the health problems. Herbal medicine can help to reduce excess weight and regulate appetite.

Traditional medicine vis-à-vis modern medicine [10]

There is a difference in the mode of action of traditional herbal medicine (THM) and modern synthetic medicine (MSM). Whereas the latter attacks the disease directly and helps the patient through the destruction of the disease, the former approaches the patient directly, over the head of the disease and destroys it by a process of reinforcement or bolstering up of the patient's body resistance and immune system. Also, modern medicine attacks the target blindly, thus affecting several

related metabolic systems of the body including the immune system. Herbal medicine works selectively and gently without disturbing the other systems of the body.

Isolation and Characterization of Active Principles [11,12]

The work of isolation of active principles from medicinal plants and characterization can be traced to the beginning of 19th century. From crude drug Ma Huang (*Ephedra* spp) of China, ephedrine was isolated in 1887 and later introduced as drug in 1925. Likewise, from opium (*Papaver somniferum*), morphine was isolated in 1804 and introduced as drug in 1818, from *Cinchona* spp. of Peru, quinine was isolated in 1820 and introduced as drug in 1825. emetine was isolated in 1894 and introduced as drug in 1912.

Large number of drugs from medicinal plants were discovered and introduced in modern pharmacopoeias during 1850-1950. Some of the important crude drugs are Belladonna (*Atropa belladona*), Cascara (*Rhamnus purshiana*), Digitalis (*Digitalis purpurea*), Rauwolfia (*Rauwolfia serpentina*). From 1950 onwards, there was research of ethno therapeutics in search of new drugs from higher plants and marine flora. During this period there was outstanding contribution of drugs from higher plants like reserpine, deserpidine and rescinnamine from *Rauwolfia serpentina*; and vinblastine and vincristine from *Catharanthus roseus.*

POLYCYSTIC OVARIAN DISEASE

Polycystic ovarian syndrome or Polycystic ovarian disease (PCOS or PCOD) in humans is also known as the Stein - Leventhal syndrome. It is a hormonal endocrine disorder of child bearing age recognized as the primary cause of infertility.

Polycystic ovarian disease is a heterogenous, multisystem endocrinopathy in women of reproductive age with the ovarian expression of various metabolic disturbances and a wide spectrum of clinical features such as obesity, menstrual abnormalities and hyperandrogenism. Women (5% - 10%) of reproductive age are affected by PCOD.

Long term consequences include increased risk of endometrial cancer, type 2 diabetes mellitus, dyslipidemia, hypertension and cardiovascular disorders. The etiology of PCOD is not clearly understood, but lipid imbalance, oxidative stress, insulin resistance and genetics are some of the contributing factors.

PCOD is frequently diagnosed by gynaecologists and it is therefore important that there is a good understanding of the long-term implications of the diagnosis in order to offer a holistic approach to the disorder. PCOD is characterized by multiple small ovarian cysts less than 1cm, LH is raised and LH/FSH ratio is ≥2. Current incidence of PCOS (5-6%) is fast increasing lately due to change in lifestyle and stress. Amongst infertile women, about 20% infertility is attributed to anovulation caused by PCOD.

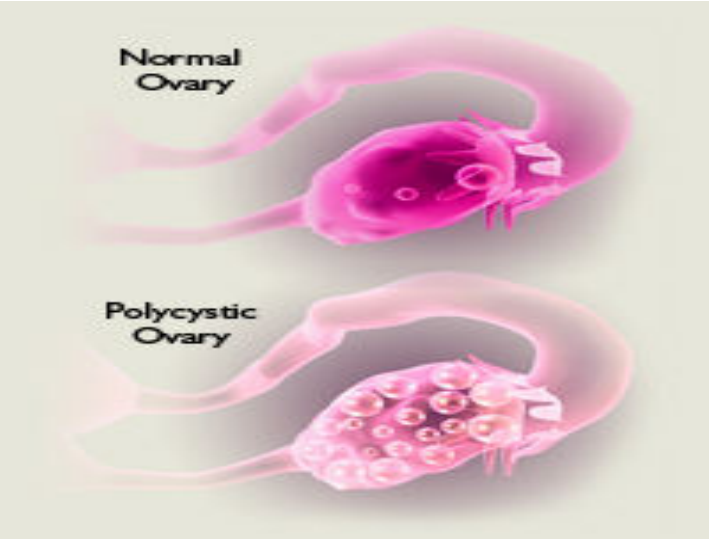


Fig. 1 Normal Ovary and Polycystic Ovary

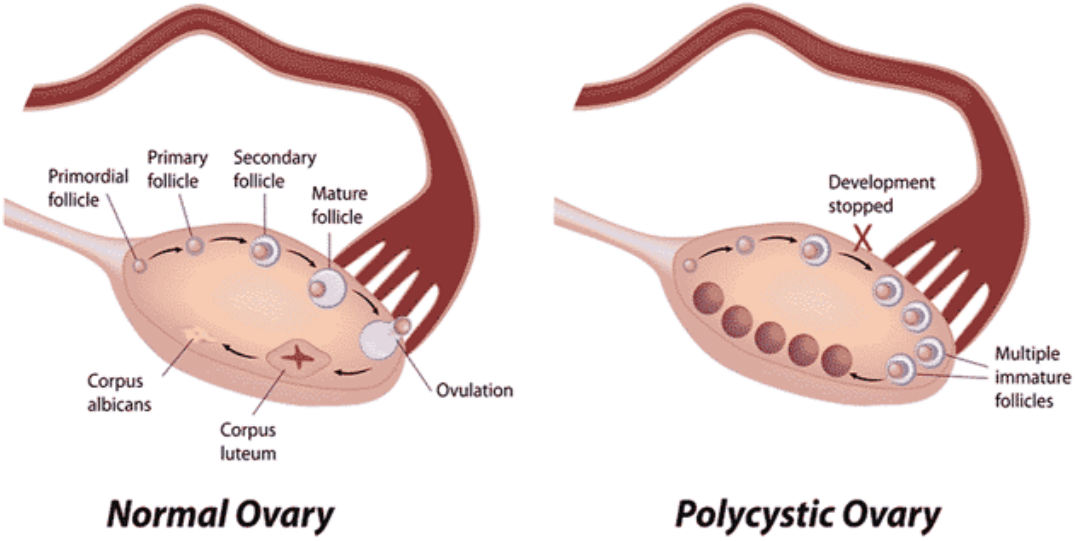


Fig. 2 Pathological difference between Normal Ovary and Polycystic Ovarian Disease

SYMPTOMS

Common symptoms, signs & metabolic abnormalities of PCOD [13]

Not all women with PCOD share the same symptoms

1) Oligomenorrhea or amenorrhea, and / or irregular bleeding.
2) Male pattern baldness or thining of scalp hair, Hirsutism involving face, chest, stomach, back, thumbs or toes.
3) Acne vulgaris – moderate to severe (treatment resistant / cystic).
4) Acanthesis nigricans (patches of thickened, dark brown or black skin) on the neck, arms, breasts or thighs.
5) Skin tags in the armpits or neck area.
6) Depression.
7) Sleep apnea syndrome.
8) Overweight or obesity, usually central.
9) Insulin resistance glucose intolerance and type II diabetes.
10) Hyperlipidemia.
11) Nonalcoholic fatty liver disease.
12) High blood pressure.
13) Anovulation and infertility.
14) High risk of coronary artery disease.
15) Prothombotic state.

CAUSES [13 - 21]

While exact cause of PCOD is unknown, doctors believe that hormonal imbalances and genetics play a role. Women are more likely to develop PCOD if their mother or sister also has the condition. Over production of the hormone androgen may be another contributing factor. Androgen is a male sex hormone that women's bodies also produce. Women with PCOD often produce higher-than-normal levels of androgen. This can affect the development and release of eggs during ovulation. Excess insulin (a hormone that helps convert sugars and starches into energy) may cause high androgen levels.

Fig. 3 Symptoms of PCOD

PATHOLOGY [18,22]

The ovaries are enlarged. Ovarian volume is increased ≥10 cm^3. Stroma is increased. The capsule is thickened and pearly with in color. Presence of multiple (≥12) follicular cysts measuring about 2 – 9 mm in diameter are found crowded around the cortex.

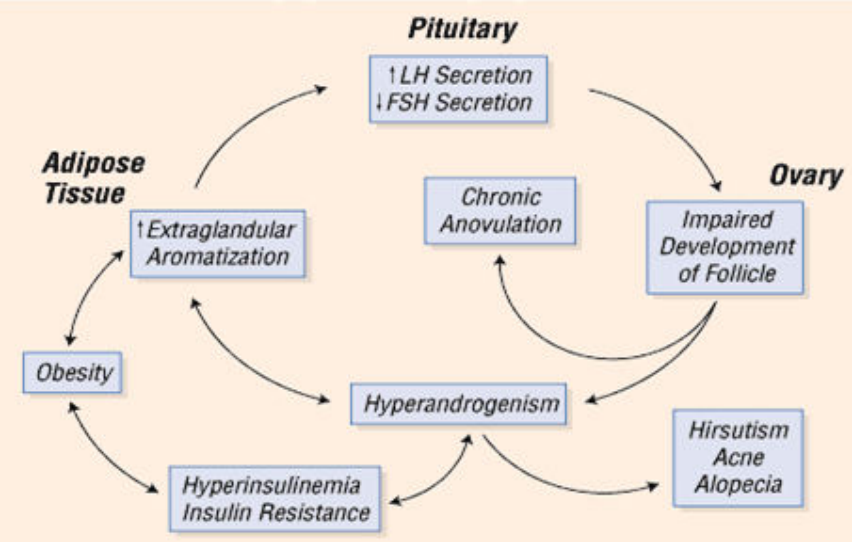


Fig 4. Pathophysiological changes of PCOD

MORPHOLOGY [18]

The ovaries are usually twice normal size and have a smooth, grey-white outer cortex studded with subcortical cysts 0.5 to 1.5 cm in diameter. On histologic examination, there is a thickened fibrotic superficial cortex beneath which are innumerable follicle cysts associated with hyperplasia of the theca interna (follicular hyperthecosis), Corpora lutea are frequently but not invariably absent.

CLINICAL FEATURES [26-29]

The pathogenesis appears to be initiated in utero or early adolescents period. Early adrenarche in the form of early pubertal hair and early menarche is observed in a few girls. Menstruation for a couple of years may be normal, but clinical features of PCOD develop early with Oligomenorrhoea (87%) or with a short period of am enorrhoea (26%) followed by prolonged or heavy periods (a common compliant in a majority of cases). Dysmenorrhoea is absent.

In the reproductive years, infertility accounts for about 20% cases. This is due to anovulatory cycles. During pregnancy, if the women conceives, carbohydrate intolerance, diabetes and hypertension may develop. Pregnancy loss occurs in 20-30%. Hyperandrogenism appears in the form of acne (30%) and hirsutism. Facial hair appears over the upper lip, chin, breasts and thighs. Baldness is sometimes noted, but virilism does not develop.

Family history of diabetes and hypertension should be asked. Excessive exercise, history of tuberculosis and thyroid are important in menstrual disorder.

Table 1 : FEATURES OF POLYCYSTIC OVARIAN DISEASE[13]

MECHANISM	MANIFESTATIONS
Pituitary dysfunction	High serum LH High serum prolactin.
Anovulatory Menstrual cycles	Oligomenorrhoea, Secondary amenorrhoea, Cystic ovaries, Infertility.
Androgen excess	Hirsutism, Acne
Obesity	Hyperglycemia, Elevated androgen
Insulin resistance	Dyslipidaemia, Hypertension

EXAMINATION [30-39]

- Obesity, especially waistline. Waist over hip ratio >0.85 is abnormal; 50% women are obese.
- Body mass index between 25 and 30 – overweight; and above 30 – obesity.
- Thyroid enlargement.
- Hirsutism and acne.
- Hyperinsulinaemia which may manifest as acanthosis nigra (5%) over the nape of the neck, axilla and below the breasts; 75% obese women reveal hyperinsulinaemia.
- Blood pressure in obese women.

Pelvic findings are normal, and it is not common to palpate the enlarged ovaries.

For the diagnosis of PCOD, the Rotterdam criteria (2003) suggests that at least two out of three criteria should be present. These criteria are:

- Oligo / Amenorrhoea, Anovulation, Infertility
- Hirsutism – Acne
- Ultrasound findings.

DIAGNOSIS [31-39]

The diagnosis of PCOD is traditionally made on clinical history and endocrine assessment and there is now an ongoing discussion on the merits of classifying women with the disorders on the basis of endocrine and metabolic criteria versus ovarian Ultrasound based criteria. In many cases, PCOD may be identified solely on the ultrasonographic morphology of the ovaries, however the metabolic disorder may be present in a women with normal-appearing ovaries, and women with ovaries

profoundly suggestive of PCOD on ultrasound examination may appear to be phenotypically normal.

There is no specific test for PCOD but your doctor will consider your symptoms and usually complete a physical examination, blood tests and a transvaginal ultrasound.

Physical examination

The doctor will ask numerous questions about your menstrual cycle, symptoms, weight and examine you for physical signs of PCOD, e.g. – acne, excess hair growth and darkened skin.

Blood tests

Blood may be tested for high cholesterol, blood sugar levels (insulin resistance) and for changes in LH (luteinizing hormone) or FSH (follicle stimulating hormone).

Transvaginal ultrasound

A long term slender probe is inserted into the vagina to determine the presence of ovarian cysts or enlarged ovaries and also to examine the reproductive organs or any irregularities. If would prefer not to have a vaginal scan, the doctor may conduct an ultrasound of abdomen – done externally while you have a full bladder.

INVESTIGATIONS [35-39]

Ultrasound is diagnostic of PCOD

- It confirms the enlarged ovaries, their size and increased stroma. Ovarian volume will be more than 10 mm.
- It shows 12 or more small follicles each of 2-9 mm in size placed peripherally.
- It rules out ovarian tumor.
- It shows endometrial hyperplasia if present.

- Hormonal study mentioned earlier is not performed routinely, but specific hormonal studies are undertaken in a women as and when required. All hormonal studies are not needed as a routine.
- Thyroid function tests in obese woman.
- Laproscopy is reserved for therapeutic purpose, now that the diagnosis can be confirm ultrasound findings. Laproscopy reveals enlarged bilateral ovarian cysts.

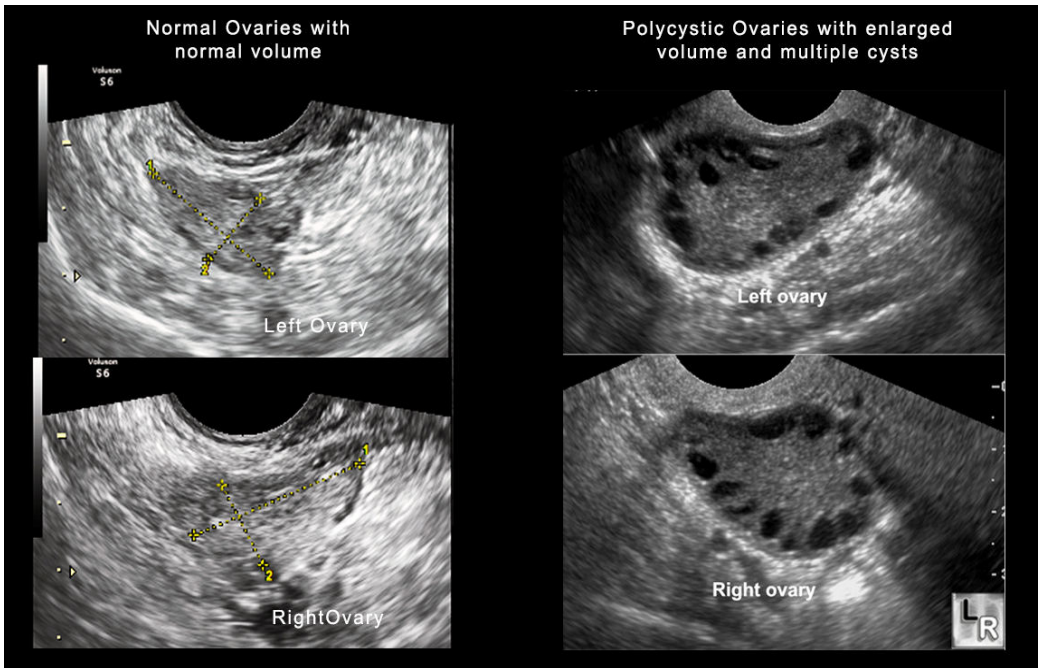


Fig 5. Ultrasound examination of PCOD

PHARMACOTHERAPY

This includes many agents which may be beneficial in addressing the individual components, however the most popular theraphy now a days is insulin sensitizers, as this is supposed to break the root cause of syndrome, the insulin resistance.

Insulin sensitizers

Metformin

A biguanide that primarily inhibits hepatic gluconeogenesisand lipogenesis and also enhances peripheral glucose uptake, at doses of 1000 – 2500 mg daily, appears valuable in increasing menstrual cyclicity and pregnancy rate. Metformin is preferred given its long history of safe use in PCOD.

Thiazolidinediones are associated with weight gain, fluid retention and heart failure.

Clomiphene citrate

This is an oral oestrogen antagonist that raises circulating concentrations of FSH and induces follicular ultrasound examination and luteal progesterone level. Combination of clomiphene citrate and metformin has been used successfully in a cubset for ovulation induction. Clomiphene with dexamethasone improves fertility rate.

Gonadotrophin treatment

Patients start on low dose recombinant FSH administered subcutaneously. Human chronic gonadotrophin is given when one follicle reaches 16 – 20 mm in size, multiple gonadotrophin cycles may be required to achieve pregnancy.

In – vitro fertilization

Ovulation induction by a skilled reproductive endocrinologist is preferable to *in – vitro* fertilization because of the risks of hyper – stimulation and multiparity with the latter procedures. As clomiphene citrate rapidly blocks steroid negative feedback with 10% multiparity, whereas metformin gradually reduces hyperinsulinemia with low multiparity. Clomiphene citrate may be preferable when time to conceive is essential.

Surgical treatment

Ovarian wedge resection is the surgical removal of part of an ovary. This is done to help regulate menstrual cycles and start normal ovulation. It is rarely used now because of the possibility of damaging the ovary and creating scar tissue.

Laproscopic ovarian drilling is a surgical treatment that can trigger ovulation in women who have PCOD and who have not responded to weight loss and fertility medicine. Electrocautery or a laser is used to destroy portions of the ovaries.

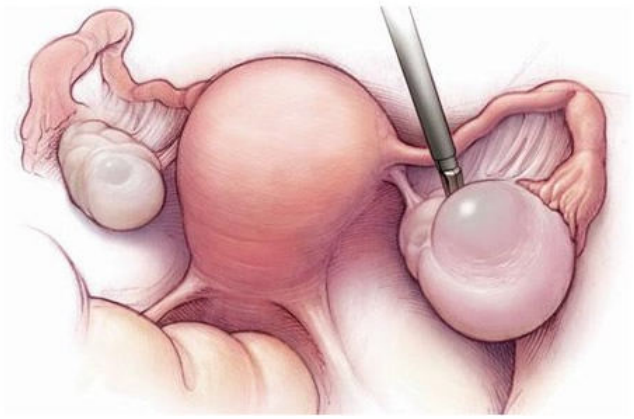


Fig. 6 Ovarian drilling

RISK FACTORS OF PCOD [37-38]

Cardiovascular Disease

They should hence be regularly monitored and advised to consume less fat and cholesterol. PCOD is characterized by endothelial dysfunction and resistance to the vasodilating action of insulin. An increased risk of myocardial infarction in PCOD women than age – matched controls has also been reported.

Obesity

Obesity is also a feature observed and estimated to effects 50% of PCOD women, classically presented in patients with upper body obesity which has been

associated with menstrual disturbances. It amplifies biochemical and clinical abnormalities of PCOD. Previously obesity was thought to be the cause of PCOD but now understood as a modifier of the condition.

Infertility

Infertility is the complicating feature of PCOD is the effects it has on ovulation and fertility with >75% of women with anovulation infertility and treatment is based upon the patient's characteristics.

Complication of PCOD

Endometrial cancer

Recent interest in the long term risks of PCOD has also focused on its possible associations with endometrial cancer. Prolonged anovulation which characterizes the syndrome is considered to be the main mechanism responsible for continual unopposed secretion of estrogens and consequent increased risk of endometrial carcinoma. The known factors which increase the risk of developing endometrial cancer are obesity, long term use of unopposed oestrogens, nulliparity, infertility, hypertension and diabetes.

Complications in pregnancy

Women with PCOD have a greater risk of complications viz. Gestational diabetes, pre – eclampsia, pre – term labor, small for gestational age, pregnancy induced hypertension, spontaneous abortions.

Sleep Apnoea

Women with PCOD have increased Sleep Disordered Breathing (SDB) and daytime sleepiness. Depression there was a higher prevalence of depression in PCOD patients associated with higher body mass index and greater insulin resistance.

NEED FOR NATURAL SOURCES AS POLYCYSTIC OVARIAN DISEASE

Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate suggests that about 80% of people in developing countries still rely on traditional medicine based largely on species of plants and animals for their primary health care.

The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications.

Table – 2 MEDICINAL PLANTS USED FOR POLYCYSTIC OVARIAN DISEASE[41]

S.No	Botanical name	Part used	Family	Chemical constituent	Medicinal uses
1.	*Aloe barbadensis*	Aloe juice	Liliaceae	Aloin	Dysmenorrhoea, menstrual suppressions.
2.	*Curcuma longa*	Rhizome	Zingiberaceae	Cucumin	Decrease the cholesterol level.
3.	*Erythrina variegata*	Leaves	Fabaceae	Steroids	Emmenagogue.
4.	*Astragalus officinalis*	Flower	Fabaceae	Polysaccharides	Insulin resistance
5.	Cinnamomum zylanicum	Bark	Lauraceae	Essential oil	Decrease insulin resistance.
6.	*Saraca indica*	Leaves & Flower	Fabaceae	Tannin	Uterine tonic
7.	Glycyrrhiza glabra	Root	Leguminosae	Glycyrrhizic acid	Reduce serum testerone.
8.	Lepidium sativum	Seeds	Curciferaceae	Oestrogenic activity	Emmenagogue.
9.	Linum usitatissiumum	Seeds	Linaceae	Chlorogenic acid	Reduce the hirsutism
10.	Tribulus terrestris	Fruit	Zygophyllaceae	Alkaloids, fixed oil	Hypoglycemic, hypolipidemic, diuretic.

Literature reveals that the above mentioned plants are used in Polycystic Ovarian Disease. The plant *Erythrina variegata* Linn., claimed to be useful for Polycystic Ovarian Disease has not been evaluated scientifically.

REVIEW OF LITERATURE

Pharmacognostical Review

1) **Verma et al (2016)** Phyto – Pharmacognostical investigations and evaluation of anti – inflammatory and sedative hypnotic activity of the leaves of *Erythrina variegata* lam[41].
2) **Ramila Devi et al** (2011) characteristics of Pharmacognostical significance of *Erythrina variegata* var Bark[42].
3) **Kumar et al (2010)** *Erythrina variegata* linn: A review on Morphology, Phytochemistry and Pharmacological aspects[43].

Phytochemical Review

4) **Subramanian et al (2016)** Phytochemical screening and HPTLC Fingerprinting analysis of ethanolic extract of *Erythrina variegata* L.flowers[44].
5) **Valli et al (2015)** *Erythrina variegata* leaves extract Assisted Synthesis of Titanium Dioxide Nanoparticles in an Ecofriendly Approach[45].
6) **Valli et al (2015)** Molecular properties and bio – activity of alkaloids in *Erythrina vaiegata* leaves to find lead compound[46].

Pharmacological review

7) **Jagdish et al (2015)** reported the Hypoglycemic and Hypolipidemic activity of Root extracts of *Erythrina variegata* in Alloxan induced diabetic rats[47].
8) **Mangathayaru et al (2014)** reported the Estrogenic effect of *Erythrina variegata* L. in perpubertal female rats[48].
9) **Murugalakshmi et al (2014)** reported the Analgesic and Anti – inflammatory activities of *Erythrina variegata* leaves extracts[49].
10) **Sahoo et al (2012)** reported the *In – vitro* analysis of antimicrobial activity of stem extracts of *Erythrina variegata* L : A useful medicinal plant[50] .
11) **Baskar et al (2010)** reported the Anti – cancer activity of methanol extract of root bark of *Eytrina variegata* Linn[51].
12) **Ajay kumar et al (2010)** *In – vitro* antioxidants and anti – inflammatory activities of *Erythrina variegata* bark[52].

ETHNOBOTANICAL SURVEY

Ethnobotanical Survey of *Erythrina variegata* Linn.,

PLANT PROFILE [53-64]

Plant name	:	*Erythrina variegata* Linn.,
Common name	:	Indian coral tree
Synonym	:	*Eryhtrina indica*
Family	:	Fabaceae

VERNACULAR NAMES [59-65]

Tamil	:	Kaliyana Murungai
English name	:	Indian coral tree, Moochy wood tree.
Sanskrit	:	Mimbataru, Manalia.
Bengali	:	Palita-madar, Palidhar.
Hindi	:	Ferrud, Mandar, Pangra.
Telugu	:	Barijiamu , Modugo, Badchipa-chettu.
Gujarati	:	Panarawas, Pararoo.
Malayalam	:	Mooloomogrikah.

TAXONOMIC CLASSIFICATION [53-54]

Botanical name	:	*variegata*
Family	:	Fabaceae
Sub family	:	Papilionaceae
Kingdom	:	Plantae
Divisions	:	Mangoliophyta
Classs	:	Mangnolipsida – Dicotyledons
Genus	:	*Erythrina* L- coral tree
Species	:	*Erythrina variegta* L.

Fig 7. *Erythrina variegata* Linn., tree

Table 3. Erythrina variegata Linn., Leaves

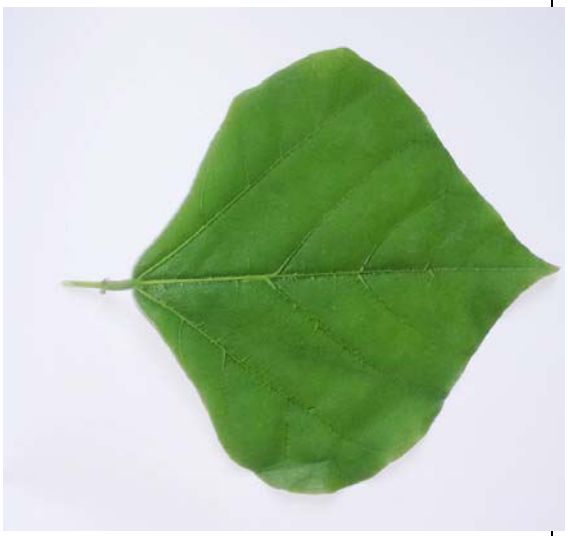
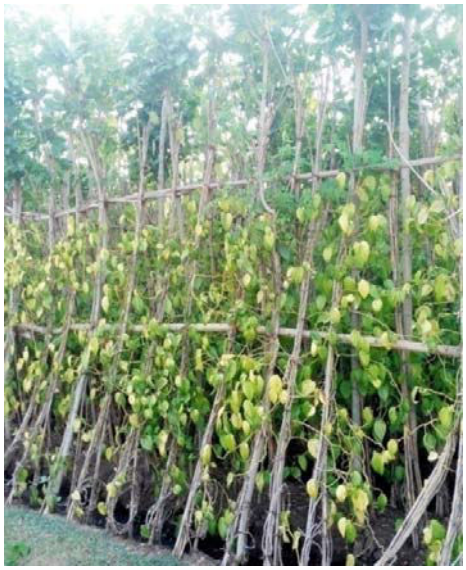
Size of the matured leaves	Size of the young leaves
	
Dorsal surface	**Ventral surface**
	

Table 4. Habitat and Height of the plant

Habitat of the plant	Height of the plant
	

PLANT DESCRIPTION [53]

Erythrina variegata Linn., belongs to the family *Fabaceae* commonly known as the 'Indian coral tree' in Asia or 'tropical coral' in the Pacificis an important multipurpose tree species and thrives well in arid and semiarid region. *Erythrina* variegata Linn., is found in many tropical and subtropical regions.

DESCRIPTION [54]

Erythrina variegata Linn., is a medium – sized, spiny, deciduous tree normally growing to 6 – 9 m (occasionally 28 m) tall and 60cm.

Table 5. Plant Description

S.NO	PART	DIAGRAM	FEATURES
1	LEAVES		Leaves trifoliate, alternate, bright emerald – green, on long petioles 6 – 15 cm, rachis 5 – 30 cm long, prickly; leaflets smooth, shiny, broader than long, 8– 20 by 5–15cm, ovate to acuminate with an obtusely pointed end.
2	BARK		Bark is smooth and green when young, exfoliating in papery flakes, becoming thick, corky and deeply fissured with age.
3	FLOWER		Flowers in bright pink to scarlet red, erect terminal racemes 15 – 20 cm long; stamens slightly protruding from the flower.

4	STEM		*Erythrina variegata* is a compact shrub with prickly stems.
5	FRUIT		Fruit a cylindrical torulose pod, green, turning black and wrinkly as they ripen, thin – walled and constricted around the seeds
6	SEED		Seeds are 1 – 8 smooth, oblong, dark red to almost black seeds per pod

ETHNOBOTANICAL USES [40-51]

Different parts of the plant have been used in the traditional system of medicine as a nervine sedative, collyrium in opthalmia, anti-asthmatic, antiepileptic, antiseptic and as an astringent.

LEAVES

- They are applied externally for depressing veneral buboes and for relieving pain in joints'.
- The leaves are laxative, diuretic, anthelnmintic, galactagogue, emmenagogue.
- The fresh juice of the leaves is used for the relief of ear ache and as anodyne in tooth-ache.
- The juice is also used for killing worms in sores. The paste of the leaf is applied externally to relieve pain in hernia and hydrocele.

- The paste of leaf is applied for ulcers.
- Leaf juice said to cured long - standing dysmenorrhoea and also removed sterility in fatty women by gradually reducing fat and produce natural menstrual flow, if the medicine is being continued for two or three months

BARK

- Bark is boiled for preparation of tears and baths for skin diseases and impetigo.
- Cooked rice soaked in steam bark extract for a day and then made into paste is given internally in menorrhagia.
- A decoction of the bark scrapings with lemon juice for the treatment of infertility. The juice of the bark and that of *Moringa oleifera* lam mixed with common salt is given internally in acute conditions of stomachache.
- Haemagglutinating activity has been reported in seed extract which specifically inhibits galactose and lactose.

SEEDS

- The seed trypsin inhibitors inhibit plasmin involved in the blood coagulation and fibrinolytic system.
- The root extracts exhibits antimicrobial activity *in vitro* against Staphylococcus aureus and Mycobacterium megmatis.

RATIONALE FOR SELECTION

- The plant *Erythrnina variegatea* Linn., belonging to the family Fabaceae was selected for the present work.
- The Ethnomedicinal uses were a nervine sedative, collyrium in opthalmia, anti-asthmatic, antiepileptic, antiseptic, astringent, toothache, emmenagogue and galactagogue. *Erythrina variegata* Linn. is an adulterant to the ayurvedic drug Rohitaka.
- The Polycystic Ovarian Disease activity was not scientifically validated.
- So the leaves of the plant *Erythrina variegata* Linn., was selected for evaluation of Polycystic Ovarian Disease activity.

AIM AND OBJECTIVES

- To objective of the study is to explore the Phamacognostical, Phytochemical and Pharmacological profile on the leaves of *Erythrina variegata* Linn.,
- Collection and Authentication of the plant specimen.
- Establishing Pharmacognostical profile of the plant.
- Extraction of plant material by using, Soxhlet apparatus with solvents of increasing polarity such as Petroleum ether > Chloroform > Ethyl acetate > Ethanol and Aqueous (cold maceration).
- Phytochemical screening and determination of bioactive constituents.
- Evaluation of Polycystic Ovarian Disease activity by *In – vitro* and *In – vivo* models.
- Isolation of compound using Column Chromatography.
- Survey of Polycystic Ovarian Disease.

PLAN OF WORK

PHARMACOGNOSTICAL STUDIES

- Macroscopical studies
- Microscopical studies
- Powder Microscopy
- Quantitative Microscopy- Leaf constants.
- Quantitative Microscopy -Linear Measurements of Calcium Oxalate Crystals
- Histo-chemical studies
- Determination of Physico-chemical constants.
    - Ash values
        - Total ash value
        - Acid insoluble ash value
        - Water soluble ash value
        - Sulphated ash value
    - Extractive values
        - Alcohol soluble extractive value
        - Water soluble extractive value
        - Ether soluble extractive value
    - Determination of crude fiber content
    - Determination of Loss on Drying
    - Determination of Swelling index
    - Determination of Foaming index
    - Determination of Volatile oil content
- Qualitative and Quantitative Estimation of Heavy metals and Inorganic element

II. PHYTOCHEMICAL STUDIES

- Preparation of Extracts
- Preliminary Phytochemical screening of extracts
- Quantitative Estimation of Phytoconstituents
- Fluorescence analysis of Plant powder and extracts
- Thin Layer Chromatography.
- High Performance Thin Layer Chromatography-Finger Print profile.

III. SELECTION OF ACTIVE EXTRACT

- *In – vitro* DPPH assay
- *In – vitro* Hydrogen Peroxide Scavenging Assay.

IV. PHARMACOLOGICAL STUDIES

- Acute toxicity studies
- *In vivo* Letrazole induced Polycystic Ovarian Disease
    - Body weight
    - Food intake
    - Vaginal exfolitative cytology
    - Blood Glucose level
    - Lipid profile
    - Reproductive organ weight (Uterus and Ovary).
    - Histopathological study of uterus and ovary

IV. ISOLATION OF COMPOUND USING COLUMN CHROMATOGRAPHY

- Infra red spectroscopy
- Gas chromatography-Mass spectroscopy studies

V. SURVEY OF POLYCYSTIC OVARIAN DISEASE

MATERIALS AND METHODS

PHARMACOGNOSTICAL STUDIES

Collection of Plant Material

The leaves of *Erythrina variegata* Linn., was collected from Karur district Pillapalayam village, Tamilnadu in August – 2016.

Identification and Authentication of Plant Material

The collected specimens was botanically identified and authenticated by Dr. R. Jayaraman Ph.D., Director, Institute of Herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai-45. It was identified as *Erythrina variegata* linn Fabaceae family.

MACROSCOPY

Fresh leaf of *Erythrina variegata* of were collected and different organoleptic features viz shape, size, colour, type, odour, taste were observed. These parameters are considered useful in the qualitative control of the crude drug and evaluated as per standard WHO guidelines.

MICROSCOPY [65-79]

Staining method

Fixation of plant material

The sample or leaves was cut fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 90ml of 70% Ethanol). The specimen was dehydrated after 24 hours of fixing. The seeds were graded with series of tertiary butyl alcohol, as per the standard method.

Infiltration of the specimen

It was carried out by gradual addition of 58 – 60^0 C of melting pointed paraffin wax until Tertiary butyl alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 – 12µ. Dewaxing of the sections were done by customary procedures. The sections were stained Toludine blue. Since toludine blue is a polychromatic stain, the sections were stained as per the method published by O'Brein et al. The staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to subrein, violet to mucilage and blue to the protein bodies. Whenever necessary sections were also stained with safranin, fast green and iodine for starch.

PHOTO MICROGRAPHS

Microscopic descriptions of tissues were supplemented with photo micrographs whenever necessary. Photographs of different magnifications were taken with Nikon lap photo 2 microscopic units. For normal observations bright field was used.

For the study of crystals and lignified cells, polarized light was employed. Since, these structures have birefringent property under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books.

POWDER MICROSCOPY

Shade dried leaf of *Erythrina variegata* were powdered well, then the powder was passed through sieve no.60 and used or powder analysis and organoleptic characters such as nature, colour, odour and taste were studied. Powder analysis using

various staining reagents viz 1% phloroglucinol in 90% ethanol, conc Hcl and N/50 iodine. Slide were observed under the microscope.

QUANTITATIVE MICROSCOPY [81]

LEAF CONSTANT:

DETERMINATION OF STOMATAL NUMBER AND STOMATAL INDEX

Stomatal number is the number of stomata per sq.mm of epidermis of the leaf. Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawing to scale. Draw a square of 1mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata, also the number epidermal cells in each field. Calculate the stomatal index and stomatal number. Determine the values for upper and lower surface (epidermis) separately.

DETERMINATION OF VEIN ISLET NUMBER AND VEINLET TERMINATION NUMBER:

For studying the venation pattern, leaf was cleared with 5% Sodium hydroxide solution and epidermal peeling by partial maceration employing Jeffrey's maceration were prepared. Glycerin mounted on temporary preparations were made for macerated materials and used for the study of vein islet number and vein termination number.

Linear Measurements: Measurement of Calcium oxalate Crystals

Little quantity of powdered drug was cleared with 5% caustic soda and transferred to a watch glass. The calcium oxalate crystals were stained using phloroglucinol on a glass slide and mounted in glycerin, observed under low power. The intact stained calcium oxalate were focused and the entire length and width of the calcium oxalate crystals was measured by rotating the scale of eye piece micrometers. Each value was multiplied by the calibration factor. Minimum, average and maximum measurements of 25 observations were taken.

HISTOCHEMICAL STUDIES [82-83]

Portions of fresh fruit peels of the plant of *Erythrina variegata* Linn., were used. The peels were soaked in water before taking the sections. The sections were stained using specific reagents (N/50 iodine, dilute ferric chloride, phloroglucinol and con.hydrochloric acid, picric acid, ortho toludine blue and dragendroff reagent) to observe and locate starch, lignin, tannin, protein, flavanoid and alkaloid respectively as per the protocols. The stained sections were then washed in water to remove the excess stain and observed under a microscope.

PHYSIO-CHEMICAL CONSTANTS [84 – 86]

Shade dried powdered plant materials of the fruits of *Erythrina variegata* Linn., was used for the determination of the physio chemical constants in accordance with the WHO guidelines.

DETRMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or delibility added to it, as a form of adulteration.

Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which complies of inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination of the quality and purity of the crude drug in the powdered form.

TOTAL ASH:

Total ash method is designed to measure the total amount of material remaining after ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0c until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

WATER SOLUBLE ASH:

The difference in weight between the total ash and the residue after treatment of the total ash in water.

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter were collected in a ashless filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 450^0. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

$$\text{Water soluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

$$\text{Acid insoluble ash} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These test are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible are heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly chared. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25^{o} until all black particles have disappeared. Crucible are allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. This process is repeated until two successive weighing differ by more than 0.5 mg.

$$\text{Sulphated ash} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of phyto constituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of water soluble extractive

5gm of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for rest eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent

and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value was calculated with reference to the air dried drug.

$$\text{Water soluble extractive value} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

Determination of alcohol soluble extractive

5gm of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

$$\text{Alcohol soluble extractive} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

Determination of ether soluble extractive

2gm of powdered sample was accurately weighed and extracted with anhydrous diethyl ether in a continuous extractive apparatus for 20hours. The ether solution was transferred to tarred porcelain dish and evaporated spontaneously, dried over phosphorous pent oxide for 18hours and the total ether extract was weighed. The extract was heated gradually and dried at 105°C to constant weight. The loss in weight represents the volatile portion of the extract.

$$\text{Volatile ether soluble extractive} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

LOSS ON DRYING

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Use of high speed mill in preparing the samples are avoided. The sample in the tarred evaporating dish were placed in the drying chamber (105°C) for 5 hours and weigh. Drying and weighing is continued every one hour interval until the difference between the two successive weight is not more than 0.25 percent. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air dried sample.

$$\% \text{ Moisture content} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

DETERMINATION OF FOAMING INDEX

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80-90°C for about 30min. It was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The tubes were stoppered and shaked in a length wise motion for 15 seconds, two shakes per second. Allowed to stand for 15min and the height of the foam was measured. The results are assessed as follows.

If the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula:

$$\text{Foaming index} = 1000/a$$

Where, a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

DETERMINATION OF SWELLING INDEX

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

DETERMINATION OF VOLATILE OIL IN THE DRUG:

Determination of the volatile oil in a sample is done by distilling the drug with a mixture of water and glycerine, collecting the distillate in a graduated tube in which the aqueous portion is automatically separated and returned to the flask and the volume of oil collected is measured.

The content of the oil is expressed as percentage v/w.

It is performed in the Clevenger's apparatus.

Procedure:

Weighed amount of sample is distilled with the 75 ml of glycerine and 175 ml of water in the one litre distilling flask with few pieces of earthen ware and one filter paper 15 cm cut into small head. Condenser is placed above the flask and water is allowed to run through the condenser. Heating is discontinued after 3 to 4 hours.

Volume of oil collected is read in the graduated tube. The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

Percentage of volatile content = $\frac{\text{Amount of volatile oil obtained}}{\text{Weight of peel}}$ X 100

QUALITATIVE AND QUANTITATIVE ESTIMATION OF HEAVY METALS AND INORGANIC ELEMENTS[87]

Plant minerals play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non essential elements which may be beneficial or harmful to living things. Non essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium, silver bring about toxic effects resulting in intoxification. Hence, qualitative and quantitative estimation of inorganic elements in the plant *Citrus maxima*. (J. Burm.) Merr. were carried out.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS

To the ash of the drug material 50%v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.

Calcium: Solution of calcium salts, when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute HCL.

Borate: The mixture obtained by the addition of sulpuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Potassium: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Carbonate: Carbonate, when treated with dilute acid effervescence, liberating carbon dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.

Sulphates: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS[88]:

Inductive coupled plasma-Optical emission spectroscopy (ICP-OES)

It is an excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly. This technique utilizes the plasma as an ion source or light emission source are capable of producing values.

QUANTITATIVE ESTIMATION OF HEAVY METALS BY ICP-OES:

Instrumentation parameters:

Instrument name: Inductive coupled plasma-Optical emission spectroscopy

Instrument Model: PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial

Detector system: Charge coupled detector, (UV-Visible detector which is maintaining at -40º C) to detect the intensity of the emission line.

Light source (Torch): Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow gem tip.

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to

the volume with distilled water. Blank solution was prepared as above without sample.

The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the calcium, sulphate, borate, silver, aluminium, copper, potassium, chloride by Inductively Coupled Plasma Emission Spectrometry.

PHYTOCHEMICAL STUDIES

PRILIMNARY PHYTOCHEMICAL SCREENING [89-91]

Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the Phyto constituents qualitatively and quantitatively.

Extraction

The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Erythrina variegata* was first extracted with Petroleum ether (60-80°C) in Soxhlet apparatus and then with solvents of increasing polarity like Chloroform, Ethyl acetate and Ethanol at 60 - 70ºC. They were then followed with maceration in aqueous solvent. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts

were recorded and preceded for further detailed phyto chemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING

The chemical tests for various Phyto constituents in the dried powder and extracts of leaves of *Erythrina variegata* Linn., were carried out as described below and the results were recorded.

Detection of Flavonoids

- Shinoda test :

  To the solution of extract, few piece of magnesium turnings and concentrated Hcl was added drop wise, pink to crimson red, occasionally green to blue color appears after few minutes indicates the presence of flavonoids.

- Alkaline reagent test :

  To the test solution few drops of sodium hydroxide solution was added, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids.

Detection of Alkaloids

- Dragendorff's reagent :

  The substance was dissolved in 5ml of distilled water, to this 5ml of 2M HCL was added until an acid reactions occurs, then 1ml of Dragendorff's reagent was added and examined for an immediate formation of an orange red precipitate.

- Mayer's reagent:

The substance was mixed with little amount of dilute hydrochloric acid and Mayer's reagent and examined for the formation of white precipitate.

- Wagner's reagent :

The test solution was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

Detection of Carbohydrates

- Molisch's test :

To the test solution few drops of alcoholic alpha napthol and few drops of conc. sulphuric acid were added through the sides of test tube, purple to violet color ring appears at junction.

- Fehling's test :

The test solution was mixed with Fehling's I and II and heated and examined for the appearance of red coloration for the presence of sugar.

Detection of Gum and Mucilage

- Small quantities of test substances was dissolved in 5 to 10ml of acetic anhydride by means of heat, cooled and add 0.05ml of concentrated sulphuric acid; it is examined for the formation of bright purplish red color.

Detection of Proteins

- Biuret test :

The sample was treated with 5-8 drops of 10% w/w copper sulphate solution, violet color is formed.

Detection of fats and fixed oils:

- Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

Detection of Steroids:

- Libermann Burchards Test:

The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc.sulphuric acid was added from the sides of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids and formation of deep red color indicates presence of tri terpenoids..

Detection of Glycosides

- Borntrager's test :

The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

- Modified Borntrager's test :

The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

. **Detection of Phenols**

- Ferric chloride test :

A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green color.

Detection of Saponins

- A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT[93-95]

TOTAL ALKALOID CONTENT

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2ml) of atropine standard solution were transferred to different separating funnels. Then 5ml of Ph 4.7 phosphate buffer and 5ml of BCG solution were added the mixture was shaken with 1, 2, 3 and 4ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust the volume with chloroform was measured at 470nm against blank prepared as above but without atropine.

TOTAL FLAVONOID CONTENT

Total flavanoid content was determined by calorimetric method, using quercetin as a standard. The test samples were individually dissolved in DMSO. Then the sample solution (150 µl) was mixed with 150 µl of 2% aluminium chloride. After 10min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435nm using spectrophotometer. Three replicates were made for each test sample. The total flavanoid content was expressed as quercetin equivalent in mg/gm extract (mg QRT/gm extract).

TOTAL PHENOLICS CONTENT (Folin – Ciocalteu's assay)

Total phenolic content of the extracts were determined using Folin – Ciocalteu's assay. 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted Folin Ciocalteu's reagent and 2.5ml of 7.5% sodium carbonate. After incubation at 40°c for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrophotometer. Three replicates were made for each test sample. Gallic acid was used as a standard and total phenolic content of the extract was expressed in mg of Gallic acid equivalents (mg GAE/g extract).

FLUORESCENCE ANALYSIS[92]

Fluorescence analysis was carried out in day light and in UV light. The leaf powder and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light.

CHROMATOGRAPHY [96-98]

Chromatography methods are important analytical tool in the separation, identification and estimation of components present in the plant.

THIN LAYER CHROMATOGRAPHY

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of

stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

TLC Plate Preparation

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase:

Solvent mixture was selected on the basis of the phyto constituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents. From the vast analysis, best solvent was selected which showed good separation with maximum number of components.

$$Rf = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

HIGH PERORMANCE THIN LAYER CHROMATOGRPHY [99]

The alcoholic extracts of leaf of the plant were subjected to HPTLC method for identification for the phytoconstituents.

Solvent system

The solvent system optimized for HPTLC was

Chloroform : Methanol : Formic acid : Water (65:25:10:5)

Application of sample

A small quantity of extract was dissolved in Methanol and sample was applied in precoated plate with the help of Linomet V applicator.

Development of Chromatogram

A rectangle twin trough glass chamber was used in the experiment. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth filter paper was placed in the glass chamber and was allowed to be soaked in the developing solvent. The moistened paper was pressed against the walls of the chamber so that it adheres to the walls. The chamber was allowed to saturate for 15min before use. The experiment was carried out at room temperature in diffused day light.

Procedure

The plate was dipped in a saturated chromatographic chamber containing the solvent system and was allowed to elute up to 8 cm and was air dried. The spots were scanned in CAMAG TLS scanner-3.

HPTLC of alcoholic extract of *Erythrina variegata* leaf

Sample	:	Alcoholic extract
Stationary phase	:	HPTLC precoated, with silica gel G 60 F254 as adsobent (Merck, Germany)
Mobile Phase	:	Chloroform : Methanol : Formic acid : Water (65:25:10:5)
Sample Concentration	:	10 mg/ml
Applied volume	:	5.0 and 10.0 µl
Size of the plate	:	10 × 10 cm
Developing chamber	:	Twin trough glass chamber
Mode of application	:	Band
Band size	:	5 mm
Separation technique	:	Ascending
Temperature	:	60^0 C
Saturation time	:	5 min
Scanning wavelength	:	254 nm/ 366nm
Scanning mode	:	Absorbance / Reflectance

SELECTION OF ACTIVE EXTRACT

IN VITRO ANTI OXIDANT ACTIVITY [103]

DPPH Assay: 1, 2-DIPHENYL-2-PICRYL HYDRAZYL RADICAL (DPPH)

Initial volume 0.1 mL of various concentrations of samples was mixed with 0.4 mL of 0.3M DPPH reagent prepared in ethanol. The mixture was shaken thoroughly and incubated in the darkness at room temperature for 30 min. The absorbance of the reaction was measured spectrophotometrically at 517nm, immediately after mixing and then after incubation as well. The scavenging effect of DPPH free radical was calculated by using the following equation.

% scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100

Where control is the absorbance of the blank (a reaction with all the reagents except the test extract), and absorbance of sample is the absorbance of the test extract. Tests were carried out in triplicates to obtain 50% inhibition (IC50). Using Butylated hydroxy Toulene.

HYDROGEN PEROXIDE SCAVENGING ASSAY (HPSA) [104-105]

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch. A solution of Hydrogen peroxide (2mmol/l\lt) was prepared in phosphate buffer (PH 7.4). Various concentrations of extracts (10-100µg/ml) were added to hydrogen peroxide solution (0.6ml). Absorbance at 230nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide.

% Scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100

PHARMACOLOGICAL STUDIES:

ACUTE ORAL TOXICITY STUDY (OECD 423 GUIDELINES)[47]

Literature survey showed that acute toxicity of the extracts was determined according to the OECD guideline No. 423. Male albino mice weighing 27-30 g were used for ethanolic Extract of *Erythrina variegata Linn.,* was given to four groups (n = 5) of animals each at 5, 50, 300 and 2000 mg kg-1 b.w. p. o. The treated animals were under observation for 14 days, for mortality and general behaviour. No death was observed till the end of the study. The test sample was found to be safe up to the dose of 2000 mg/kg. So, $1/10^{th}$ and $1/5^{th}$ of the dose (200 and 400mg/kg) were selected for this study.

***IN VIVO* EVALUATION OF LETROZOLE INDUCED POLYCYSTIC OVARIAN DISEASE**

The protocol for conducting the *In Vivo* study in female adult albino wistar rat was approved by the Institutional Ethical Committeee (ICE) of the Madras Medical College, Chennai – 600003 India.

Approval no : IAEC/MMC/06/2016. Dated : 21.11.2016

EXPERIMENTAL DESIGN [27, 106,107]

Materials and Methods

Plant extract

Ethanolic extract of leaves of *Erythrina variegata* Linn.,

Animal selection and procurement

Healthy young female wistar albino rats (weighing about 150 – 200 gm) were procured from the Madras Medical College animal house.

The procured animals were kept in a clean, dry polycarbonate cages and maintained in a well-ventilated animal house. The temperature of experimental animal room was maintained at 22ºC (± 3ºC) and the relative humidity was maintained from 50-60%. Lighting was artificially maintained for 12hrs dark and 12hrs light. All the animals were kept in the cages for at least 5days prior to dosing for acclimatization to the laboratory conditions. The animals were fed with standard pellet diet and water was given ad libitum. Before starting the dose, the animals were fasted overnight but allowed to access water.

PCOS induction

All the experimental animals except Control group, were orally administered with LETROZOLE at a dose of 1mg / kg dissolved in 0.5% Carboxy Methyl Cellulose (CMC) once daily for 21 days. Control group received vehicle only (0.5 % CMC). Vaginal Smears were collected daily and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS.

STUDY DESIGN

The study consisted of 30 female wistar albino rats equally divided into five groups designated as Group I (served as control group), Group II (served as PCOD induced group), Group III (served as standard group), Group IV and V served as treatment groups. Following LETROZOLE administration, standard group was administered with Metformin at a dose of 1 mg/kg in per oral route and treatmennt groups IV and V were administered Ethanolic extract of *Erythriva variegata* Linn., with the dose of 200mg/kg (Group IV) and 400 mg/kg (Group V) body weight respectively in 0.5% CMC per oral for 22 to day 52.

The animals received following treatment

A total of 30 Adult female wistar albino rats are divided into 5 groups of 6 animals each

Table 6. GROUPING OF ANIMALS

S.NO	GROUP	INDUCTION	TREATMENT for 30 days	NO OF ANIMALS
1	I Control	Normal saline orally	Normal saline orally	6
2	II Disease control	1 mg/kg Letrozole OD for 21 days	Normal saline orally	6
3	III Standard	1 mg/kg Letrozole OD for 21 days	Metformin 100 mg/kg orally	6
4	IV Test group (200 mg/kg)	1 mg/kg Letrozole OD for 21 days	200 mg/kg ethanolic extract orally	6
5	V Test group (400 mg/kg)	1 mg/kg Letrozole OD for 21 days	400 mg/kg ethanolic extract orally	6

EVALUATION PARAMETERS

- Body weight
- Food intake
- Vaginal exfoliative cytology
- Determination of blood glucose level
- Lipid profile
- Weight of Reproductive organ (Uterus and Ovary)
- Histopathological evaluation (Uterus and Ovary)

Body weight

Initial body weight of animals in all groups were determined on the first day. The body weight of different groups of rat were noted weekly for 2 months.

Food intake

100 g of weighed rat feed was given to six groups and the residue of food intake was weighed.

Vaginal exfoliative cytology

Smear technique – Lavage or Washing with saline or Water from pipette

A small amount (approximately 0.2ml) of saline or distilled water is drawn up into the pipette tip. The rat is held around the thorax, ventral surface uppermost, with one hand whilst the hand holding the pipette is used to restrain the tail, to provide additional support and help prevent the animal struggling.

The tip of the pipette is pushed gently into the entrance of the vagina to a depth of 2-5 mm and the fluid is flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. A small amount of the cell suspension is then expelled onto a labelled glass slide. Slides should be labelled with the female identification numbers; the data and study number may also be shown but as the smears are discarded immediately after reading this is not usually considered necessary as long as the tray holding the slides is identified appropriately.

Crystal violet stain was added to the slide to cover the smear. The slide was kept covered in petridish for 5 min. Distilled water was added to the crystal violet stain and gently rocked. A violet scum appeared on top of the slide. The slide was stained for 10 min in dilute crystal violet stain. The stained slide was dried and then washed in tap water. The washed slide was air dried and observed under the microscope in 40× objective.

Stages of estrus cycle in rat

During the study, vaginal smears were observed microscopically using Crystal violet stain for determination of estrus cyclicity.

4 stages of estrus cycle is seen in female rat

Proestrus

It is a preparatory phase to the next estrus phase. Vaginal smear shows nucleated epithelial cells. This stage is last for about 12 hrs.

Estrus

The vaginal smear shows 100% cornified epithelial cells. This stage is last for 9-15 hrs.

Metestrus

It follow after estrus stage, the vaginal smear shows many leucocytes with a few cornified cells. This stage is last for 20 hrs.

Diestrus

It is the longest phase, consists of mainly leucocytes in vaginal smear. This stage is last for 57 hrs.

Preparation of Blood Serum

After 21 days, PCOS control group and after 52 days, animals from other groups were fasted overnight and anaesthetized with diethyl ether. Blood was collected by retino orbital puncture then serum was separated by centrifugation and was used for estimation of blood glucose, lipid Profile .

Determination of Blood Glucose Level

The blood glucose level of each group were monitored at the end of the study.

Lipid Profile

Lipid profile which includes total cholesterol (TC), triglycerides TGs), low-density lipoprotein (LDL) and high density lipoprotein (HDL) were estimated by autoanalyzer microlab 200 using Ecoline-kits.

Weight of Reproductive organ

Uterus and ovaries were dissected out from each animal and weighed by a digital electronic weighing balance to evaluate the effect of the extract.

Histopathological evaluation

The isolated samples of ovaries and uterus in each group were selected for histopathological evaluation. The tissues placed in 10% buffered formalin. The fixed tissues were stained with Haematoxylin and Eosin. Slides were reviewed for the evaluation of histopathological changes like follicles, carpus luteum and cysts.

Statistical analysis

Results were expressed as Mean ± SEM. The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test. P values < 0.01 were considered as Significant.

COLUMN CHROMATOGRAPHY FOR ISOLATION AND IDENTIFICATION OF PHYTOCONSTITUENTS

Ethanol extract was selected for the isolation and identification of phytoconstituents.

A suitable column of 2.5 cm in diameter and 60 cm in length was selected and thoroughly washed with water, dried and rinsed with acetone and then dried completely. Little pure cotton was placed into the bottom of the column with the help of a big glass rod up to neck to avoid the leakage of smaller particles of the adsorbent.

Ethanol extract (3 gm) was mixed with silica gel (200 to 400 mesh size) in the ratio of 1: 2 to form a free flow of the extract. It was loaded in the column by wet packing method using n-Hexane. Silica gel (200 to 400 mesh size) was made in to

slurry with n-Hexane and it was packed in the column. Pure cotton was placed over the silica gel. The extract was packed in the column through the funnel. Again pure cotton was placed over the extract.

The column was developed by elution with n-Hexane and then followed by n-Hexane : Chloroform (75:25, 50:50, 25:75), 100 % Chloroform, Chloroform : Ethyl acetate (75:25, 50:50, 25:75), 100 % Ethyl acetate, Ethyl acetate : Ethanol (75:25, 50:50, 25:75) in gradient manner and finally exhausted using 100 % Ethanol. The liquid level was always kept above the surface of the column to prevent the cracking of the column. Fractions of 50 ml were collected and monitored by TLC on silica gel with various mobile phases and visualized in iodine chamber as well as in UV light.

TLC

As soon as the fraction eluted, it was analyzed by using ready made TLC plate with suitable mobile solvent according to the polarity of elute.

SOLVENT

Chloroform : methanol (9:1).

Observation

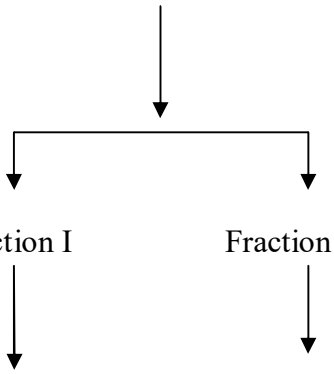
The developed chromatogram was observed under UV, Fluorescence and also derivatised using detecting agent.

Ethanolic extract of

Erythrina variegata Linn., 3g

Column chromatography silica gel.

Hexane, ethyl acetate, ethanol gradient elution.



Fraction I	Fraction II
Very little and had very several spots	Narrow band, single spots.

CHARACTERISATION OF ISOLATED COMPOUNDS

PHYSICAL EVALUATION:

1. Physical properties of the isolated compounds are evaluated, such as

   - Colour
   - Nature
   - Solubility
   - Molecular weight
   - Molecular formula

2. Further the isolated compounds were characterized by following Spectroscopic methods. Such as

   IR Spectroscopy

   Gas Mass spectroscopy

IR SPECTROSCOPY [100]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The possible characteristic bands of the nucleus are

1. 3540-3300 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration
3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration
5. 3100-3000 cm-1 C-H Aromatic Stretching Vibration

GAS – MASS CHROMATOGRAPHY [101 - 102]

It is a combined technique, used for molecular weight determination. Gas chromatography and mass spectroscopy combined to form GC-MS.

GC-MS information

Make	:	Perkin Elmer
GC model	:	Clarus 680
Mass Spectrometer	:	Clarus 600 (EI)
Software	:	TurboMass ver 5.4.2
Library ver	:	NIST-2008

ACQUISITION PARAMETERS

Oven	:	Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min,
Total Run Time	:	32.00 mint
Inj auto	:	260°C,
Volume	:	1 µL,
Split	:	10:1,
Flow Rate	:	1 mL/mint
Carrier Gas	:	He,
Column	:	Elite-5MS (30.0m, 0.25mmID, 250µm df)

MASS CONDITION (EI)

Solvent Delay : 2.00 min,

Transfer Temp : 240°C,

Source Temp : 240°C,

Scan : 50 to 600Da,

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

SURVEY OF POLYCYSTIC OVARIAN DISEASE [16,17]

OBJECTIVE OF THE STUDY

- Identify the adolescent girls who are at high risk for PCOS.
- Find association between PCOS risk status with selected variable general health status.
- The major purpose of this study is to create awareness among adolescent college girls. This will help them to modify their life style and to have better reproductive life later.

CRITERIA USED FOR DIAGNOSING PCOS [20, 21]

NH / NICHHD 1990 or NHI STATEMENT

To include all of the following

1) Hypernandrogenism and / or Hyperandrogenemia
2) Oligo-ovulation
3) Exclusion of hypothyroidism, Hypoprolactinemia, Cushing's syndrome, NCAH, Androgeen secreting tumors and exogenous androgen intake.

ROTTERDAM 2003 or ESHRE / ASRM statement

To include all of the following in addition to exclusion of related disorder

1) Clinical and / or biochemical hyperandrogenism
2) Oligo-ovulation or anovulation
3) Polycystic ovaries (exclusion of other endocrinopathies)

AE – PCOS 2006

To include all of the following

1) Clinical and / or biochemical hyper androgenism with either.
2) Oligo-anovulation
3) Polycystic ovaries (exclusion of other androgen excess of related disorders.

MATERIALS AND METHODS

The medical history was recorded, with Anthropometric, clinical and biochemical parameters by using the questionnaire on whether the participant had been previously diagnosed with PCOS. Menstrual irregularity was assessed as a usual cycle of less than 21 days or more than 35 days. Clinical hyprandrogenism was assessed on the basis of the self-reported degree of hirsutism by using self assessment method. Results were kept confidential. The selected participants likely to have PCOS asked to go or further clinical and ovarian ultra sound examination.

This is a questionnaire based study on the awareness of polycystic ovary syndrome. The participants who undertook the students of a college and working womens. A total of 29 questions were asked to 232 girls of age group between 17-34 yrs. Individuality was assured when the subjects filled the survey. The questionnaire is filled in paper and pen method. After the data collection, statistical measurements were applied. The questions included are ;

The questionnaire

Anthropometric, clinical and biochemical parameters

(1) Age _____yrs.

(2) Height _____ cms.

(3) Weight _____kg.

(4) Body Mass Index (BMI) _____kg/m^2 (calculated).

(5) Waist circumference_____ (cm).

(6) Hip circumference _____ (cm).

(7) Age at menarche _____.

(8) Regularity of the menstrual cycle _____(yes or no).

(9) Pelvic pain during menstruation _____(yes or no).

(10) Marital status _____ If Married (yes or no). I yes answer 12 & 13.

(11) Fertility problems _____(yes or no).

(12) Use of oral contraceptive Pills _____(yes or no).

(13) Presence of PCOD_____ If Yes, Answer the following

(14) Diet _____(what type of diet is taken).

(15) Craving for carbohydrates and sugar _____(yes or no).

(16) Obesity_____(yes or no).

(17) Blood pressure_____ (mmHg)

(18) Symptoms of hypoglycemia _____(yes or no) .

(19) Serum FSH and LH level_____ and _____(ng/dl)

(20) Serum Testosterone level_____(ng/dl)

(21) Hirsutism (Unwanted hair growth) of face and body_____

(22) Acne_____(yes or no).

(23) Stretch marks (Stria) _____

(24) Hair loss_____(yes or no).

(25) Acanthosis nigricans (Skin disorder) _____(yes or no).

(26) Other systemic disorders (✓ or ×)

Diabetes_____ , Hyperthyroidism_____ ,

Hypertension _____, High Cholesterol _____,

Depression and mood change_____, Stress _____.

(27) Family History : Your Mother with PCOD_____, Your siblings with PCOD_____.

(28) Have you everbeen come across the term PCOD? _____.

(29) If yes, how did you know about PCOD? _____.

Fig 8.Questionnarie filling,

Fig 9. Height determination

Fig 10. Weight Checking

RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Organoleptic characters :

Nature	–	Coarse powder
Taste	–	No characteristic
Odour	–	Unpleasant
Colour	–	Dark green

MACROSCOPY

Type	–	Trifoliate, Alternate, Compound
Shape	–	Rhomboid, Heart shaped
Margin	–	Entire
Apex	–	Acuminate
Base	–	Obtuse
Size	–	14-16 cm in length
		13-15 cm in breadth
Surface	–	Upper – Smooth dark green
Lower	–	Smooth pale green
Texture	–	Fine
Venation	–	Reticulate
Leaflets	–	Triangular medium

MICROSCOPIC FEATURES

Transverse section of leaf:

In transectional view, the leaf exhibits thick midrib and uniformly thick and smooth lamina (fig 11). The midrib consists of wide, conical, short thump and wide semicircular abaxial part of the midrib. The midrib is 1.1mm thick and the abaxial semicircular part in 1mm wide.

T.S of Midrib - enlarged

The vascular system of the midrib includes adaxial, median collateral vascular bundles, two lateral vascular bundles and a wide are of three discrete abaxial median bundles (fig 12).

T.S of Midrib – Upper portion

The adaxial median vascular bundles has a cluster of large angular, thick walled vessels and three vertically elongated phloem units to eated other adaxial side, there is a thick and wide sclerenchyma cells placed above the adaxial medium bundle (fig 13).

T.S of Midrib – Lower portion

The epidermis of the midrib consists of small squarish the cell walled cells 2mm squarish epidermis occur six in seven layers of fairly wide, angular, thick walled and compact parenchyma cells (fig 14). There is a wide bowl shaped layer of schlerenchyma cell enclosing. The schlerenchyma layer is two to four cells thick.

The lateral and abaxial are of vascular bundles are all collateral with wide mass of randomly placed thick walled angular vessels and many independent units of small mass of phloem elements.

Calcium Oxalate Crystals (Druses) in the Adaxial part of the Midrib

Calcium oxalate crystals fairly widely distributed in the midrib and leaf (fig 15). In the midrib the crystals are drusen located in the parenchyma cells inside the adaxial epidermal layer.

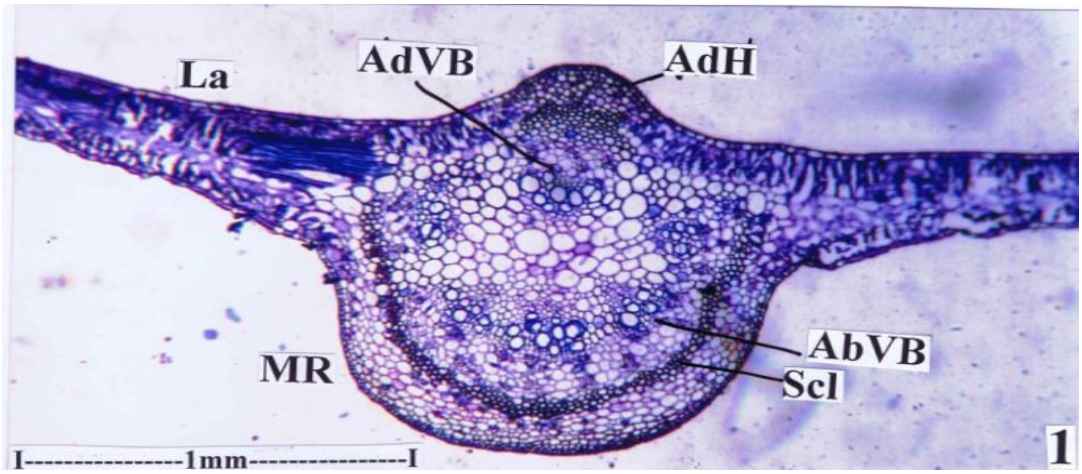
Prismatic crystals in Lamina

The leaf crystals are mostly prismatic type and are seen along the veins of the lamina (fig 16).

MICROSCOPIC FEATURES OF THE LEAVES

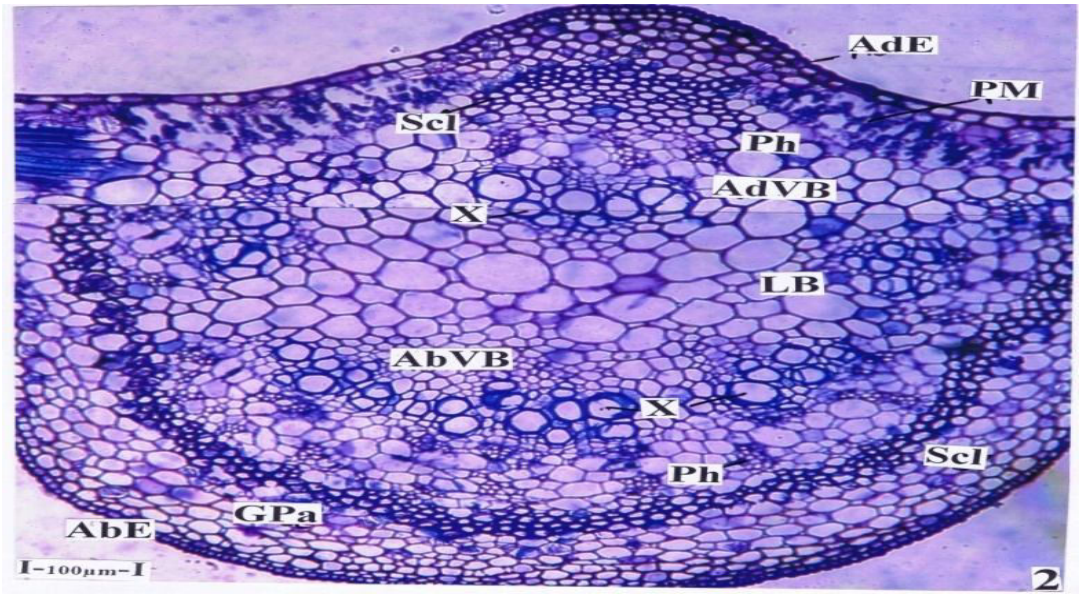
TRANSVERSE SECTION OF THE LEAVES

Fig 11. T.S of leaf through midrib



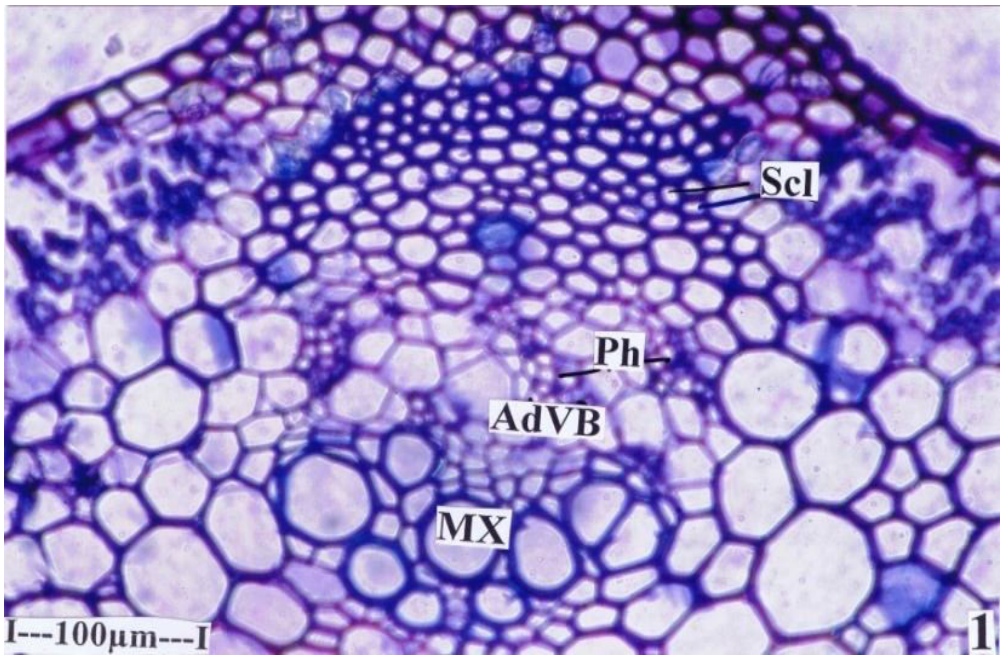
La-Lamina, AdVB-Adaxial vascular bundle, AdH-Adaxial Hump, MR-Midrib, AbVB-Abaxial vascular bundle, Scl-Sclerenchyma.

Fig 12 : T.S of Midrib – enlarged



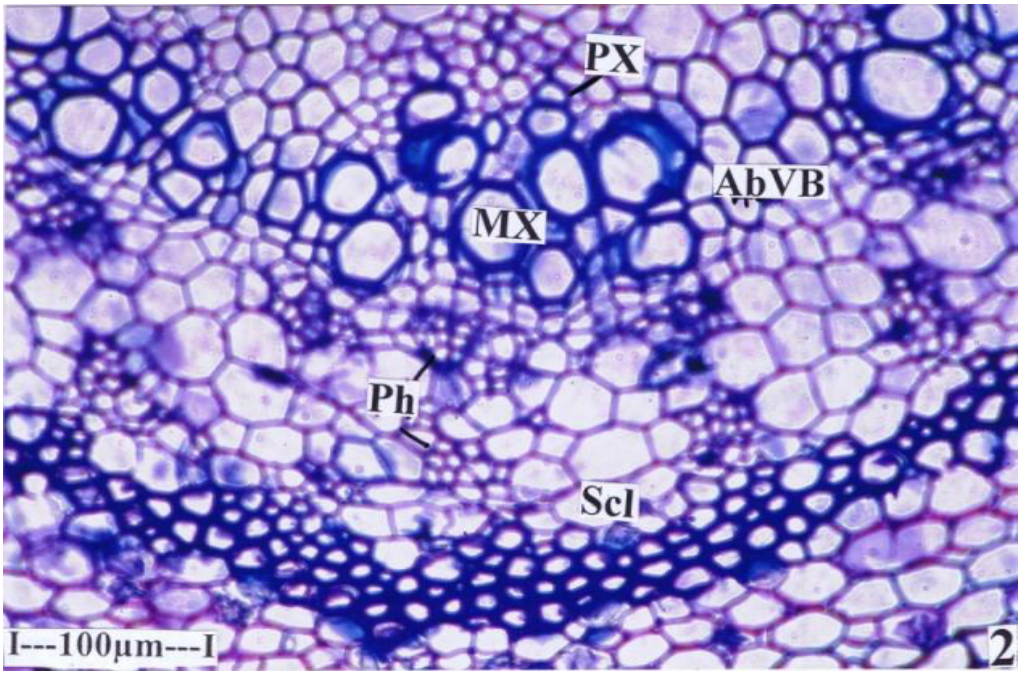
AdE-Adaxial Epidermis, PM-Palisade Mesophyll, Ph-Phloem, X-Xylem, LB-Lateral bundle, GPa-Ground Parenchyma.

Fig 13. T.S of Midrib – Upper portion



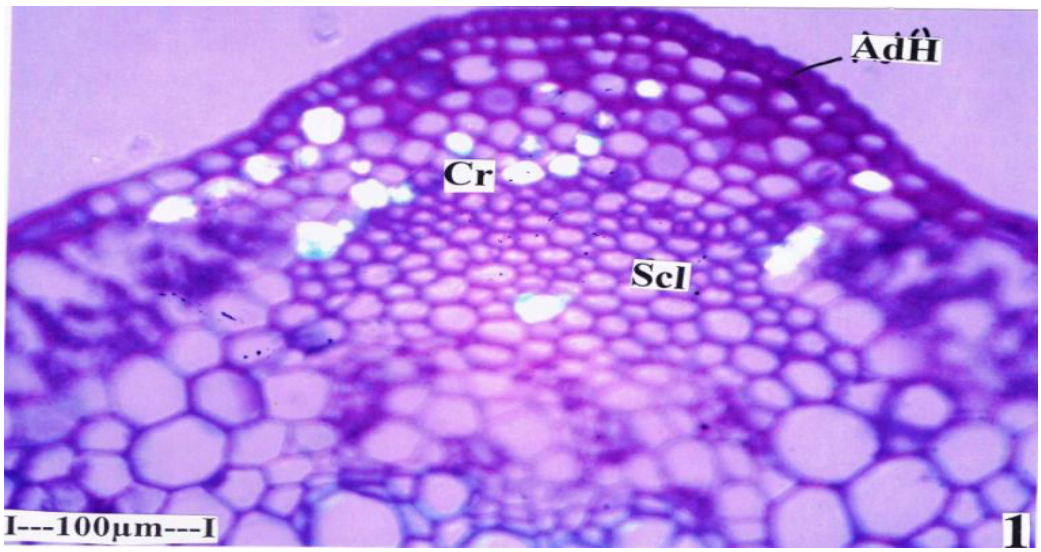
AdVB-Adaxial vascular bundle, Scl-Sclerenchyma, Ph-Phloem, MX-Meta Xylum

Fig 14. T.S of Midrib – Lower portion



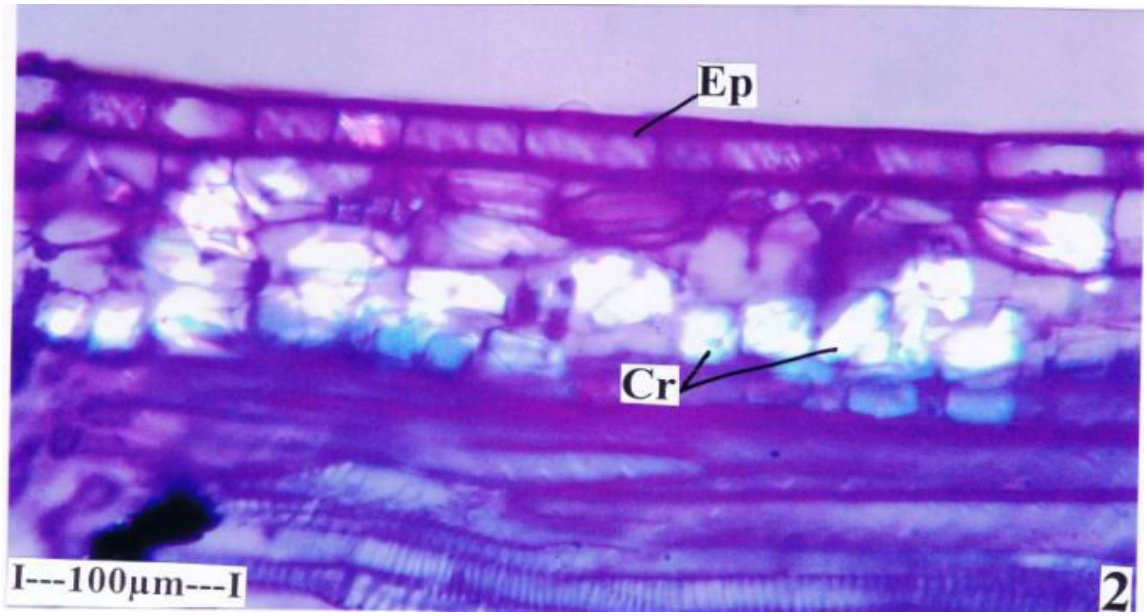
AbVB-Abaxial vascular bundle, Scl-Sclerenchyma, Ph-Phloem, MX-Meta Xylum

Fig 15. Calcium Oxalate Crystals (Druses) in the Adaxial part of the Midrib



AdH – Adaxial Hump, Cr – Crystals , Ep – Epidermis, Scl - Sclerenchyma

Fig 16. Prismatic crystals in Lamina

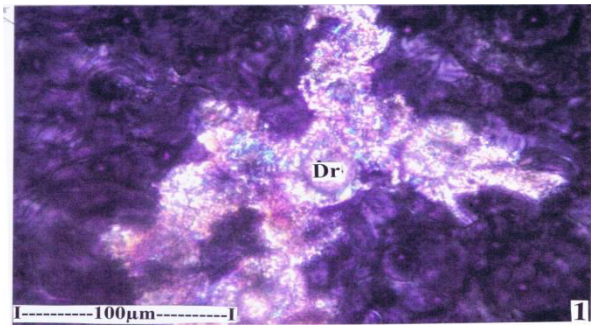


Ep – Epidermis, Cr – Crystals

POWDER MICROSCOPY

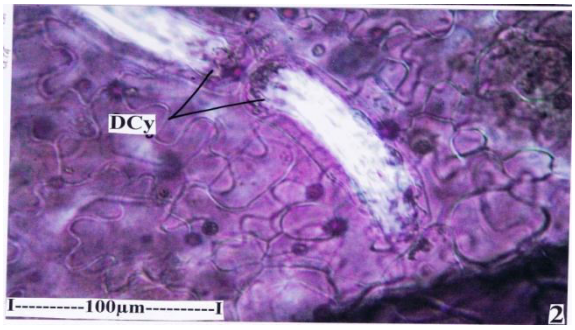
It was also observed that wide drusan occur in large clusters in the mesophyll tissue of the lamina (Fig 17). Apart from druses and prismatic crystals, these also another type of crystals called double – cystolith (Fig 18). These crystals are elongated, cylindrical and occur in pairs, end to end.

Fig. 17: Druses in leaf mesophyll



Dr – Druses

Fig. 18: Double Cystolith in lamina



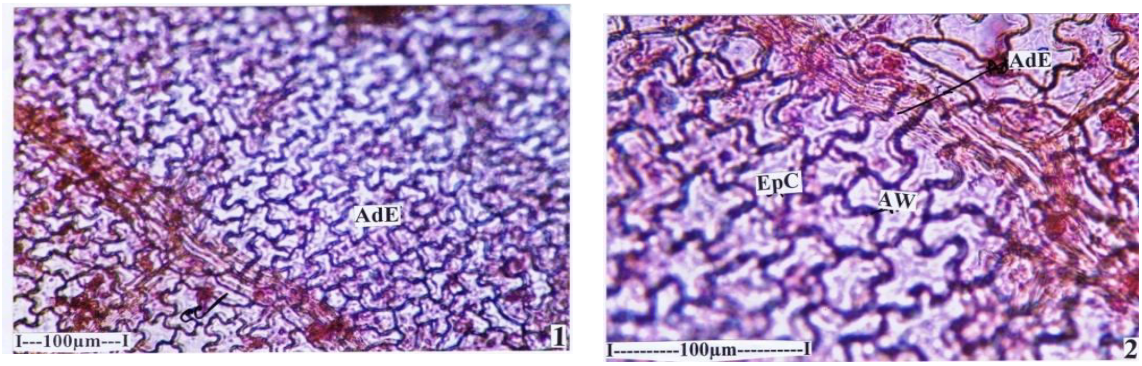
DCy – Double Cystolith

EPIDERMAL TISSUE :

Adaxial epidermal cells in surface view

Epidermal cells and stomatal morphology were studied in surface view of the epidermal peeling. The adaxial epidermis is nonstomatic the epidermal cells have slightly wavy undulate anticlinal walls which are thin and smooth. The costal cells forming the veins of the lamina, are vertically elongated with straight walls (Fig.19).

Fig 19. Adaxial epidermal cells in surface view



AdE-Adaxial Epidermis, AW - Anticlinal Wall, EpC- Epidermal Cells, CC- Coastal Cells

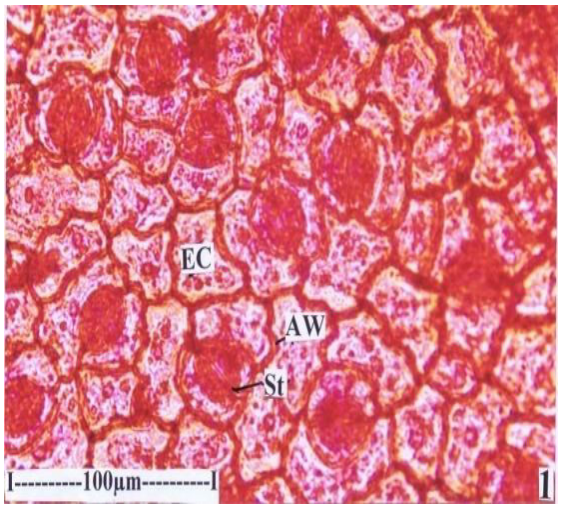
Abaxial epidermis

The abaxial epidermis is densely stomatierous. The epidermal cells have thick undulate anticlinical walls. The cells possess prominent nuclei and other cell inclusions (Fig 20). The stomata are broadly elliptical and have thick walls. The stomatal pore in narrow and slit like the guard cells are 20-25µm long and 20µm thick.

Paracytic Stomata – enlarged

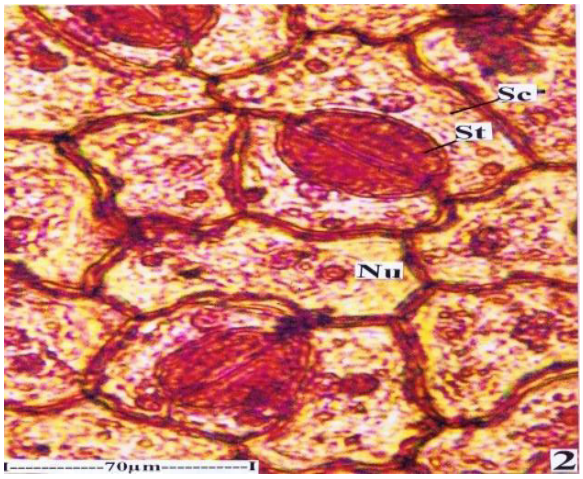
The stoma has two subsidiary, one on either side of the guard cells and parallel of the long axis of the stoma. The stomata are paracytic type. (Fig 20, 21)

Fig 20. Abaxial epidermis showing stomata



EC - Epidermal cells, AW–Anticlinal Walls, St - Stomata,

Fig 21. Paracytic stomata enlarged

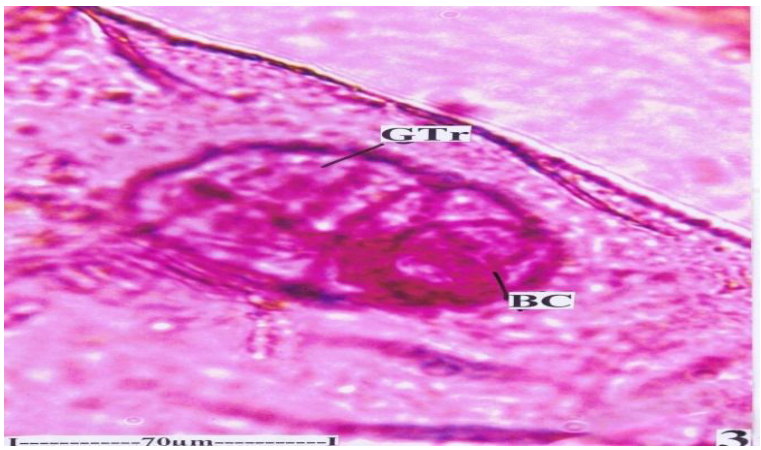


Sc - Subsidiary cells, Nu-Nucleus

Glandular trichome

Thick, short, elliptical much cellular glandular trichomes (Fig. 22) are common on the lamina. The gland has a short wide stalk cells of the gland stand erect. The gland has about row of four cells, the cells being arranged one above the other.

Fig. 22 Glandular trichome



GTr – Glandular Trichome, BC – Basal cells

LEAF CONSTANTS

Table 7 : Leaf constants of *Erythrina variegata* Linn.,

S.NO	PARAMETERS	Values in sq mm
1	Stomatal no (upper epidermis)	6 – 8
2	Stomatal index (upper epidermis)	18.18 – 22.32
3	Stomatal no (lower epidermis)	10 – 12
4	Stomatal index (lower epidermis)	15.05 – 20.01
5	Vein islet number	24 – 28
6	Veinlet termination number	12 – 14

LINEAR MEASUREMENT OF CALCIUM OXALATE CRYSTALS

Table 8 : Quantitative microscopy – linear measurement of calcium oxalate crystals

PARAMETERS	MINIMUM (µm)	AVERAGE (µm)	MAXIMUM (µm)
Length	125	298	572
Width	12.8	16.4	26.8

HISTOCHEMICAL STUDIES

Table 9 : Histochemical Color Reaction *Erythrina variegata* Linn.,

S.no	Chemicals	Test for	Nature of change	Histology	Degree of change
1	**Phloroglucinol + HCl**	Lignin	Pink	Xylem	+
2	**N / 50 Iodine Solution**	Starch	Blue	Middle layer near oil globules	+
3	**Dil. Ferric Chloride**	Tannin	Black	Vascular bundles	+
4	**Picric acid**	Protein	No yellow color	--	-
5	**Dragendroff's reagent**	Alkaloid	Orange color	Middle layer	+

PHYSIOCHEMICAL CONSTANTS

Table 10 : Physiochemical Constants of *Erythrina variegata* Linn., leaves

S.NO	PARAMETERS	VALUES (%W/W)
I	**ASH VALUE**	
1.	Total ash	9.48±0.67
2.	Water insoluble ash	6.9±0.26
3.	Acid soluble ash	0.73±0.75
4.	Sulphated ash	4.2±0.42
II	**EXTRACTIVE VALUE**	
1.	Water soluble extractive	4.37±0.82
2.	Alcohol soluble extractive	6.65±0.45
3.	Ether soluble extractive	7.25±0.58
4.	Non volatile ether soluble extractive	12.17±0.17
IV	**LOSS ON DRYING**	5.11±0.52
V	**FOAMING INDEX**	Nil
VI	**SWELLING INDEX**	Nil
VII	**VOLATILE OIL CONTENT**	Nil

INORGANIC ELEMENTS AND HEAVY METALS

Table 11 : Qualitative Estimation of Inorganic Elements of *Erythrina variegata* Linn.,

S.No	INORGANIC ELEMENTS	OBSERVATIONS
1.	Aluminium	+
2.	Chloride	+
3.	Calcium	+
4.	Iron	+
5.	Borate	+
6.	Potassium	+
7.	Carbonate	-
8.	Sulphates	-
9.	Silver	+
10.	Nitrate	+

Table 12 : Quantitative Estimation Of Inorganic Elements of *Erythrina variegata* Linn.,

S.NO	INORGANIC ELEMENTS	TOTAL AMOUNT (%W/W)
1.	Aluminium	0.016
2.	Chloride	0.043
3	Calcium	0.034
4.	Borate	0.006
5.	Potassium	0.046
6.	Nitrate	0.012
7.	Iron	0.034
8.	Silver	0.008

Quantitative Estimation Of Heavy Metals By ICP OES Method

The quantification of the individual heavy metals was analyzed for the powdered mixture of *Erythrina variegata* Linn., by ICP – OES technique the following metals like arsenic, lead, cadmium were detected and quantified , results are given in the following table.

Table 13 : Quantitative Estimation Of Heavy metals

S.No	Element	Results (ppm)	Specification as per WHO Guidelines
1.	Mercury	Not detected	Not more than 0.5ppm
2.	Arsenic	0.023	Not more than 5.0ppm
3.	Lead	0.032	Not more than 10ppm
4.	Cadmium	0.001	Not more than 0.3ppm

PHYTOCHEMICAL STUDIES

Table 14 : Percentage Yield Of Successive Extraction of *Erythrina variegata* Linn.,

S.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	**Petroleum ether**	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Yellowish green	6.8
2.	**Chloroform**		Semisolid	Dark green	4.7
3.	**Ethyl acetate**		Semisolid	Dark brown in color	3.7
4.	**Ethanol**		Semisolid	Brown in color	5.6
5.	**Aqueous**	Cold maceration	Sticky	Brown color	4.8

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Table 15 : Qualitative Phytochemical Analysis

S.NO	TEST OF CONSTITUENS	PET ETHER	CHLORO FORM	ETHYL ACETATE	ETHANOL	AQUEOUS
1	FLAVONOIDS	+	+	+	+	+
2	Alkaloids	+	+	+	+	+
3	Carbohydrates	-	-	-	-	-
4	Gums & Mucilage	-	-	-	-	-
5	Proteins & Amino acids	-	-	-	-	-
6	Fats and fixed oils	+	+	-	-	-
7	Steroids	-	-	+	+	-
8	Glycosides	-	-	-	-	-
9	Phenols	-	-	+	+	+
10	Saponins	-	-	-	-	-

Note : + indicates presence, - indicates absence.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The *Erythrina variegata* Linn., was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total Alkaloids, total Flavonoids and Phenolic content in the ethanol extract were decided to be taken as parameters. Samples were drawn from three random samples of *Erythrina variegata* Linn., was drawn and the content present in them were estimated.

Table 16 : Quantitative estimation of Phytoconstituents

S.NO	PARAMETERS	VALUES (µg/ml)
1.	Total Alkaloids	8.72µg/ml
2.	Total Flavonoids	5.46µg/ml
3.	Total Phenolics	6.86µg/ml

FLUORESCENE ANALYSIS

Table 17 : Fluorescence Characteristics Of Powdered Sample Of *Erythrina variegata* Linn., in Short UV and Long UV.

S.NO	TREATMENT	DAY LIGHT	SHORT-UV (254nm)	LONG-UV(365nm)
1	Powder	Green	Brown	Dark brown
2	Powder + Water	Dark green	Brown	Greenish brown
3	Powder + Ethanol	Dark green	Brown	Dark brown
4	Powder + 1N HCl	Yellow	Yellow	Greenish brown
5	Powder + 1N H_2SO_4	Light brown	Brown	Dark brown
6	Powder + 1N NaOH	Green	Dark green	Dark green
7	Powder + 1N alcoholic KOH	Dark green	Brown	Dark brown
8	Powder + $FeCl_3$	Greenish brown	Brown	Dark brown
9	Powder + Acetic acid	Greenish yellow	Brown	Dark brown
10	Powder + Ammonia	Green	Brown	Greenish brown
11	Powder + Iodine	Green	Brown	Bluish brown

Table 18 : Fluorescence Analysis Of Various Extracts Of

***Erythrina variegata* Linn.,**

S.NO	EXTRACTS	DAY LIGHT	SHORT-UV (254nm)	LONG-UV (365nm)
1	Petroleum ether	Dark green	Dark green	Dark green
2	Chloroforum	Pale green	Dark green	Dark green
3	Ethyl acetate	Light brown	Brown	Brown
4	Ethanol	Brown	Dark brown	Dark brown
5	Aqueous	Brown	Dark brown	Dark brown

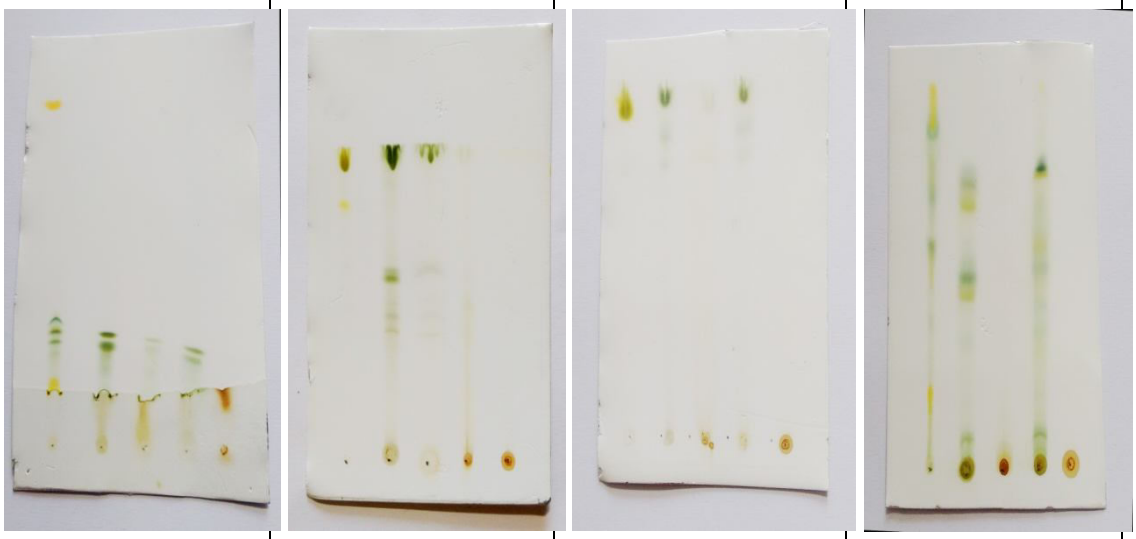
The powdered leaves and extracts of *Erythrina variegata* Linn., showed the absence of any fluorescence chromophore.

THIN LAYER CHROMATOGRAPHY OF EXTRACTS

Table 19. TLC Solvents And Rf Value Of Various Extract

S. no	Chemical constituents	Solvent system	Extract	No of spots	R_f value
1.	Alkaloids	Toluene : Ethylacetate : Diethylamine (70:20:10)	Pet ether Chloroform Ethyl acetate Ethanol Aqueous	5 3 3 3 1	0.2,0.32,0.34,0.36,0.43 0.22,0.31,0.33 0.22,0.32,0.34 0.23,0.34,0.34 0.23
2.	Flavonoids	Ethyl acetate : Formic acid : Glacial Acetic acid : Water (100:11:11:26)	Pet ether Chloroform Ethyl acetate Ethanol Aqueous	1 5 3 2	0.73 0.29,0.33,0.42,0.46,0.74 0.3,0.47,0.76 0.42,0.82
3.	Phenolic compounds	Toluene : Ethyl acetate (93:7)	Pet ether Chloroform Ethyl acetate Ethanol Aqueous	1 3 2 3	0.88 0.77,0.84 0.77,0.93 0.76,0.84
4.	Steroids	Chloroform : Methanol (27:3)	Pet ether Chloroform Ethyl acetate Ethanol Aqueous	3 4 1 5	0.52,0.71,0.79 0.4,0.48,0.6,0.72 0.54 0.39,0.48,0.52,0.71,0.87

Table 20 : TLC Of Various Extracts

TLC of five extracts			
TLC of Alkaloids	**TLC of Flavonoids**	**TLC of Phenolic compounds**	**TLC of Steroids**
			

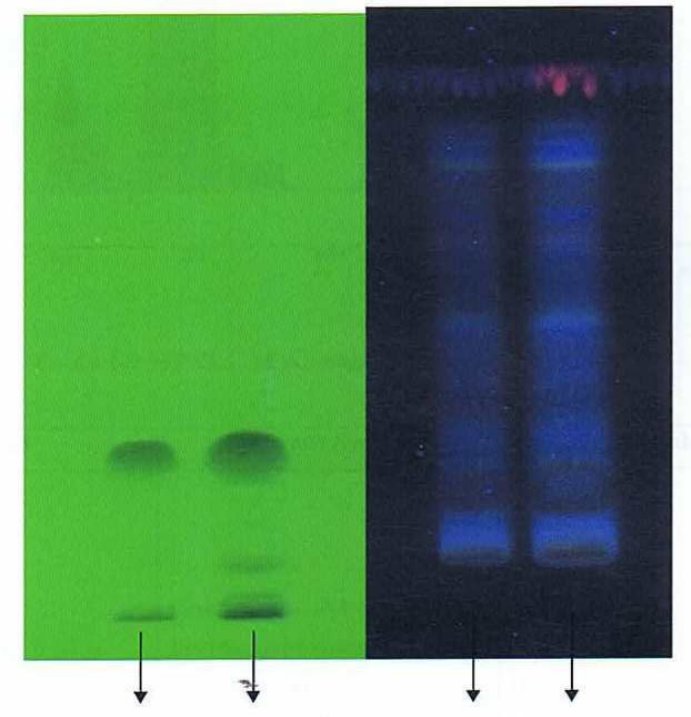
HPTLC FINGER PRINT PROFILE

HPTLC Finger print Data of Ethanolic Extract of *Erythrina variegata* Linn., High performance thin layer chromatography (HPTLC) finger printing was performed with the ethanol extract of ***Erythrina variegata Linn.,***

Table No 21: Solvent System Of HPTLC analysis

EXTRACT	SOLVENT
Ethanolic extract	Chloroform : Methanol : Formic acid : Water (65:25:10:5)

Fig. 23 HPTLC finger print of Acoholic extract at 254 and 366nm



5µl 10µl 5µl 10µl

at 254 nm at 366 nm

Fig 24. HPTLC Chromatogram of Alcoholic Extract (5µl) at 254 nm

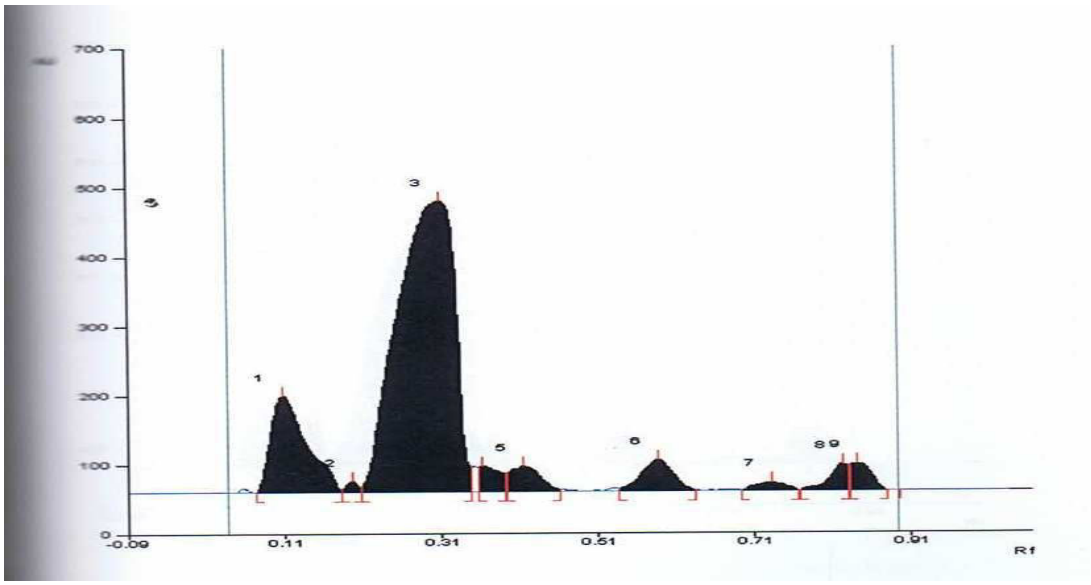


Table 22 : HPTLC of Alcoholic Extract (5µl) at 254 nm

Extract	Solvent system	Rf values
Alcoholic extract 5µl	Chloroform : Methanol : Formic acid : Water (65:25:10:5)	0.11 0.20 0.31 0.37 0.42 0.59 0.74 0.83 0.85

Fig. 25 HPTLC of Alcoholic Extract (5µl) at 366 nm

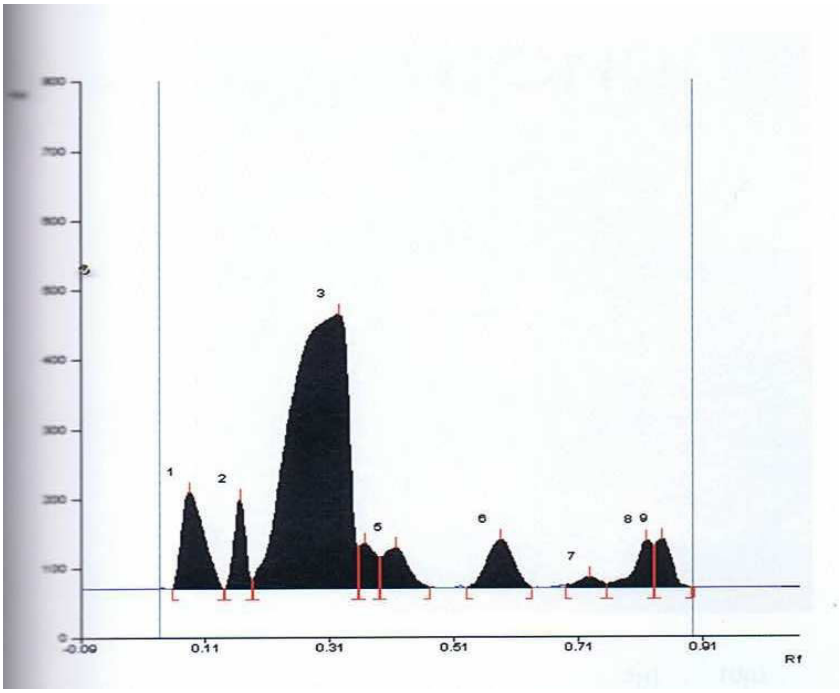


Table 23 : HPTLC of Alcoholic Extract (10µl) at 366 nm

Extract	Solvent system	Rf values
Alcoholic extract 10µl	Chloroform : Methanol : Formic acid : Water (65:25:10:5)	0.09 0.17 0.33 0.37 0.42 0.59 0.73 0.82 0.85

SELECTION OF ACTIVE EXTRACT

IN – VITRO ANTI – OXIDANT ACTIVITY :

Table 24 DPPH ASSAY

DRUGS	% INHIBITION AT VARIOUS CONCENTRATION					IC_{50} (µ g/ml)
	100 g/ml	200 g/ml	400 g/ml	800 g/ml	1000 g/ml	
Diclofenac sodium	26.17±0.45	34.72±1.86	41.88±0.54	61.23±1.56	73.02±0.09	578
Petroleum ether	15.45±0.15	17.52±0.71	22.76±0.56	25.04±2.43	38.48±0.65	1549
Chloroform	4.64±0.52	7.68±1.06	19.53±0.67	26.48±1.46	39.99±1.84	1309
Ethyl acetate	17.22±0.90	24.45±1.43	36.24±0.43	45.59±0.96	51.15±1.73	916
Ethanol	24.23±2.80	35.07±1.08	47.95±0.67	63.64±1.87	65.86±2.34	489
Aqueous	1.78±1.62	5.75±1.87	14.18±1.73	28.3±1.43	35.67±2.54	1388

Fig. 26 *In vitro* DPPH Assay

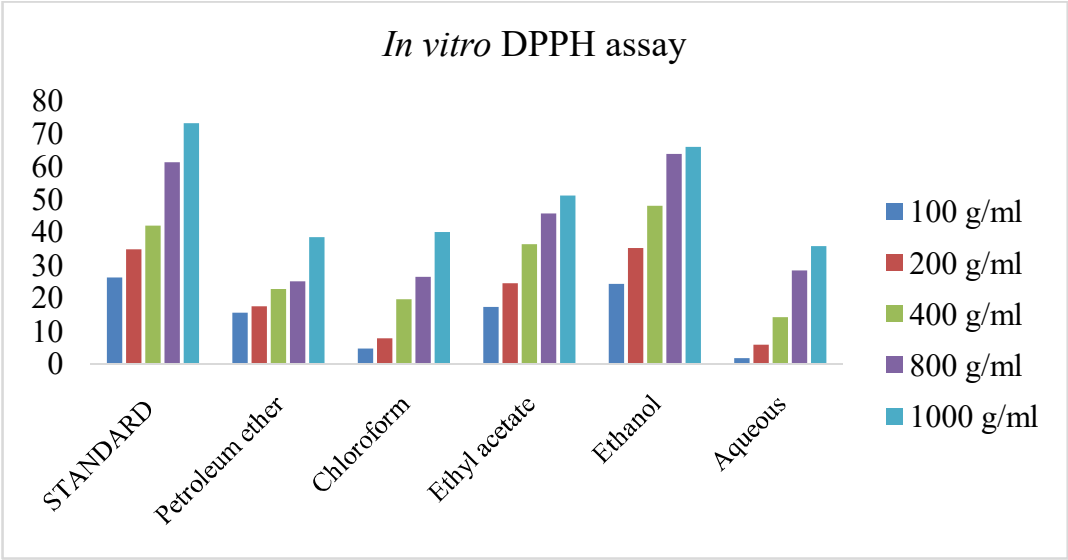
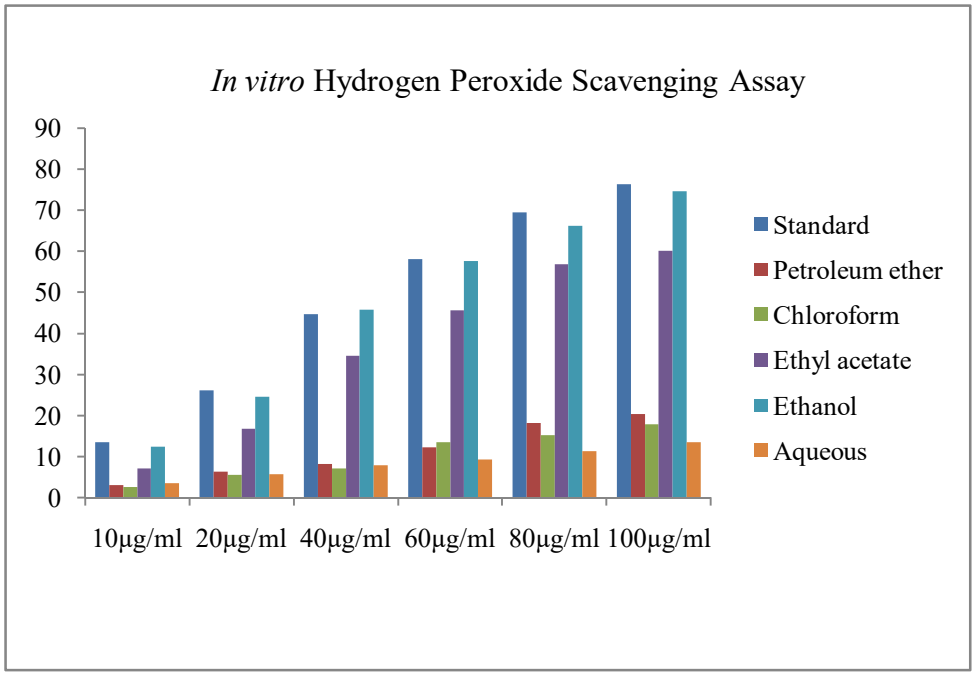


TABLE 25 : HYDROGEN PEROXIDE SCAVENGING ACTIVITY

EXTRACTS	% INHIBITION AT VARIOUS CONCENTRAT						IC_{50} (µ g/ml)
	10 µg/m	20 µg/m	40 µg/m	60 µg/m	80 µg/ml	100 µg/ml	
Standard (Ascorbic acid)	13.59	26.18	44.62	58.12	69.48	76.28	56.82
Petroleum ether	3.14	6.32	8.24	12.36	18.23	20.35	252.53
Chloroform	2.65	5.58	7.18	13.52	15.27	17.85	309.32
Ethyl acetate	7.16	16.82	34.57	45.61	56.83	60.12	78.48
Ethanol	12.48	24.63	45.81	57.59	66.18	74.56	59.78
Aqueous	3.54	5.72	7.94	9.34	11.34	13.57	365.74

Fig. 27 *In vitro* Hydrogen Peroxide Scavenging Assay



Body weight

Table 26 : Changes in the body weight (g).

Groups	Initial weight	14th day	28th day	42nd day	56th day
Group I Control	157±1.04	165.67±0.82	173.67±0.74	176±0.73	172.34±0.68
Group II Disease contro	154±0.47	173±0.27	190.34±0.53	197.34±0.40	205.45±0.46
Group III Standard	158.64±0.72	174.67±0.5	185±0.62	177.34±0.89	176.67±0.91
Group IV 200 mg/kg	158.67±0.72	171.67±0.85	184.54±0.36	188.67±0.4	187.16±0.35
Group V 400 mg/kg	159.34±0.4	170.34±0.61	179±0.59	184.34±0.57	182.67±0.39

Fig. 28 Changes in body weight

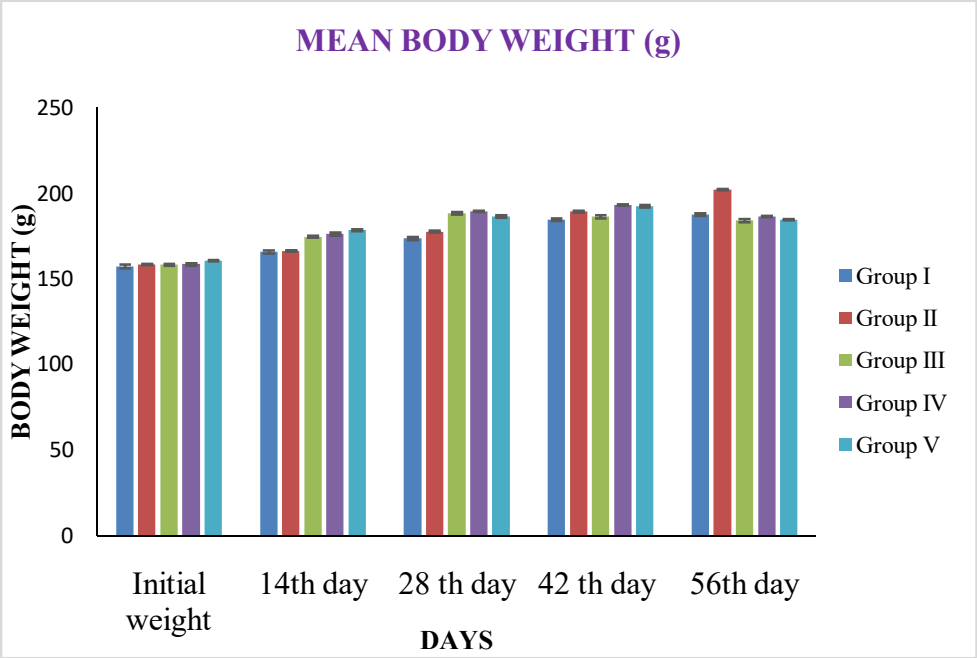
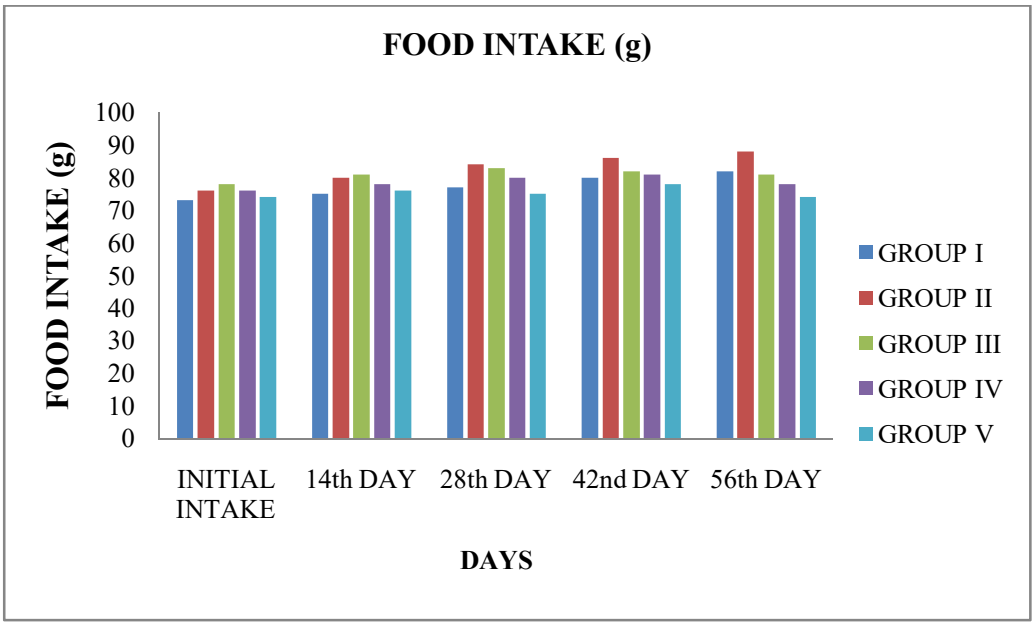


Table 27 : Changes In The Food Intake (g)

Groups	Initial Weight	14th day	28th day	42nd day	56th day
Group I Control	73	75	77	80	82
Group II Disease control	76	80	84	86	88
Group III Standard	78	81	83	82	81
Group IV Low dose	76	78	80	81	78
Group V High dose	74	76	75	78	74

Fig. 29 Changes In Food Intake



VAGINAL EXFOLIATIVE CYTOLOGY

Fig. 30 Working Table – Method Of Taking Vaginal Smear

Fig. 31 Vaginal Smear Taking In Rat Using Pipette Method

Fig. 32 Preparing Vaginal Smear In Room Temperature

TABLE 28 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 1 to 10

Groups	Days / S.no	1	2	3	4	5	6	7	8	9	10
Group I	1	P	M	D	P	E	M	M	D	M	D
	2	P	E	M	D	E	M	M	D	P	P
	3	M	D	EP	E	M	D	EP	P	P	M
	4	P	--	D	P	E	M	D	D	P	M
	5	P	E	M	D	D	E	M	M	EP	P
	6	P	M	M	D	EP	E	M	D	D	P
Group II	7	P	E	M	P	LP	E	M	D	M	D
	8	D	E	M	D	D	P	P	--	P	M
	9	--	D	D	P	E	M	M	D	M	P
	10	D	D	P	--	D	P	M	D	D	P
	11	D	P	E	M	D	P	E	M	M	D
	12	P	E	M	D	LP	E	--	D	P	M
Group III	13	M	D	D	E	M	D	--	M	D	P
	14	P	--	D	D	P	E	D	D	D	P
	15	E	M	D	D	P	E	D	P	P	D
	16	P	M	D	P	E	M	D	M	P	M
	17	M	M	D	E	M	D	EP	P	P	M
	18	P	E	M	P	E	M	D	D	M	D
Group IV	19	--	P	E	M	D	D	P	P	P	D
	20	D	D	E	M	D	D	P	D	P	M
	21	--	E	M	E	M	D	D	M	M	D
	22	D	D	M	--	D	D	D	M	M	D
	23	P	P	--	--	E	M	M	P	P	M
	24	P	--	--	P	E	M	D	M	D	D
Group V	25	--	--	--	--	--	--	D	D	P	--
	26	D	E	M	D	D	P	E	M	D	P
	27	--	--	--	--	--	--	M	D	P	E
	28	M	D	E	--	M	D	D	P	E	M
	29	--	--	--	--	--	--	--	D	D	D
	30	P	E	--	D	P	E	M	D	P	E

TABLE 29 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 11 TO 19

Groups	Days / S.no	11	12	13	14	15	16	17	18	19
Group I	1	D	M	P	P	P	P	P	M	M
	2	M	D	P	D	P	P	P	P	P
	3	D	M	P	D	D	D	P	P	P
	4	M	D	P	D	D	D	D	P	P
	5	D	D	M	P	P	P	M	D	D
	6	D	D	D	P	P	D	P	P	P
Group II	7	P	D	M	M	D	P	P	P	M
	8	D	M	D	P	M	D	D	D	D
	9	D	P	P	M	D	D	D	P	P
	10	M	D	D	P	M	D	D	D	P
	11	D	P	M	P	P	M	D	D	D
	12	D	D	P	P	P	M	M	D	P
Group III	13	M	M	D	P	D	D	D	P	P
	14	P	M	D	E	M	D	D	D	D
	15	D	M	D	D	P	P	M	M	D
	16	D	P	M	M	M	D	P	P	M
	17	M	P	D	--	--	M	M	D	D
	18	D	P	M	M	D	P	P	M	M
Group IV	19	D	D	M	P	D	P	P	M	M
	20	D	P	M	P	D	D	P	P	M
	21	P	M	M	M	D	P	--	D	P
	22	D	D	P	M	M	M	D	D	P
	23	M	D	D	D	P	P	P	P	M
	24	D	P	P	--	P	M	M	D	P
Group V	25	D	D	M	M	D	D	D	E	M
	26	E	M	D	--	D	D	P	E	M
	27	M	D	D	P	D	E	M	D	P
	28	D	D	P	E	M	D	P	E	--
	29	P	E	M	D	P	D	P	E	M
	30	M	M	D	M	M	D	P	P	E

TABLE 30 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 20 TO 29

Groups	Days / S.no	20	21	22	23	24	25	26	27	28	29
Group I	1	D	D	D	D	P	P	P	M	P	E
	2	M	M	M	D	D	D	P	D	D	E
	3	P	P	M	M	M	D	D	D	E	M
	4	P	M	M	D	D	D	P	P	P	E
	5	D	D	P	P	D	D	D	D	D	D
	6	D	D	P	P	P	D	D	D	D	EP
Group II	7	D	D	D	P	P	M	M	D	P	LP
	8	P	M	M	D	D	D	D	P	D	D
	9	P	M	D	D	D	D	P	P	P	E
	10	P	P	M	D	D	D	P	P	--	D
	11	D	D	P	P	M	M	D	D	M	D
	12	P	D	D	D	D	P	P	P	D	LP
Group III	13	P	P	P	M	M	M	M	D	E	M
	14	P	P	P	P	M	M	M	D	D	P
	15	D	D	D	D	P	P	P	M	D	P
	16	M	D	D	D	D	P	P	P	P	E
	17	D	D	P	P	P	--	M	D	E	M
	18	M	D	D	D	D	P	--	P	P	E
Group IV	19	D	D	--	D	P	P	M	M	M	D
	20	M	M	M	D	D	P	P	P	M	D
	21	P	M	M	M	D	D	D	P	E	M
	22	P	P	P	D	M	D	D	D	--	D
	23	M	M	D	P	D	P	P	M	--	E
	24	D	D	P	P	D	D	P	P	P	E
Group V	25	D	M	D	E	P	E	M	D	--	--
	26	D	D	P	E	M	D	D	P	D	D
	27	E	D	P	P	--	D	P	E	--	--
	28	D	D	D	D	E	M	D	D	--	M
	29	D	E	M	P	D	P	E	M	--	--
	30	M	D	D	P	E	M	D	D	D	P

TABLE 31 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 30 TO 38

Groups	Days / S.no	30	31	32	33	34	35	36	37	38
Group I	1	D	P	--	M	--	D	D	P	M
	2	M	M	M	D	--	D	P	--	--
	3	D	D	M	D	--	M	M	D	D
	4	P	--	M	D	--	D	D	P	M
	5	D	D	D	D	P	M	D	D	P
	6	M	--	D	D	M	--	D	P	--
Group II	7	M	--	D	D	--	P	E	M	M
	8	--	M	D	D	D	P	M	M	--
	9	M	D	D	P	M	D	D	P	M
	10	P	D	M	M	P	M	D	D	P
	11	D	--	E	M	M	D	D	D	E
	12	P	P	D	D	P	M	M	--	--
Group III	13	M	M	D	P	E	M	M	D	D
	14	P	D	P	LP	E	M	D	P	P
	15	--	P	P	M	D	D	D	P	M
	16	M	M	D	P	M	D	D	D	P
	17	M	--D	D	--	D	M	M	D	P
	18	M	P	P	M	M	D	D	D	--
Group IV	19	P	D	D	D	P	D	M	M	D
	20	M	P	P	P	P	M	M	D	D
	21	D	D	D	P	P	M	M	D	P
	22	E	D	D	P	M	D	D	D	D
	23	M	D	D	D	P	D	D	D	M
	24	P	D	--	M	M	--	--	D	P
Group V	25	D	P	E	M	M	D	D	D	P
	26	D	D	E	M	M	D	D	--	P
	27	M	M	D	E	M	D	D	P	E
	28	E	M	M	D	D	E	E	M	D
	29	D	D	--	E	--	D	D	D	P
	30	E	E	D	EP	E	M	M	D	D

TABLE 32 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 39 TO 47

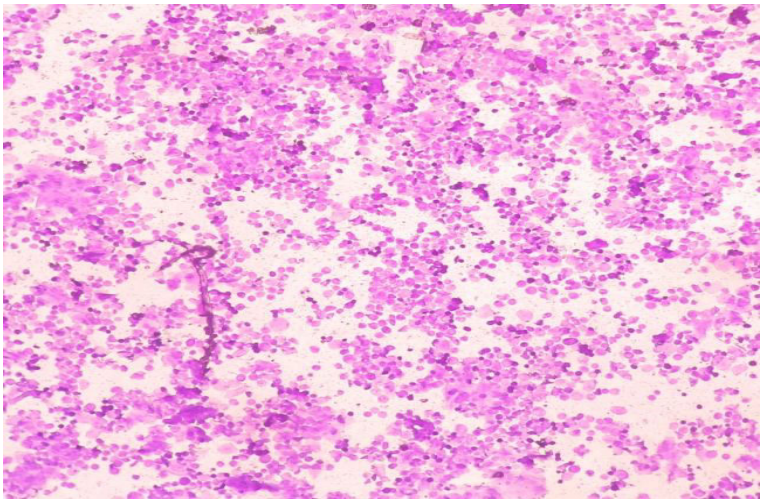
Groups	Days / S.no	39	40	41	42	43	44	45	46	47
Group I	1	M	M	D	M	P	E	M	D	D
	2	P	E	M	D	D	P	E	M	M
	3	D	D	E	M	M	D	D	P	E
	4	D	E	M	D	D	P	E	M	M
	5	E	M	D	D	P	D	E	M	D
	6	M	D	D	D	E	M	D	D	D
Group II	7	P	E	M	M	D	D	P	E	M
	8	D	--	D	P	E	M	D	D	D
	9	D	D	M	D	D	D	P	E	M
	10	M	D	D	D	M	E	E	M	M
	11	M	M	M	M	D	D	E	M	M
	12	D	M	D	P	E	M	D	D	P
Group III	13	P	E	M	M	D	D	D	P	E
	14	E	E	M	M	D	D	D	P	P
	15	P	E	M	D	D	P	E	M	M
	16	E	M	--	M	D	D	D	P	M
	17	E	M	M	D	D	P	E	M	M
	18	P	E	M	M	D	D	D	P	E
Group IV	19	D	D	P	P	M	D	D	P	M
	20	P	E	M	M	D	D	D	P	E
	21	D	D	D	P	D	M	M	M	D
	22	D	P	D	D	D	D	P	P	E
	23	M	E	P	D	D	P	E	M	P
	24	P	D	D	M	M	D	D	D	--
Group V	25	E	M	D	--	--	M	D	D	P
	26	E	M	D	E	M	D	D	P	E
	27	M	D	D	--	D	P	E	M	M
	28	P	E	M	D	D	P	P	E	M
	29	E	M	D	--	P	E	M	D	D
	30	P	E	M	M	D	D	D	P	E

TABLE 33 : VAGINAL EXFOLIATIVE CYTOLOGY DAY 48 TO 56

Groups	Days / S.no	48	49	50	51	52	53	54	55	56
Group I	1	P	E	E	M	M	P	D	D	P
	2	D	D	D	P	P	D	M	M	M
	3	M	D	D	P	P	D	E	D	D
	4	D	D	P	P	E	M	D	P	--
	5	D	D	P	E	E	M	D	D	D
	6	P	P	P	M	M	P	E	M	--
Group II	7	P	P	P	P	D	E	D	M	--
	8	P	P	E	M	M	P	D	--	M
	9	M	P	D	P	M	P	E	M	D
	10	P	D	P	P	D	E	D	P	D
	11	D	D	D	P	P	D	E	D	--
	12	E	E	E	M	P	P	D	P	P
Group III	13	M	M	D	D	P	P	M	M	M
	14	E	M	M	D	D	E	M	P	D
	15	D	D	P	P	D	P	D	--	P
	16	M	D	D	P	P	D	M	M	M
	17	D	D	P	P	D	E	D	M	D
	18	M	M	P	P	D	E	M	M	P
Group IV	19	M	D	D	D	E	M	P	P	D
	20	M	M	D	D	E	M	M	M	P
	21	D	D	P	--	D	E	P	D	D
	22	M	M	P	P	D	E	D	E	D
	23	M	D	D	P	P	D	D	M	D
	24	P	P	E	E	M	P	M	P	D
Group V	25	E	M	M	P	P	D	--	D	P
	26	M	D	P	M	M	P	E	D	D
	27	D	D	E	M	P	D	--	M	M
	28	D	D	P	P	D	D	D	E	M
	29	P	E	M	M	P	D	--	D	D
	30	M	M	D	D	E	M	M	E	E

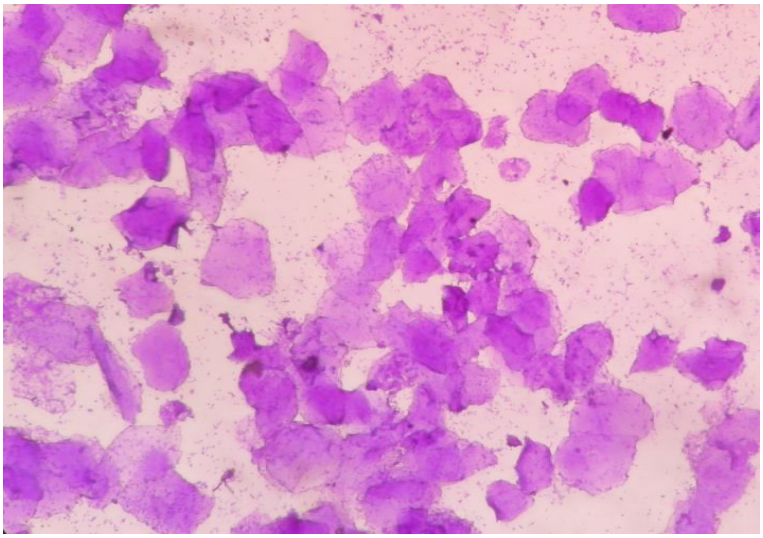
STAGES OF ESTRUS CYCLE

Fig 33. Proestrus phase



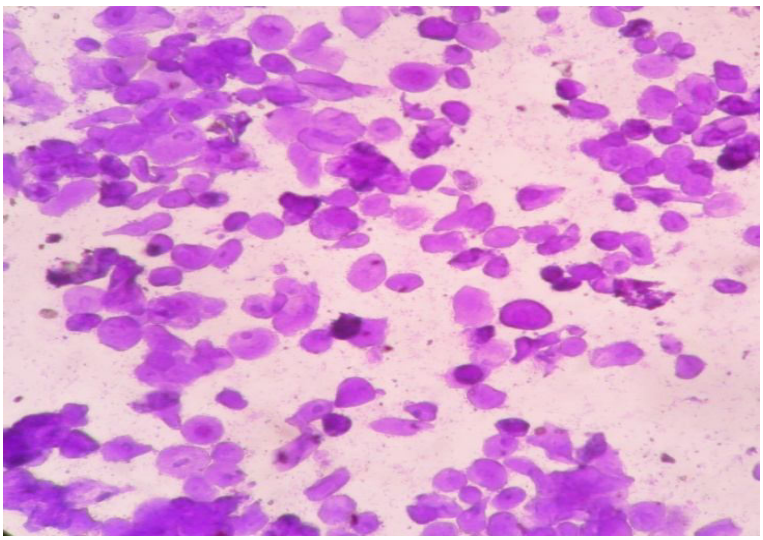
Proestrus smears are characterized by rounded, nucleated, epithelial cells are present.

Fig. 34 Estrus phase



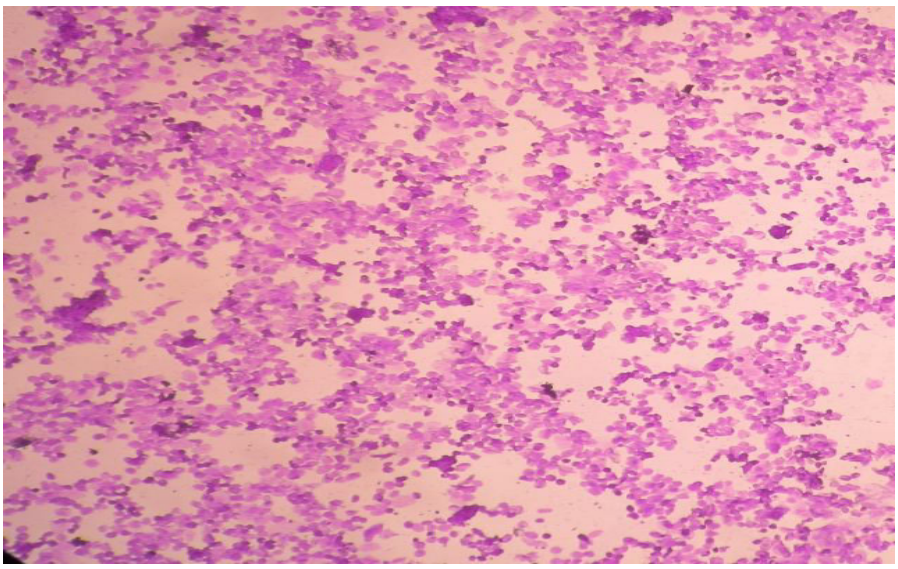
Estrus smears consist entirely of cornified cells and often non – nucleated.

Fig. 35 Met estrus phase



Met estrus smears consists of large number of leucocytes and smaller numbers of mostly large, non – granular and non – nucleated epithelial cells.

Fig. 36 Diestrus phase

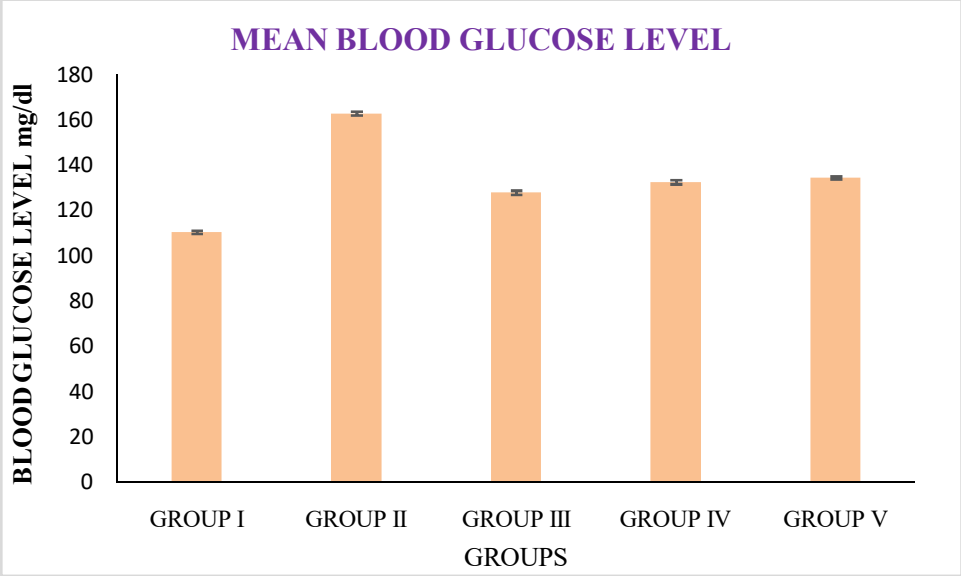


Di – estrus smears consists mainly of Lecocytes but with quite variable numbers of epithelial and small cornified cells.

TABLE 34 : BLOOD GLUCOSE

Groups	Mean value
Group I Control	110.34±0.64***
Group II Disease control	152.83±0.92
Group III Standard	118.83±0.94****
Group IV 200mg/kg	125.5±0.94****
Group V 400mg/kg	122.5±0.67****

Fig. 37 Blood Glucose Level



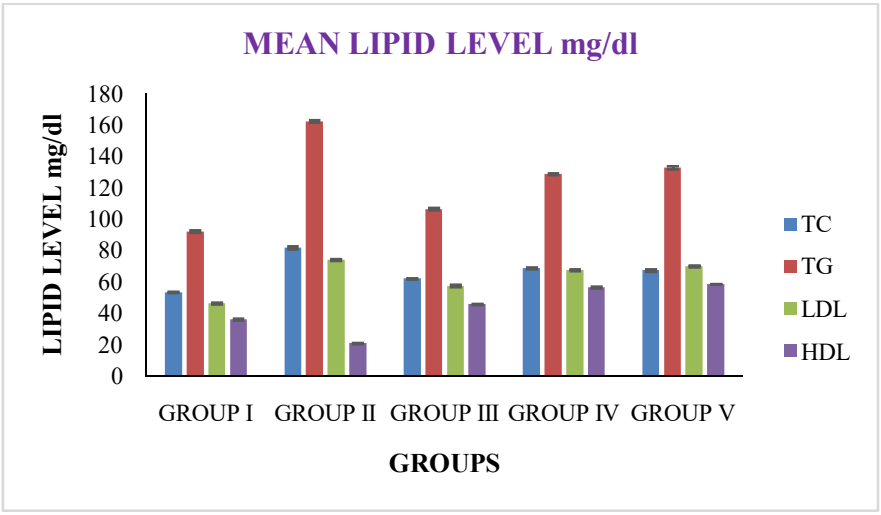
Values are means ±SEM, (n=6) ****p<0.0001 versus disease control, Group I – Control, Group II – Disease control, Group III – Standard, Group IV – Low dose (200mg/kg body weight of extract), Group V – High dose (400mg/kg body weight of extract), SEM – Standard error of mean.

TABLE 35 : LIPID PROILE

Groups	TC	TG	LDL	HDL
Group I Control	53.34±0.43**	126.17±0.89**	46.25±0.56***	35.84±0.6***
Group II Disease control	61.83±0.92	142±0.78	43.91±0.49	20.67±0.36
Group III Standard	29±0.35***	102.167±0.79***	27.34±0.67****	25.67±0.37*
Group IV 200mg/kg	28.67±0.69***	100.67±0.62****	27.5±0.48****	26.34±0.43*
Group V 400mg/kg	27.34±0.72***	105.84±0.9****	27.91±0.47****	28.5±0.31**

Values are mean ±SEM, (n=6) ****p<0.0001, ***p<0.001,**<0.005 versus disease control, Group I – Control, Group II – Disease control, Group III – Standard, Group IV – Low dose (200mg/kg body weight of extract), Group V – High dose (400mg/kg body weight of extract), SEM – Standard error of mean.

Fig. 38 Lipid Profile



ISOLATED ORGANS

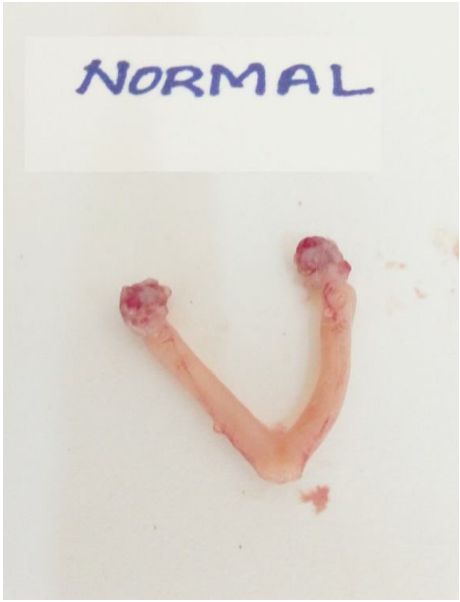


Fig. 39 Ovary and Uterus of Group I

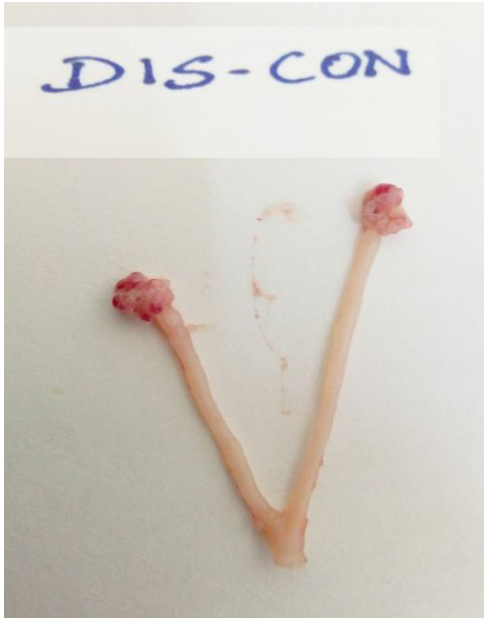


Fig. 40 Ovary and Uterus of group II

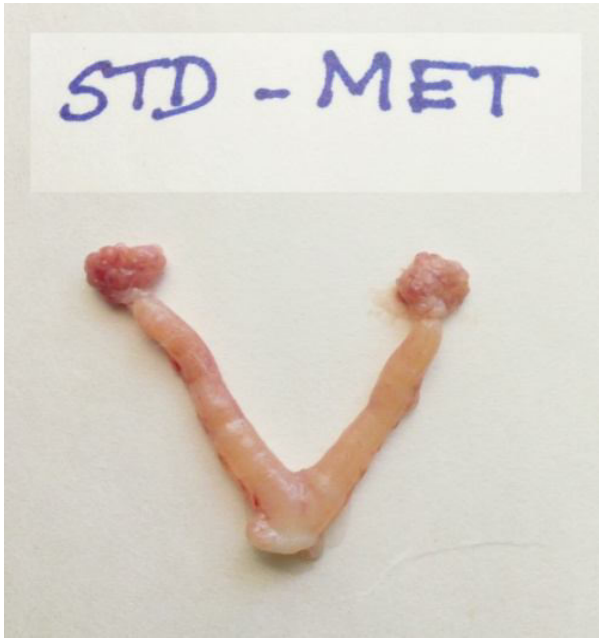


Fig. 41 Ovary and Uterus of Group III

Fig. 42 Ovary and Uterus of Group IV

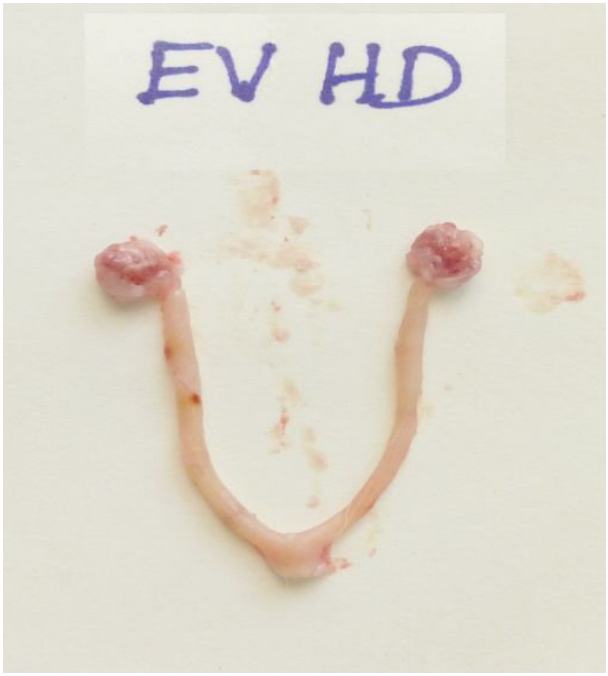


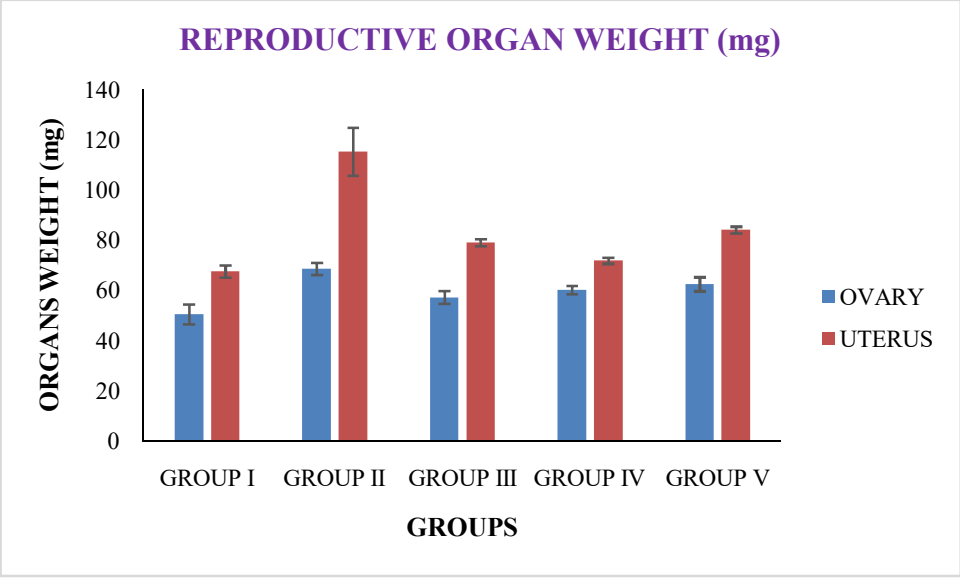
Fig. 43 Ovary and Uterus of Group V

WEIGHT OF REPRODUCTIVE ORGAN :

TABLE 36 : CHANGES OF OVARY AND UTERUS WEIGHT IN RAT

S.NO	GROUUPS	OVARY WEIGHT	UTERUS WEIGHT
1.	Group I Control	50.45±4.02	67.54±2.48
2.	Group II Disease control	68.58±2.44	115.31±9.57
3.	Group III Standard	57.21±2.62	79.06±1.42
4.	Group IV 200 mg/kg	60.12±1.7	71.8±1.18
5.	Group V 400 mg/kg	62.45±2.81	84.04±1.36

Fig. 44 Reproductive Organ Weight



HISTOPATHOLOGICAL EXAMINATION OF GROUP I : (CONTROL)

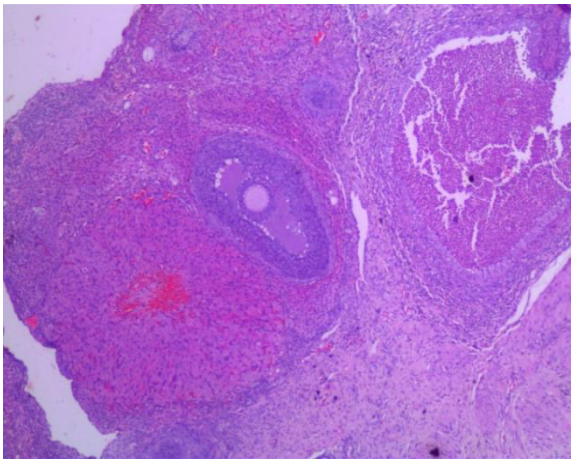


Fig. 45 Section of Ovary

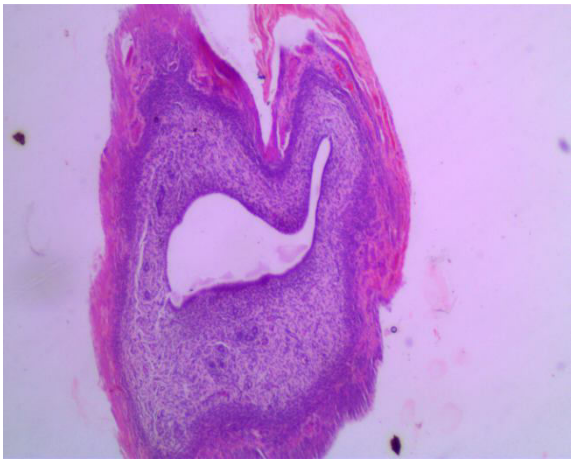


Fig. 46 Section of Uterus

HISTOPATHOLOGICAL EXAMINATION OF GROUP II

(DISEASE CONTROL)

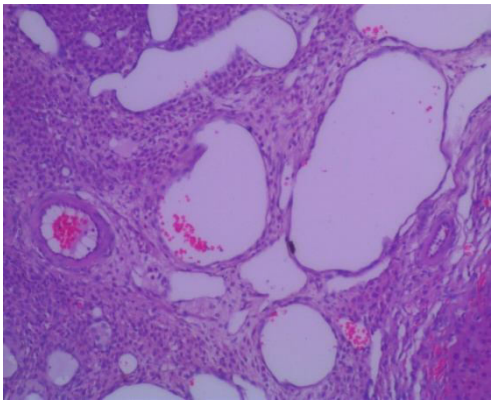


Fig. 47 Section of Ovary

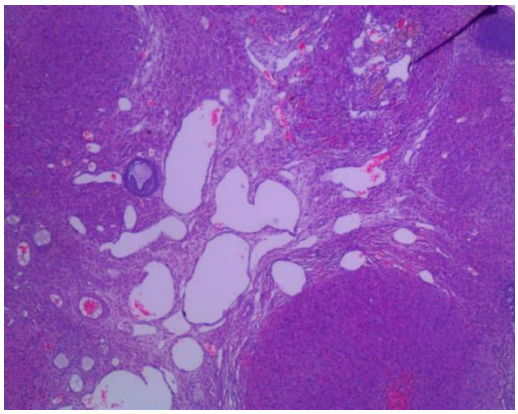


Fig. 48 Section of Uterus

HISTOPATHOLOGICAL EXAMINATION OF GROUP III (STANDARD)

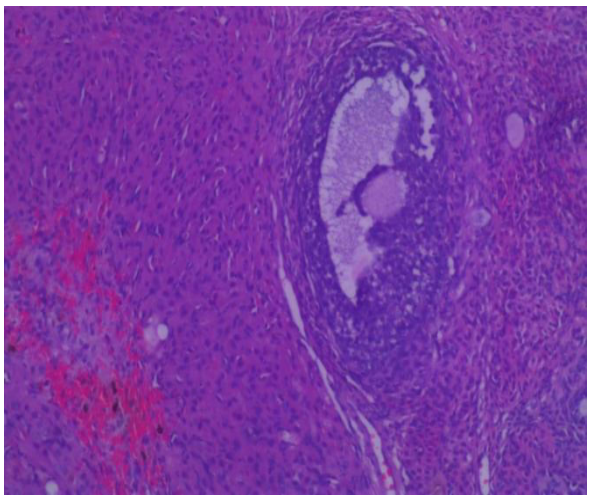


Fig. 49 Section of Ovary

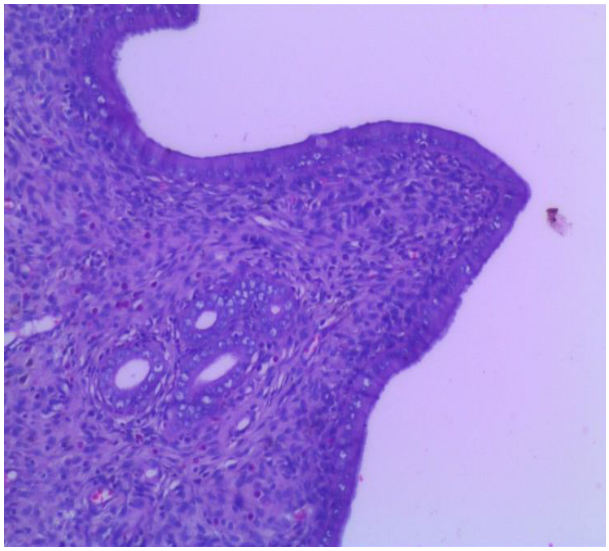


Fig. 50 Section of Uterus

HISTOPATHOLOGICAL EXAMINATION OF GROUP IV (200 mg/kg)

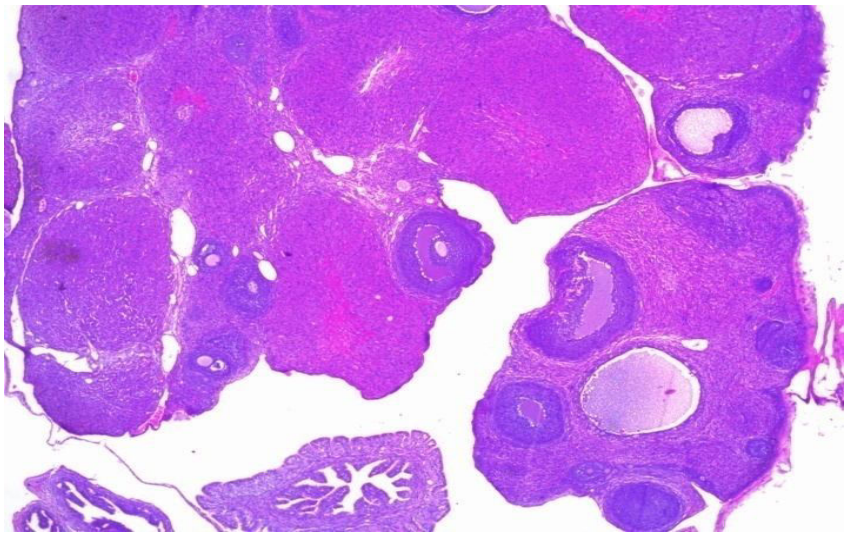


Fig. 51 Section Of Ovary Overview

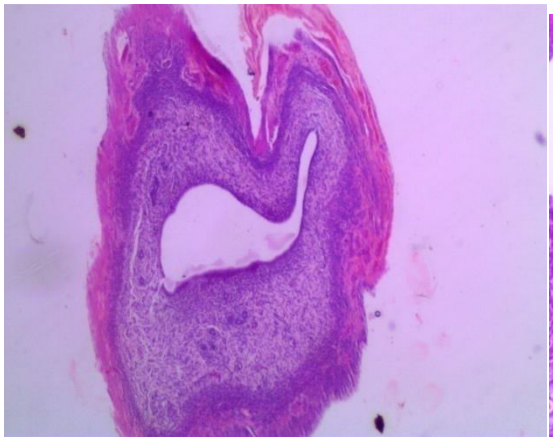


Fig.53 Section of Uterus

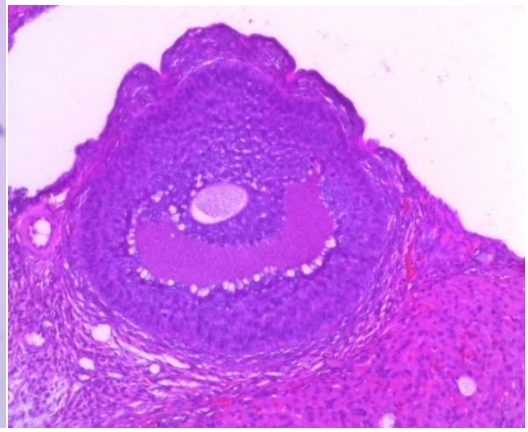


Fig.52 Section Ovary Showing Secondary Follicles

HISTOPATHOLOGICAL EXAMINATION OF GROUP V (400 mg/kg)

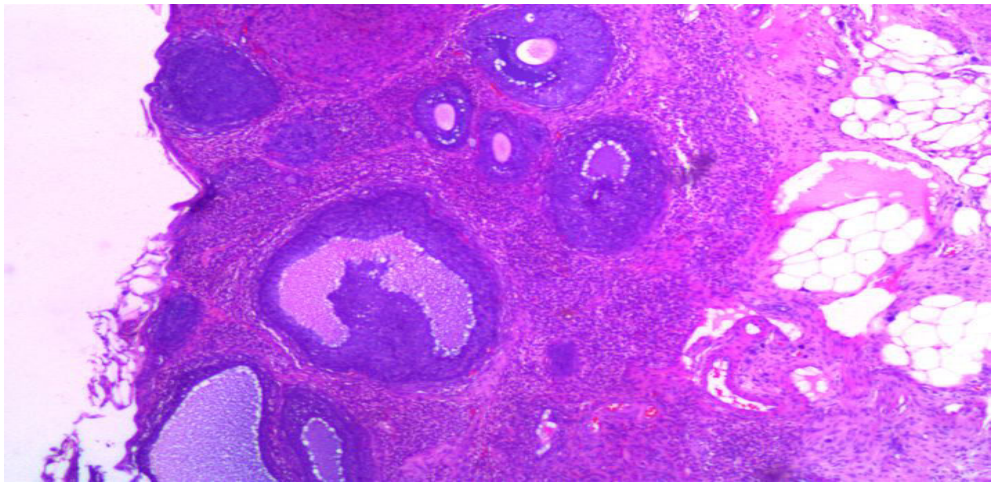


Fig. 54 Section Ovary

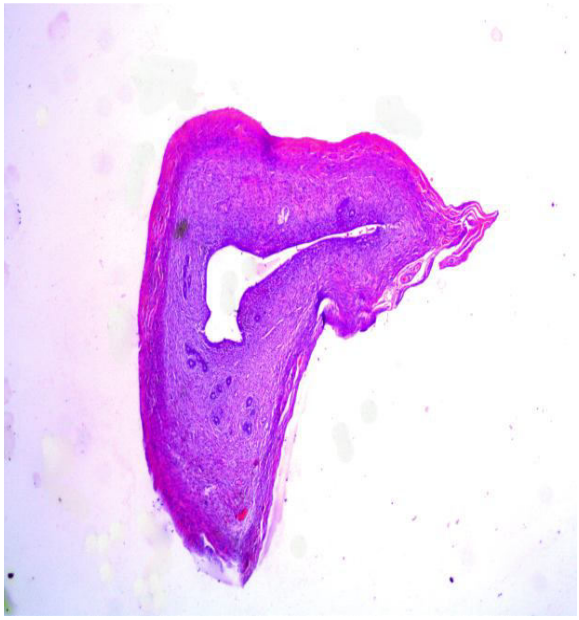


Fig. 55 Section Ovary

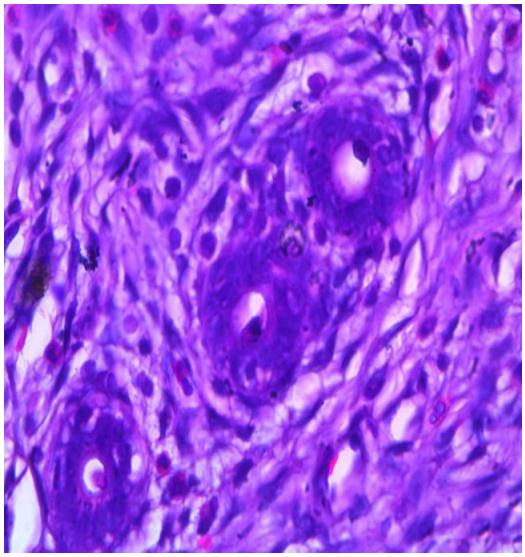
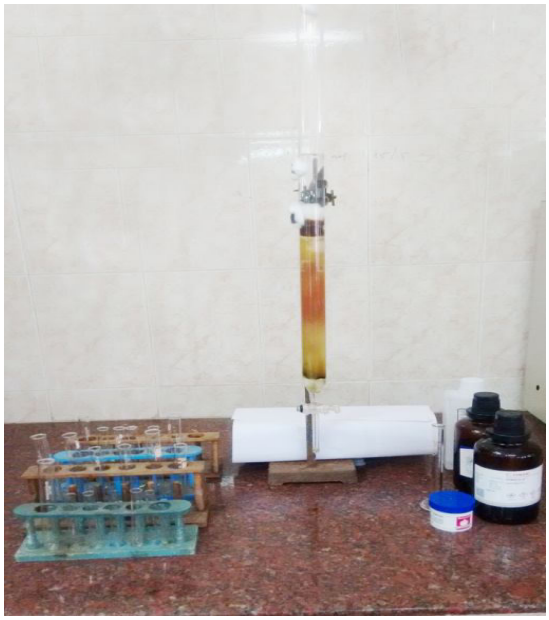


Fig. 56 Section of Uterus

ISOLATION OF PHYTOCONSTITUENTS USING COLUMN CHROMATOGRAPGY

Fig. 61 Column Chromatography Experiment In Lap



ISOLATION OF COMPOUND BY COLUMN CHROMATOGRAPHY:

COLUMN CHROMATOGRAPHY

Characterization of isolated compound

Physical properties of the isolated compounds are evaluated, such as

- Colour : Light Brown
- Nature : Semisolid
- Solubility : Soluble in Ethanol and Water A
- TLC Rf value : 0.73
- Chemical test : Yellow to colourless on addition of acid (Alkaline reagent test)

Spectral analysis:

The compound (C-1 and C-2) obtained from ethanolic extract have identified and spectral datas were depicted in the figure below.

Compound 1

IRυ^{cm-1}: Nujol mull FIG 62. IR SPECTRUM

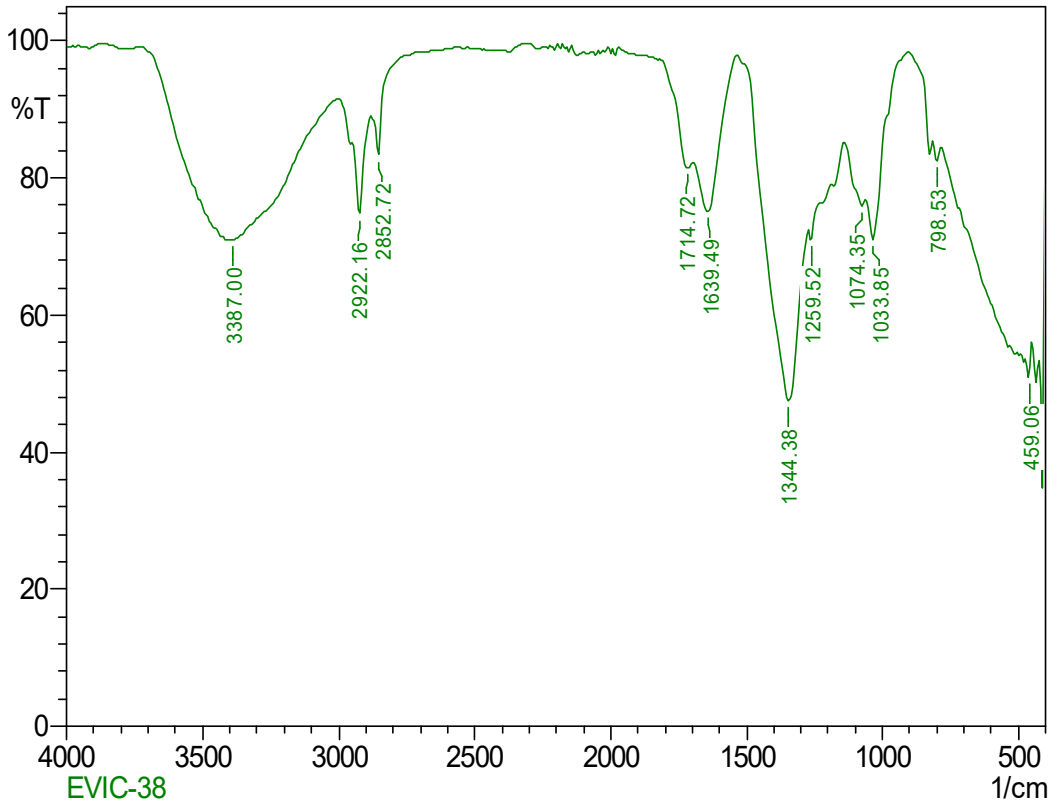


TABLE 41. INTERPRETATION OF IR SPECTRUM

WAVE NUMBER	INTENSITY	TYPE
3387.00	Broad	C = O Stretching
2922.16	Sharp	C = H Stretching
2862.72	Sharp	C = H Stretching
1714.72	Weak band	C = N Stretching
1639.49	Moderate	N - H Bending
1344.38	Sharp	C – H Bend in plane
1259.52	Weak band	O – H Bending
1074.35	Moderate	C – O Stretching
1033.85	Moderate	C – O Stretching
798.53	Sharp	C – H Rocking
459.06	Moderate	C = O Stretching

GC-MS ANALYSIS

FIG 63. STRUCTURE OF N-HEXADECANOIC ACID

Compound name: N-Hexadecanoic acid

Molecular weight: 256

Molecular formula: $C_{16}H_{32}O_2$

FIG 64. STRUCTURE OF ERUCIC ACID ‘

Compound name: Erucic acid

Molecular weight: 338

Molecular formula: $C_{22}H_{42}O_2$

FIG 65. MASS SPECTRUM OF N- HEXADECONIC ACID

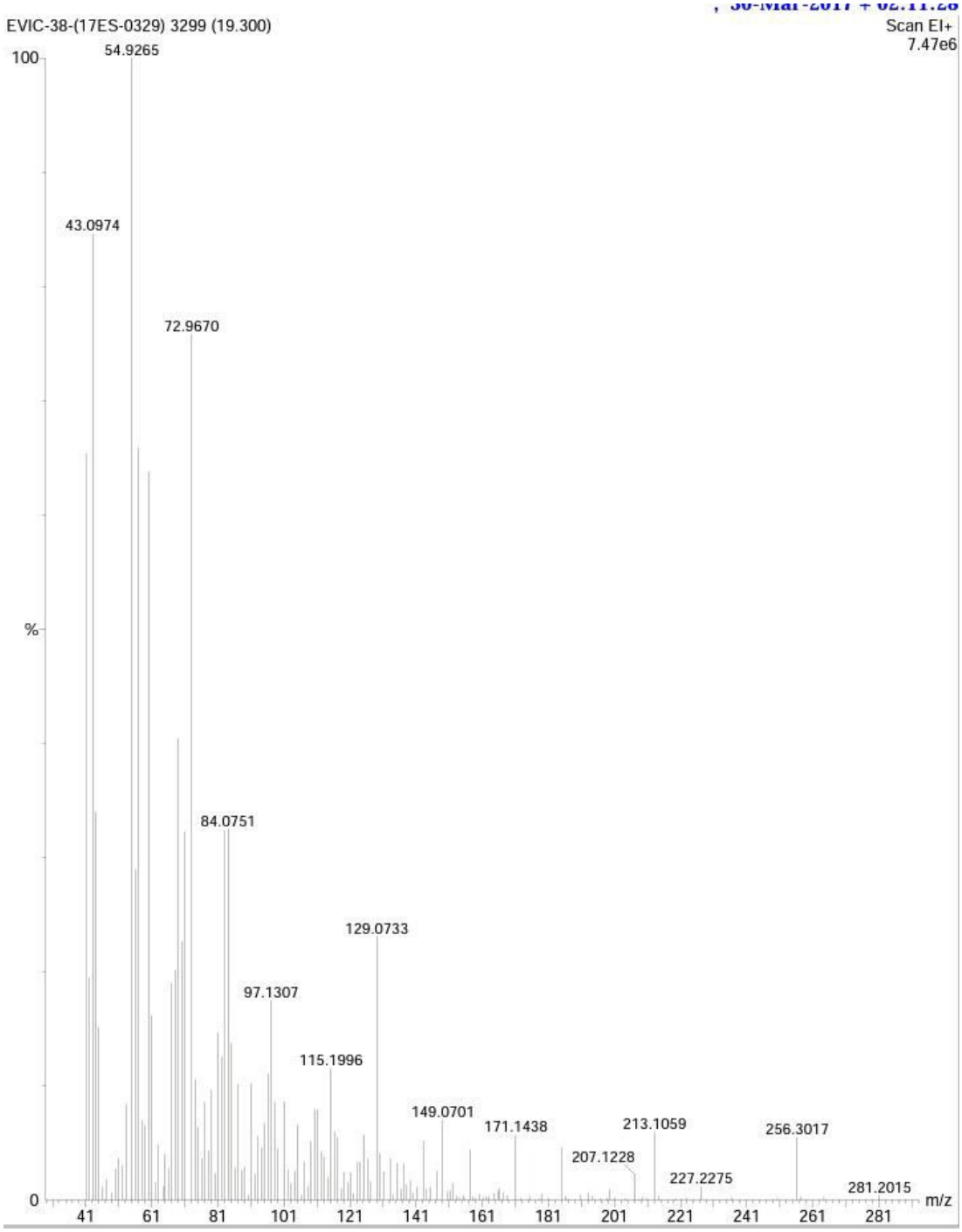
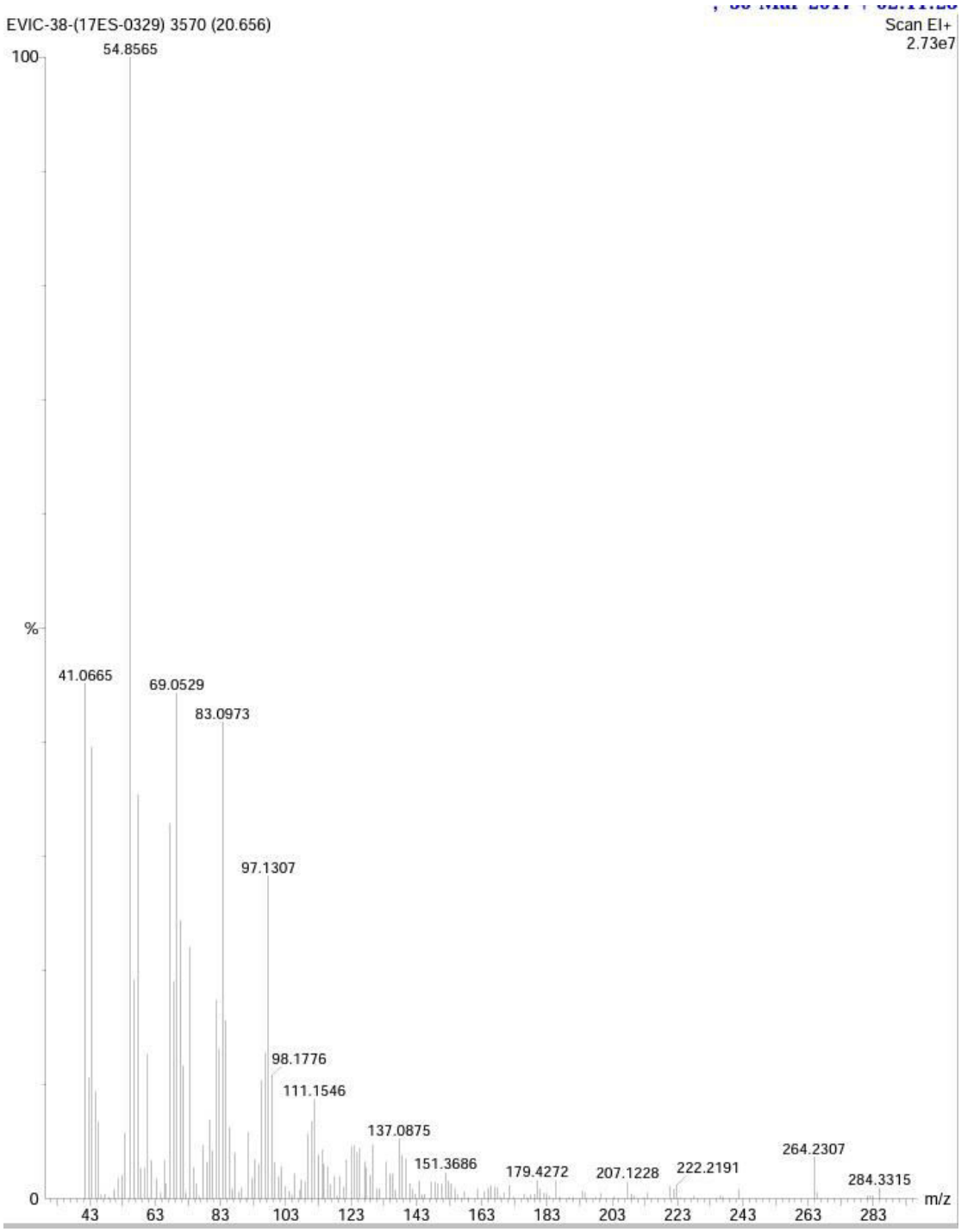


FIG 66. MASS SPECTRUM OF ERUCIC ACID



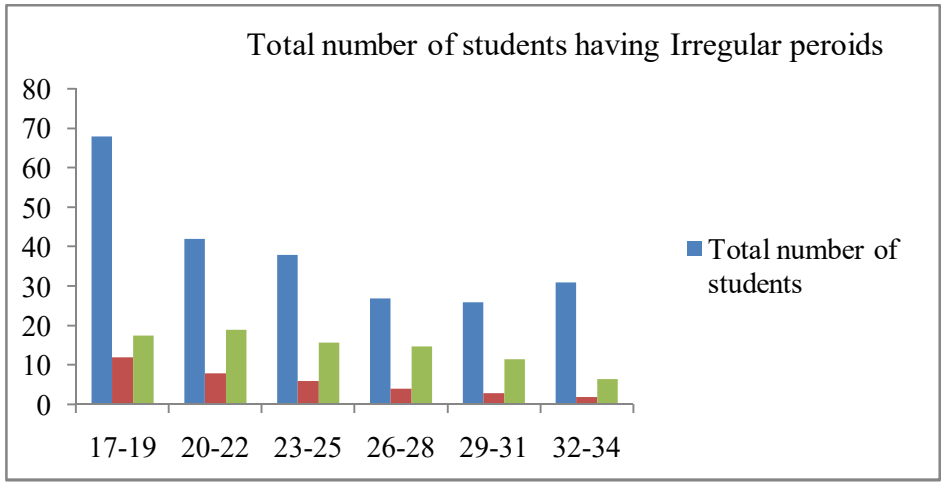
SURVEY OF POLYCYSTIC OVARIAN DISEASE

The study population comprised about 232 girls. The study reveal that among 68 girls of 17-19 years of age group 8 of them were with symptoms of PCOD. About 42 girls of 20-22 years age group 7 girls were with symptoms of PCOD. About 35 girls of 23-25 years age group 5 girls were with symptoms of PCOD. About 27 girls of 26-28 years age group 4 girls were with symptoms of PCOD. About 26 girls of 29-31 years age group 2 girls were with symptoms of PCOD. About 31 girls of 32-35 years age group 1 girls were with symptoms of PCOD.

Table 37 : Total number of Students Having Irregular Periods

S.no	Age	Total number of students studied	Total no students having irregular periods	% of students with PCOS
1	17-19	68	12	17.6
2	20-22	42	8	19
3	23-25	38	6	15.7
4	26-28	27	4	14.8
5	29-31	26	3	11.5
6	32-34	31	2	6.45

Fig. 57 Total Number Of Students Having Irregular Periods

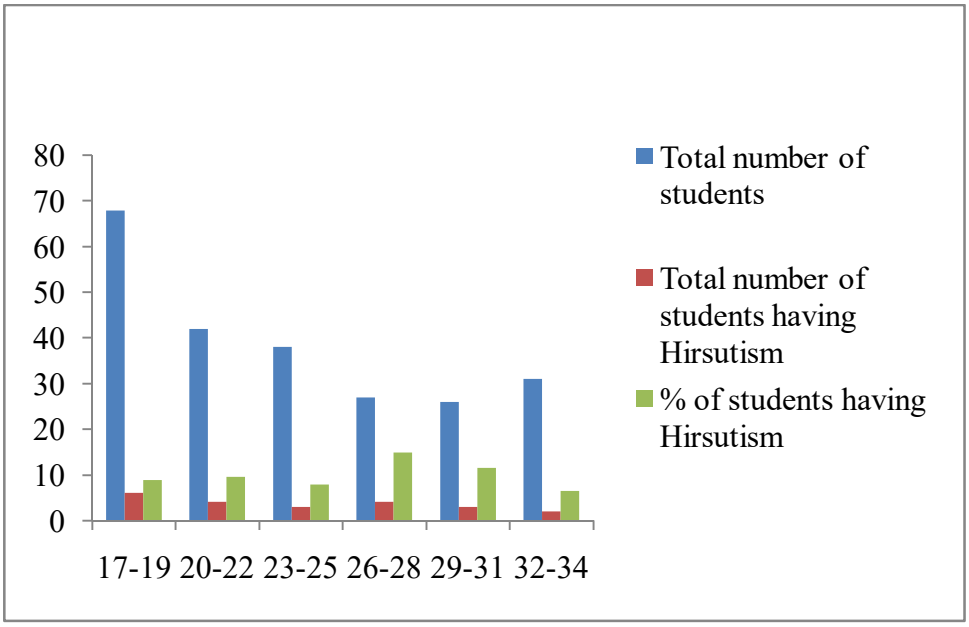


Totally 35 girls in different age group to be having irregular menstrual cycles. 27 girls were detected with polycysts in their ovaries.

Table 38 : Total Number Of Students Having Hirsutism

S.no	Age	Total number of students	Total number of students having Hirsutism	% of students having Hirsutism
1	17-19	68	6	8.8
2	20-22	42	4	9.52
3	23-25	38	3	7.89
4	26-28	27	4	14.81
5	29-31	26	3	11.5
6	32-34	31	2	6.45

Fig. 58 Total Number Of Students Having Hirsutism

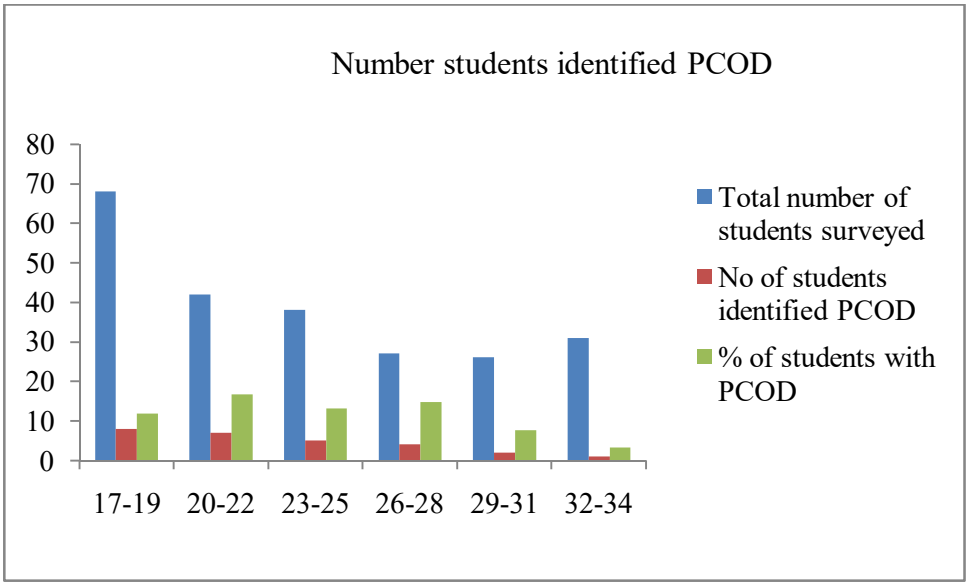


22 girls were found to be with Hirsutism

Table 39 : Number of students Identified with PCOD Syndrome:

S.no	Age	Total number of students surveyed	No of students identified PCOS	% of students with PCOS
1	17-19	68	8	11.76
2	20-22	42	7	16.6
3	23-25	38	5	13.15
4	26-28	27	4	14.81
5	29-31	26	2	7.69
6	32-34	31	1	3.22

Fig. 59 Number Of Students Identified with PCOS

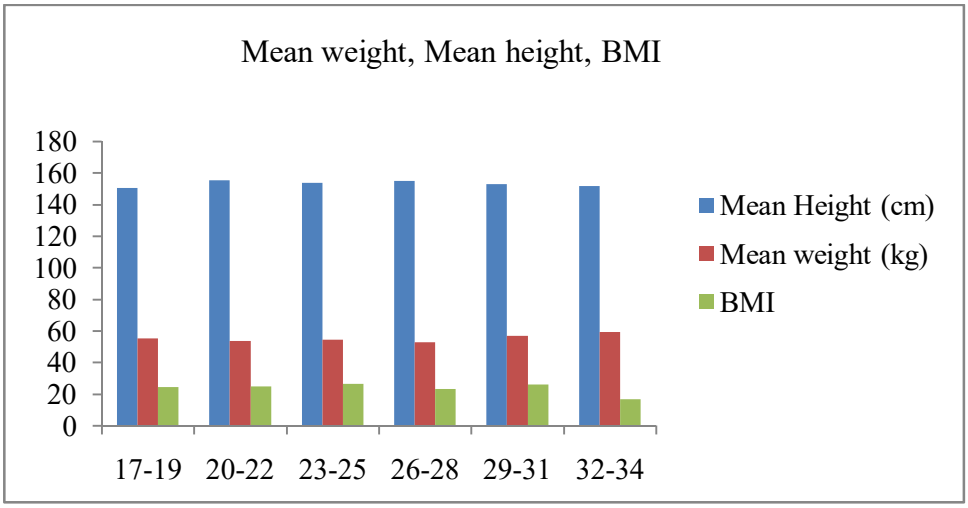


Number students identified PCOS: 27

Table 40 : Mean weight (kg), Mean Height (cm), BMI

S.no	Age	Mean Height (cm)	Mean weight (kg)	BMI
1	17-19	150.6	55.41	24.5
2	20-22	155.25	53.62	25.28
3	23-25	153.6	54.74	26.8
4	26-28	155	53	23.4
5	29-31	152.75	57.12	26.1
6	32-34	151.8	59.27	17.12

Fig. 60 Mean weight (kg), Mean Height (cm), BMI



The mean BMI of girls in age groups 17–19, 20 – 22, 23 – 25, 26 – 28,29– 31, and 32 – 34 were 24.5, 25.28, 26.8, 23.4, 26.1 and 27.12 respectively.

DISCUSSION

PHARMACOGNOSTICAL STUDIES

Pharmacognostical studies play a key factor in establishing the authenticity of the plant material. The botanical identity of the leaves was established by examining its anatomical features.

The anatomical examination of Erythrina variegata Linn., leaves exhibited important microscopical features like vascular bundle, schlernchyma cells, parenchymatous cells, trichomes, stomata and prism like calcium oxalate crystals.

The leaf exhibits thick midrib and uniformly thick and smooth lamina. The midrib consists of wide, conical, short thump and wide semicircular abaxial part of the midrib. The midrib is 1.1mm thick and the abaxial semicircular part in 1mm wide.

The upper portion of midrib shows vascular bundles which has a cluster of large angular, thick walled cells. Three vertically elongated pholem units present in adaxial side of the leaf and sclerenchyma cells placed above the adaxial medium bundle.

In lower portion epidermis of the midrib consists of small squarish the cell walled cells 2mm wide, angular, thick walled and compact parenchyma cells.

In the midrib the calcium oxalate crystals are drusen located in the parenchyma cells inside the adaxial epidermal layer.

Powder microscopy showed the presence of paracytic stomata, glandular trichome, anticlinal walls, double cystolith these features can be employed for inter specific identity of drugs.

Leaf constant values such as stomatal number, stomatal index, vein islet number and vein termination number was carried out.

The linear measurements of length and width of the calcium oxalate crystals were carried by using stage micrometer and eye piece micrometer and the range for length and width of the calcium oxalate crystals is as follows Length 125µ, 298µ, 572µ and Width 12.8µ, 16.4µ, 26.8µ.

Histochemical reactions were carried out on the different sections of the leaves using various chemical reagents. The presence of lignin, alkaloid, starch is identified.

Physiochemical parameters are mainly used in judging the purity and quality of the powdered drug. Ash values of a drug given an idea of the earthy matter or inorganic elements and other impurities present along with the drug.

Ash values are mainly used in judging the purity and quality of the drug is the indicative of contamination, substitution and adulteration. The total ash usually consists of carbonate, phosphate and silicates.

Total ash was found to be 9.48±0.56%w/w. The acid insoluble ash indicates contamination with siliceous materials like earth and sand. The values was found to be 6.9±0.48%w/w.

The water soluble ash is the good indicator of water soluble salts. The value of water soluble ash was found to be 4.73±74%w/w. Sulphated ash is obtained by treatment with dil.sulphuric acid where the oxides are converted to sulphates. The values was found to be 5.6±0.75%w/w.

The alcohol soluble and water soluble extractives were found to be 41.37%w/w and 24.65%w/w respectively. Ether soluble extractive was found to be 11.54%w/w.

The alcohol soluble extractive value indicates the presence of considerable amount of polar organic salts present in the plant. These constants would help to identify and to standardize the plant by future researchers.

Loss on drying determines the amount of moisture content of any kind that can be driven off under conditions specified.

The loss on drying was found to be 5.11%w/w. The leaf powder showed absence of foaming index, mucilage index and swelling index.

The qualitative analysis of heavy metals and in organic elements were carried out. The heavy metals are present within the limits.

The percentage compositions of different inorganic elements were determined. The presence of inorganic elements like, aluminium, chloride, calcium, iron, borate, potassium, nitrate and silver was found to be 0.016, 0.043, 0.034, 0.036, 0.006, 0.046, 0.012 and 0.008%w/w respectively.

The detailed Pharmacognostical studies on the leaves of *Erythrina vaiegata* Linn., provides information on the standardization parameters and physiochemical parameters which is essential for the identification of raw material and also used to differentiate the plant from its adulterants and substitutes.

PHYTOCHEMICAL STUDIES

Since herbal medicines are prepared from materials of plant origin they are sometimes to prone deterioration and variation in composition. Hence, before proceeding to clinical studies, scientists need a tool to authenticate plants and also to detect their potency.

A lot of analytical techniques have been developed for quality control of drugs from plant origin. Therefore it is very important to undertake Phytochemical investigations along with biological screening to understand therapeutic efficacy of medicinal plants and also to develop quality parameters.

In this analysis different polarity of Phytoconstituents were sorted out from the coarsely powdered leaves of *Eryhtrina variegata* Linn., by using solvents of increasing polarity like Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Aqueos by using successive solvent extraction.

Successive extractive values revealed the solubility and polarity participants of the metabolites in the leaf powder. Percentage yield of various extracts were as follows, Petroleum ether (6.8%w/w), Chloroform(5.7%w/w), Ethyl acetate (4.6%w/w), Ethanol (7.2%w/w) and Aqueous(3.5%w/w). Ethanolic extract showed high extractive yield among other extracts.

Qualitative Phytochemical analysis was performed initially with different respective chemical detecting agent to detect the phytoconstituents nature and their presence in each extract and powder.

Petroleum ether extract showed the presence of FLAVONOIDS, Alkaloids, fats and fixed oils. Chloroform extract showed the presence of FLAVONOIDS, Alkaloids,

fats and fixed oils, phenols. Ethyl acetate extract showed the presence of FLAVONOIDS, Alkaloids, steroids, phenols. Ethanol extract showed the presence of FLAVONOIDS, Alkaloids, steroids, phenolic compounds. The aqueous extract showed the presence of FLAVONOIDS, alkaloids.

Quantitative estimation of alkaloids, FLAVONOIDS, phenolic compounds and fluorescence analysis of extracts were carried out. Total alkaloid content, flavanoid content and phenolic content were done by ethanolic extract were done by values were found to be 8.72µg/ml, 5.46µg/ml, and 6.86µg/ml for content respectively. Fluoroscence characteristics o powdered sample and extract was done which showed absence of fluorescence chromophore.

Qualitative chromatographic analysis of all the five extracts were done using Thin Layer Chromatography to separate and identify the single or mixture of constituents present in each extract.

High Performance Thin Layer Chromatography (HPTLC) finger printing was performed with the Ethanol extract of the leaves of *Erythrina variegata* Linn., There were 9 peaks observed with different R_f values and different heights. Percentage of areas were also obtained from chromatogram.

Isolation of phytoconstituents was carried out using Column Chromatography to elute the individual compounds. They were further subjected for spectral analysis.

PHARMACOLOGICAL STUDIES

Pharmacological studies were carried out for assessing the Letrazole induced Polycystic Ovarian Disease activity of the plant *Erythrina variegata* Linn.,

The active extract was selected on the basis of *in vitro* anti oxidant activity. Accordingly the ethanolic extract with potential anti oxidant activity was selected fo*r in vivo* study.

Based on the acute toxicity studies, previously reported $1/10^{th}$ and $1/5^{th}$ (200 and 400 mg/kg) of the maximum tolerated dose (2000 mg/kg B.W) were selected for the *in vivo* studies.

The parameter studied were body weight, food intake, vaginal exfoliative cytology, Blood glucose, Lipid profile, reproductive organ weigh, histo-pathological examination of ovary and uterus was done.

The body weight of the different groups of rat were noted. The body weight of the Polycystic Ovarian Disease induced group shows in increase in body weight compared to the normal control group. After treatment the body weight of the Group IV and Group V was found to decrease in body weight. This was compared to the standard group which showed change in body weight. This shows that the ethanolic extract is effective in normalizing the enhanced body weight.

The food intake of different groups of rat were noted. The food intake of the Polycystic Ovarian Disease induced group shows in increase in food intake compared to the normal control group. After treatment the food intake of Group IV and Group V was found to decrease in food intake this was compared to the standard group which change in food intake. This shows that the ethanolic extract is effective in normalizing the enhanced body weight.

In the present study, Letrazole – aromatase inhibitor, was used to induce Polycystic Ovarian syndrome in female Wister rats. The working of this model was

confirmed by regular examination of vaginal smears and presence of persistent vaginal cornification.

The nature of cell types in the vaginal smear determines the stage of the estrus cycle. Letrazole-treated rats showed irregularity in its estrus cycle determination and which changed to normal sequence of estrous cycle after treating the rats with the Ethanolic extract of *Erythrina variegata* Linn., The restored estrus cycle indicating the recovery of anovulation to normal ovary functions.

The Blood glucose level was determined in all groups. The Blood glucose level was found to increase to a value of 152.83±0.92 in Disease control. Ethanol treated group 122.5±0.94mg/dl lowered the elevated level of glucose to 118.83±0.94 mg/dl. Which was compared to standard which showed a value of 118.83±0.94.

In the present study, lipid profile, PCOS induced groups showed notable increase in TC, TGs, LDL and decrease in HDL levels. Ethanolic extract of Erythrina variegata Linn., displayed antihyperlipidemic action by considerably decreasing the enhanced serum TC, TGs, LDL while increasing HDL levels. This showed that the ethanolic extract is effective in normalizing the enchanced lipid levels.

The ovary and uterus weight of the different groups of rat were noted. The ovary and uterus weight of Polycystic Ovarian Disease group shows in increase in value when compared to the normal control group. After treatment the Group IV and Group V was found to decrese in value. This was compared to the standard group which showed decrease in ovary and uterus weight due to the effect of standard drug. This shows that the ethanolic extract is effective in normalizing the enchanced ovary and uterus weight.

The histopathological results of control ovary show normal ovarian architecture with matured secondary follicles and oocyte. The fresh corpus luteum indicates the presence of previous ovulation.

Letrazole treated rats exhibited numerous subcapsular cysts, with a very thin or no granulosa layer. Corpora lutea were completely absent indicating anovulation and irregular estrus cycle. Few follicles were observed at their early stages of development. In addition, they were accompanied with atretic follicles containing fluid filled antrum and higher incidence of pyknotic granulosa cells.

Standared treatment led to disappearance of cysts and appearance of healthy follicles and corpora lutea.

Sections from Group IV of Ethanolic extract of Erythrina variegata Linn., (200mg/kg) group exhibited follicles large in size and many corpora lutea present. Also antral follicles with clearly differentiated oocyte, granulosa cell layer, corona radiate and theca cells were observed. Group V of Ethanolic extract of Erythrina variegata Linn., (400mg/kg) group showed secondary follicles with oocyte were visible in the histopathological results. It also showed a fresh and thick corpous luteum indicates ovulation.

In treated group decrease incidence of pkynotic granulose cells. Varying number of corpora lutea were seen suggesting ovulation and normal estrus cyclicity. Follicles at different stages of development with oocyte are clear, visible granulosa cell layer were observed. Ovarian cortex appeared normal with many follicles.

Isolation of compounds were done using Column Chromatography is done. The isolated compounds were identified using IR and GC – MS analysis and were found to be Erucic acid and N-Hexadecanoic acid.

This survey will explore the complex mechanism behind the manifestation of PCOS and how these, and other factors, may make diagnosis difficult. In order to thoroughly understand these aspects of PCOS, the causes, symptoms and treatments were explored.

SUMMARY AND CONCLUSION

Herbal medicines are found to be effective in treatment of various ailments but the major lacuna is lack of proper scientific validation. Hence the present study is aimed at investigating the selected plant *Erythrina variegata* Linn., for the Polycystic Ovarian Disease.

The plant *Erythrina variegata* Linn., belonging the family Fabaceae, is claimed to be useful for Emmenagogue, but the claim has not been scientifically validated.

Authentication of the plant material plays a key role in Pharmacognostical studies. The plant *Erythrina variegata* Linn., was collected from Karur district, Pillapalayam village and Authenticated by Prof. P. Jayaraman, Ph.D., Plant Anatomy Research Centre, Chennai.

The parameters studied were Macroscopy, Microscopy, Powder microscopy, Leaf constants, Linear measurements, Histochemical studies and Physiochemical constants to establish data for proper authentication and detection of adulterants.

The qualitative and quantitative analysis were carried out to identify inorganic elements present in the plant.

The qualitative and quantitative analysis of toxic heavy metals like Cadmium, Arsenic, Lead and Mercury were within the WHO limits and ensure the safety of the drug.

In Phytochemical analysis, extraction is the first step involved. The coarse powder was extracted by Petroleum ether, Chloroform, Ethyl acetate and Ethanol by

successive solvent extraction by hot percolation method and aqueous extract by cold maceration.

The preliminary Phytochemical screening of various extract of the plant have revealed the presence of constituents like alkaloids, flavanoids, steroids and fats & fixed oils.

Quantitative estimation of alkaloids, flavonids and phenolics compounds was done.

Fluoroscence analysis of powder and extract was done and it did not any fluorescent chromophore.

TLC and HPTLC analysis of Ethanolic extract was carried out to identify Phytoconstituents present.

In – vitro studies – DPPH assay and Hydrogen peroxide radical Scavenging assay were performed to select the most Bio – active extract. Based on this the ethanol extract was selected for *In-vivo* studies.

Acute toxicity studies revealed that the extract was safe up to the dose of 2000 mg/kg and the LD_{50} values in the range of 2000 mg/kg. Hence 200 mg/kg and 400 mg/kg was selected for the study.

Polycystic Ovarian disease was assessed by Letrazole induced Polycystic Ovarian disease method.

The parameters studied were Body weight changes, Food intake, Vaginal exolitative cytology, Blood sugar level, Lipid profile (TC, TGs, LDL, HDL), Reproductive organ weight and Histopathological studies. Ethanolic extracts showed

decrease in the elevated level of body weight, food intake, triglycerides, total cholesterol, LDL and reproductive organ weight and increase in the HDL level.

All the parameters revealed the effective role of ethanolic extract in Polycystic Ovarian disease activity which was comparable to the standard.

In the study of Polycystic Ovarian disease activity the Ethanolic extract shows significant activity in both *In – vitro* and *In – vivo* models.

Ethanolic extract of *Erythrina variegata* Linn., showed many beneficial effects similar to standard drug in treating PCOS condition and inducing ovulation.

These effects may be ascribed to its multiple pharmacological activities like estrogenic, antihyperlipidemic, antioxidant and hypoglycemic effects which could be useful in managing PCOS condition and prevent ovarian cell dysfunction, ovulation and thereby improving fertility.

Together broad spectrum biological effects of Ethanolic extract of *Erythrina variegata* Linn., make it a promising drug for treating clinical and pathological abnormalities in PCOD condition.

Isolation of compound using Column Chromatography was carried out to elute the individual compounds. They were further subjected for spectral analysis like IR and GC – MS. The isolated compound was proposed to be N-Hexadecanoic acid and Erucic acid.

Survey of Polycystic Ovarian Disease among the girls students and working women in the age group 17-34 years was done , to collect the data and statistical measurements done. The research on all of these aspects of PCOD will inform the public about how PCOD can affect them and their loved ones.

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