PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *PEDALIUM MUREX* LINN LEAVES

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MASTER OF PHARMACY

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Submitted by

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OCTOBER - 2017

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Pedalium murex* Linn Leaves,** submitted by **Ms.Vinitha. A [Reg.No.261515204]** to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** was evaluated by us during the examination held on ……………..

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Pedalium murex* Linn Leaves,** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry**, is a bonafide work carried out by **Ms.Vinitha. A [Reg.No.261515204]** during the academic year 2016-2017, under the guidance and direct supervision of **Dr. M. VIJAYABASKARAN, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutical Chemistry, J.K.K. Nattraja College of Pharmacy, Komarapalayam.

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DECLARATION

I hereby declare that the dissertation entitled **PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Pedalium murex* Linn Leaves,** has been carried out under the guidance and direct supervision of **Dr. M. Vijayabaskaran, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutical Chemistry, J.K.K. Nattraja College of Pharmacy, Komarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** during the academic year 2016-2017.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associateship and fellowship or any other similar title.

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CONTENTS

Chapter	Title	Page No.
1	**Introduction**	1
2	**Plant Profile**	16
3	**Review of Literature**	19
4	**Aim and Plan of Work**	29
5	**Materials and Methods**	31
6	**Results and Discussion**	43
7	**Summary and Conclusion**	63
8	**Bibliography**	65

LIST OF TABLES

Table No.	Title	Page No.
1	Major Plant Drugs Available for Anti-cancer	3
2	Data Showing the Extractive Values of Leaves of *Pedalium murex* Linn.	43
3	Preliminary Phytochemical Screening of *Pedalium murex* Linn. Leaves	44
4	Total Antioxidant Activity of *Pedalium murex* Linn Leaves	45
5	DPPH Radical Scavenging Activity of *Pedalium murex* Linn Leaves	46
6	Thin Layer Chromatography of EEPM	48
7	Column Chromatography of EEPM	49
8	Chemical tests for EEPM 1	50
9	Physical Characters of EEPM 1	50
10	Chemical tests for EEPM 2	50
11	Physical Characters of EEPM 2	50
12	% Cell Inhibition of EEPM in MCF-7 Cell Line	60
13	% Cell Inhibition of EEPM in HeLa Cell Line	60

LIST OF FIGURES

Table No.	Title	Page No.
1	**The Cell Cycle**	**10**
2	***Pedalium murex* Linn.**	**16**
3	**Total antioxidant activity of *Pedalium murex* Linn. Leaves**	**46**
4	**DPPH Radical Scavenging Activity of *Pedalium murex* Linn Leaves**	**47**
5	**Thin Layer Chromatography of EEPM**	**47**
6	**Column Chromatography of EEPM**	**48**
7	**Thin Layer Chromatography of EEPM 1**	**51**
8	**Thin Layer Chromatography of EEPM 2**	**51**
9	**IR Spectrum of isolated compound EEPM 1**	**52**
10	**IR Spectrum of isolated compound EEPM 2**	**53**
11	**^{1}H NMR Spectrum of isolated compound EEPM 1**	**54**
12	**^{1}H NMR Spectrum of isolated compound EEPM 2**	**55**
13	**Mass Spectrum of isolated compound EEPM 1**	**56**
14	**Mass Spectrum of isolated compound EEPM 2**	**57**
15	**% Cell Inhibition of EEPM in MCF-7 Cell Line**	**61**
16	**% Cell Inhibition of EEPM in HeLa Cell Line**	**61**

1. INTRODUCTION

HERBAL MEDICINES

Herbal medicines are "plant-derived resources or products with therapeutic or other human health benefits which contain either raw or processed ingredients from one or more plants". As per WHO definition, there are three kinds of herbal medicines: raw plant materials, processed plant materials and medicinal herbal products. The earliest recorded evidence of herbal medicine use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts on herbal medicines include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita.[1]

IMPORTANCE AND SCOPE

Herbs are staging a comeback and herbal 'renaissance' is happening all over the world. The herbal products symbolize safety in contrast to the synthetics that are regarded as unsafe to social and environment. Although herbs had been priced for their medicine, flavoring and aromatic qualities for eras, the synthetic products of the modern age beaten their importance.[2]

Herbal medications are prepared from a variety of plant material such as leaves, stems, roots, bark, etc., They generally contain numerous biologically active constituents and are used primarily for treating mild or chronic sicknesses. Herbal remedies can also be bought in the form of pills, capsules or powders, or in more concentrated liquid forms called extracts and tinctures. They can apply topically in creams or ointments, soaked into cloths and used as compresses, or applied directly to the skin as poultices.[3]

Plants are considered to be medicinal if they possess pharmacological activities of possible therapeutic use. The activities are often known as a result of millennia of trial and error, but they have to carefully investigate if we wish to develop new drugs that meet the criteria of modern treatment.[2]

The identification of the active principles of medicinal plants and investigation of the extracts in order to ensure that they are safe, effective, and of

constant activity. The isolation these active principles and determination of their structure, in order that they may be synthesized, structurally modified, or simply extracted more efficiently.[4]

Ayurveda was derived from the two Sanskrit words - Ayus, or "life" and Veda, or "knowledge". Ayurveda has been translated as "the knowledge of life" and as "the science of life". It has been suggested but one modern writer that a more appropriate translation would be "the knowledge of life span".[4]

Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and / or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, degoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, among others.[2]

In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesized economically, the product must be obtained from the cultivation plant material. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available (Table No. 1). The scientific study of traditional medicines, derivation of drugs through bio prospecting and systematic conservation of the concerned medicinal plants are thus of great importance.[2]

Table No. 1

Drug	Plant	Use
Vinblastine	*Catharanthus roseus*	Anticancer
Vinblastne	*Carathus roseus*	Anticancer
Ajmalaci	*Catharanthus roseus*	Anticancer, hypotensive
Rescinnamine	*Rawvolfia serpentine*	Tranquilizer
Reserpine	*Rawvolfia serpentine*	Tranquilizer
Quninine	*Cinchona sp*	Antimalaria, amoebic dysentery
Pilocarpine	*Pilocarpus jaborandi*	Antiglucoma
Cocai	*Erythroxylumcoc*	Topical anaesthetic
Morp	*Papaver somn*	Painkiller
Codein	*Papaver somnifer*	Anticough
Atropi	*Atropa bellad*	Spasmolytic, cold
Atropi	*Hyoscyamis niger*	Spasmolytic, cold
Cardiac	*Digitalis sp*	For congestive heart failure
Glycosides Artemisinin	*Artemesia annua*	Antimalarial
Taxol	*Taxusbaccata T.brevifolia*	Brest and ovary cancer, antitumour
Berberine	*Berberis*	For leishmaniasis
Pristimerin	*Celastru spaniculata*	Antimalarial
Ricin	*Ricinus communis*	-
Digitoxin, Digoxin	*Digitalis, Thevetia*	Cardio tonic
Thevenerin	*Thevetia*	Cardio tonic
Catechin	*Acacia catechu*	Antiulcer
Indicine N-oxide	*Heliotropium indicum*	Anticancer
Elipticine	*Ochrosia*	Anticancer

Advantages of Herbal medicine

1) Herbal medicine have long history of use and better patient tolerance as well as acceptance.

2) Medicinal plants have a renewable source, which is only hope for sustainable supplies of cheaper medicines for the world growing population.

3) Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.

4) The cultivation and processing of medicinal herbs and herbal products is environmental friendly.

5) Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.

Throughout the world, herbal medicine has provided means of the potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form and as a pure chemical upon which modern medicines are structured.[5, 6]

WHO guidelines for herbal medicines

In 1992, the WHO Regional Office for the Western Pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines (WHO, 1993). This group recognized the importance of herbal medicines to the health of many people throughout the world, stating: 'A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health.[7]

WHO has also issued Guidelines for the Assessment of Herbal Medicines (WHO, 1996). These guidelines defined the basic criteria for the evaluation of quality, safety and efficacy of herbal medicines with the goal of assisting national regulatory authorities, scientific organizations and manufacturers in assessing documentation, submissions and dossiers in respect of such products. It was recommended that such assessments take into account long-term use in the country (over at least several decades), any description in the medical and pharmaceutical literature or similar

sources or documentation of knowledge on the application of a herbal medicine, and marketing authorizations for similar products.[7]

The role of herbal medicines in traditional healing

The pharmacological treatment of disease began long ago with the use of herbs Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose.[8]

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials).

Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may include powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term “mixture herbal product” can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including

synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal.

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.

Therapeutic activity refers to the successful prevention, diagnosis and treatment of physical and mental illnesses. Treatment includes beneficial alteration or regulation of the physical and mental status of the body and development of a sense of general well-being as well as improvement of symptoms.

Active ingredients refer to ingredients of herbal medicines with therapeutic activity. Where the active ingredients have been identified, the preparation of the finished herbal product should be standardized to ensure that it always contains a defined amount of the active ingredients, providing adequate analytical methods are available. In cases where it is not possible to identify the active ingredients, the whole herbal medicine may be considered as one active ingredient.

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The terms “complementary medicine”, “alternative medicine” and “non-conventional medicine” are used interchangeably with “traditional medicine” in some countries.[3]

CANCER

Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Cancer chemotherapeutic

agents can often provide temporary relief of symptoms, prolongation of life, and occasionally cures.[9]

In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects when undergoing treatment. Synthesis of modifications of known drug continues as an important aspect of research. However, a waste amount of synthetic work has given relatively small improvements over the prototype drugs. There is a continued need for new prototype-new templates to use in the design of potential chemotherapeutic agents: natural products are providing such templates. Recent studies of tumor-inhibiting compound of plant origin have yielded an impressive array of novel structures. Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells;

- Uncontrolled proliferation
- Dedifferentiation and loss of function
- Invasiveness
- Metastasis.[9]

History[10]

Today, carcinoma is the medical terms for a malignant tumor derived from epithelial cells. It is Celsus who translated carcinos into the Latin cancer, also meaning crab. Galen used “oncos” to describe all tumors, the root for the modern word oncology. Hippocrates descrbied several kinds of cancers. He called benign tumours oncos, Greek for swelling, and malignant tumours carcinos, Greek for crab or crayfish. This name probably comes from the appearance of the cut surface of a solid malignant tumor, with a roundish hard centre surrounded by pointy projections, vaguely resembling the shape of a crab. He later added the suffixoma,

Greek for swelling, giving the name carcinoma. Since it was against Greek tradition to open to body, Hippocrates only described and made drawings of outwardly visible tumors on the skin, nose, and breasts. Treatment was based on the humor theory of four bodily fluids (black and yellow bild, blood, and phlegm). According to the patient's humor, treatment consisted of diet, blood-letting, and/or laxatives. Through the centuries it was discovered that cancer could occur anywhere in the body, but humor-theory based treatment remained popular until the 19th century with the discovery of cells.

CAUSES OF CANCER [11, 12]

Modern medicine attributes most cases of cancer to changes in DNA that reduce or eliminate the normal controls over cellular growth, maturation, and Programmed cell death. These changes are more likely to occur in people with certain genetic backgrounds (as illustrated by the finding of genes associated with some cases of cancer and familial prevalence of certain cancers) and in persons infected by chronic viruses (e.g., viral hepatitis may lead to liver cancer; HIV may lead to lymphoma). The ultimate cause, regardless of genetic propensity or viruses that may influence the risk of the cancer, is often exposure to carcinogenic chemicals (including those found in nature) and/or to radiation (including natural cosmic and earthly radiation), coupled with a failure of the immune system to eliminate the cancer cells at an early stage in their multiplication.

The immunological weakness might rise years after the exposure to chemicals or radiation. Other factors such as tobacco smoking, alcohol consumption, excess use of caffeine and other drugs, sunshine, infections from such oncogenic virus like cervical papillomaviruses, adenoviruses Kaposi's sarcoma (HSV) or exposure to asbestos. These obviously are implicated as causal agents of mammalian cancers. However a large population of people is often exposed to these agents. Consequently cancer cells continue to divide even in situations in which normal cells will usually wait for a special chemical transduction signal. The tumor cells would ignore such stop signals that are sent out by adjacent tissues. Cancer cell also has the character of immortality even in vitro whereas normal cells stop dividing after 50-70 generations and undergoes a programmed cell death (Apoptosis). Cancer

cells continue to grow invading nearby tissues and metastasizing to distant parts of the body. Metastasis is the most lethal aspect of carcinogenesis.

TYPES OF CANCERS [9]

1) Cancers of Blood and Lymphatic Systems:

a) Hodgkin's disease, b) Leukemias,

c) Lymphomas d) Multiple myeloma,

e) Waldenstrom's disease

2) Skin Cancer:

a) Malignant Melanoma

3) Cancers of Digestive Systems:

a) Esophageal cancer, b) Stomach cancer,

c) Cancer of pancreas, d) Liver cancer,

e) Colon and Rectal cancer f) Anal cancer

4) Cancers of Urinary system:

a) Kidney cancer b) Bladder cancer

c) Testis cancer d) Prostate cancer

5) Cancers in women:

a) Breast cancer b) Ovarian cancer

c) Gynecological cancer d) Choriocarcinoma

6) Miscellaneous cancers:

a) Brain cancer b) Bone cancer

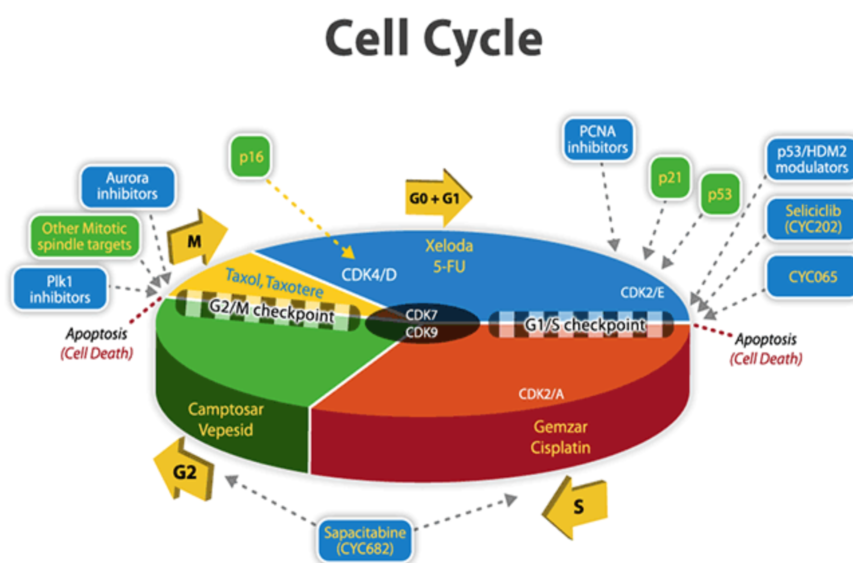
c) Carcinoid cancer d) Nasopharyngeal cancer,

e) Retroperitoneal sarcomas f) Soft tissue cancer

g) Thyroid cancer

CELL CYCLE

Fig.No.1 The Cell Cycle



Cellular multiplication involves passage of the cell through a cell cycle. The various phases of the cell cycle are characterized as : (i) the interval following cell division to the point where DNA synthesis starts, known as the pre-synthetic phase (G1). The variability in the length of the cell cycle between rapidly and slowly replicating cells is accounted by the differences in the length of (G1) phase; (ii) after mitosis some of the daughter cells pass into a resting phase or non-proliferative phase (G0), and do not re-enter the cell cycle phase G1 immediately. The may enter the G1 phase later. The G0 phase is the sub phase of G1; (iii) the DNA synthesis(s) occurs; (iv) the pre mitotic or post synthetic (G2) phase follows. In the phase RNA and protein synthesis take place, and it is shorter than the S phase; and (v) lastly

mitotic (M0 phase follows, in which the synthetic activity of the cell is low the chromosomes separate in two daughter cells through the sub phase-prophase, metaphase, anaphase and telophase. These daughter cells have the option of either entering the G1 phase or the G0 sub phase of G1 phase.

Cancers are caused by a series of mutations. Each mutation alters the behaviour of the cell somewhat. Carcinogenesis, when means the initiation or generation of cancer, is the process of derangement of the rate of cell division due to damage to DNA. Proto-oncogenes are genes which promote cell growth and mitosis, a process of cell division, and tumor suppressor genes discourage cell growth, or temporarily halt cell division in order to carry out DNA repair. Typically, a series of several mutations to these genes are required before a normal cell transforms into a cancer cell.

Proto-oncogenes promote cell growth through a variety of ways. Many can produce hormones, a "chemical messenger" between cells which encourage mitosis, the effect of which depends on the signal transduction of the receiving tissue or cells. Some are responsible for the single transduction system and signal receptors in cells and tissues themselves, thus controlling the sensitivity to such hormones. They often produce mitogens, or they are involved in transcription of DNA in protein synthesis, which creates the proteins and enzymes responsible for producing the biochemical cells use and interact with Mutation sin proto-oncogenes can modify their expression and function, increasing the amount or activity of the product protein. When this happens, they become oncogenes, and thus cells have a higher chance to divide excessively and uncontrollably. The chance of cancer cannot be reduced by removing proto oncogenes from the genome as they are critical for growth, repair and homeostasis of the body.[13]

Chemotherapy [14, 15]

Chemotherapy plays a significant role in the treatment of early stage disease, in the pro-operative period and as adjuvant therapy for the treatment of micro metastasis. As knowledge has been accumulating in the area of pharmacology, tumor biology, cytokineties and resistance, therapeutic strategies have been developed that maximize the tumor-cell kill, decrease resistance and enhance the

potential for cure by chemotherapy. The antineoplastic armamentarium currently contains over 30 drugs, with many additional agents under investigation. Human pituitary growth hormone, prostaglandins, cyclin-AMP, RNA-dependent DNA polymerase, etc also show promising results.

Since the differences between normal and neoplastic human cells are merely quantitative rather than qualitative, most antineoplastic drugs are associated with certain side effects. The toxicity usually involves attack of drugs on rapidly proliferating normal body tissues such as bone marrow, hair follicles and intestinal epithelium. In addition, individual drug may produce its own distinctive toxic effects on heart, lungs, kidneys and other organs. Hence with some exceptions it can be said that the antineoplastic agents are generally palliative and not curative. Many anti-cancer drugs (a) have a very narrow therapeutic index (b) is highly unable (c) are effective at very low concentration and (d) having unusual metabolic pathways.

Plants in the Treatment of Cancer [16]

In the face of failure to fine synthetic drugs against cancer, thousands species of plants have been screened since a long time, for antineoplastic activity, in the hope of discovering effective natural products. Compounds have been evaluated. Such work is still going on in several laboratories throughout the world. The Natural Product Drug Development Program of the U.S. National Cancer Institute has identified about 3000 species of plants and animals as useful in dealing with one or the other aspect of cancer management. Based on in vitro data, a large number of species have been identified to be of promise and taken to clinical trials. However, products of hardly a handful of plant species, such as the Vinca alkaloids, Taxol, Camptothecin.

Podophyllotoxin, etc., have passed through the rigorous tests to be officially used against certain types of cancer and are now available in the market. Yet there are severe problems associated with the use of even these largely ‘successful’ drugs, which are among the most expensive plant products. This reflects the complexity of the scenario of cancer drugs in general and plant base.

Evaluation of anticancer studies[17]

Anticancer drugs can be evaluated by *in vitro* and *in vivo* methods.

In vitro methods

Cytotoxicity: There is much pressure, both human and economic, to perform at least part of cytotoxicity testing *in vitro.* Currently it is difficult to monitor systematic and physiological effects *in vitro,* so most assay determine effects at cellular level, or cytotoxicity broadly involve the metabolic alternation of the cells, including the death of cells as a result of toxic effects of the compounds.

The choice of assay will depend on agent on study, the nature of response, and the particular target cell. In anticancer research, the *in vitro* screening involves estimation of cytotoxicity of the drug by different methods. The commonly used methods of studying cytotoxicity are.

- **Determination of cell viability by Tryphan blue dye exclusion method**

In this method, viability dyes such as tryphan blue is used to determine membrane integrity. Staining for viability assessment is more suited to suspension culture than to monolayer, because dead cells detach from the monolayer and are therefore lost from the assay. The method has been applied with equal success to solid tumor, effusions and haematological malignancies.

- **Determination of cell viability by uptake of neutral red dye by the lysosome in neutral red assay.**

The uptake of neutral red by lysosome and golgibodies has been used to quantitative cell number. The satin appears to be specific for viable cells, but the main limitation of the method is the difference in uptake between cell types. Thus some cell types. Thus some cell types, such as activated macrophages and fibroblast take up amount very rapidly where as others, such as lymphocytes, show negligible staining.

- **Determination of cell metabolic function by protein estimation**

Several methods are available for measuring the protein contents of cell monolayers. These include the use of Folin-Ciocatechu reagent according to the method of Lowry and amino black.

- **Determination of quantitative value for the loss of cell viability by measurement of lactate dehydrogenase activity by LDH assay.**

The measurement of lactate dehydrogenase in culture supernatant gives a quantitative value for the loss of cell viability.

$$\text{Pyruvate} + \text{NADH} + \text{H} + \Leftrightarrow \text{NAD}^{+} + \text{lactate}$$

The activity of LDH can be measured as the reduction of Pyruvate to lactate. The reduction is coupled to the oxidation of NADH to NAD, which is followed spectrophotometrically at 340nm.

- **Sulphoromamine B (SRB) assay.**

SRB is a pink amino xanthine dye with two sulfonic groups. Under mild acidic condition, SRB binds to protein basic amino acid residue in (trichloroacetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude. Colour development of SRB assay is rapid, stable, and visible. The developed colour can be measured over a broad range of visible wavelength in 90 – microtitre plate readers.

- **Cytotoxicity by Micro culture Tetrazolium (MTT) assay**

The ability of the cell to survive a toxic insult has been the basis of most Cytotoxic assays. This assay is based on the assumption that dead cell or their products do not reduce Tetrazolium salt (3-(4, 5 dimethyl thiazole – 2yl) –2, 5 – diphenylTetrazolium bromide) into a blue colored product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cell are found to be proportional to the extent of formazan production by cells used.

***In vivo* methods**

- Fibro sarcoma solid tumor model.
- EAC model (Ehrlich ascities carcinoma).
- Chemically induced cancer model.
- Virus induced cancer model.

2. PLANT PROFILE

Fig. No. 2 : *Pedalium murex* Linn.

Taxonomical classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Lamidae
Order	:	Caryophyllales
Family	:	Pedaliaceae
Genus	:	Pedalium
Species	:	murex
Binomial name	:	***Pedalium murex* Linn.**

Vernacular Names

Tamil	:	Yanai Nerunjil
English	:	Large Caltrops
Hindi	:	Bada Gokshur
Sanskrit	:	Brihat Gokshur
Kannada	:	Ane Neggilu
Malayalam	:	Ana Nerinnil
Oriya	:	Gokara
Marathi	:	Gokharu
Gujarati	:	Kadva Gokhru

Habitat:

It is distributed geographically in tropical Africa, Ceylon, India, Mexico and Pakistan. It is a common herb which grows throughout India but it is found commonly along the western and coromandal coasts as a weed of waste places. It is also found in Delhi, Rajasthan, Punjab, Tamil Nadu, Gujarat and Deccan peninsula.

Description:

Large Caltrops is a shrubby, stiff-stemmed herb native to India and grown for reputed medicinal and other uses. It is diffused, annual, much branched, spreading, succulent, and glandular up to 60 cm in height.

Leaves:

Leaves are simple, opposite, ovate or oblong-obovate, 1-4.5 cm long, irregularly and coarsely crenate-serrate.

Roots:

Roots are similar to turmeric in colour.

Flowers:

Yellow flowers 1.5-2 cm across, stalk 1-2 mm long, increasing up to 4 mm in fruit. Sepal's are2 mm long; Teeth linear, scaly outside, persistent. Petals are fused into a broad tube, 1-3 cm long; lobes obtuse. Stamens 0.5-1 cm long; anthers are kidney shaped.

Seeds:

The four angled seed is with 5 extremely sharp spines. It is an important famine food - leaves eaten as vegetable.

Chemical Constituents:

Fruit : Alkaloids 3.5% – 5%, stable oil, aromatic oil, resins, glycosides, carbohydrates, saponins and triterpenoids.

Stem : Saponins, phytosterols, tannins and carbohydrates.

Root : Reducing sugars, phenolic compounds, saponins, xanthoproteins, alkaloids, triterpenoids and flavonoids.

Leaves : Flavonoids, alkaloids, steroids, resins, saponins and proteins.

Medicinal Uses:

- Nervine weakness, Pains, Inflammation, Indigestion, Piles, Constipation, Heart related problems, Cough, Asthma, Epitasis, Frigidity, Impotence, Renal calculi, Dysurea, Infections.
- Leaves are antibilious. Seeds are demulcent, diuretic, tonic, mucilaginous and aphrodisiac. Used in male impotence, gonorrhoea, and incontinence.

3. REVIEW OF LITERATURE

1. **Patel PK et al., (2016)** studied the antiurolithiatic effect of *Pedalium murex* fruit extract in ethylene glycol-induced nephrolithiasis in rats. Ethanol extract of *Pedalium murex* showed significant improvement in renal function and kidney weight in prophylactic groups as compared to ethylene glycol controls. No effects were shown on urinary oxalate, urine volume and any other serological parameters. Calcium oxalate crystallization was significantly reduced in all the *Pedalium murex* treated groups ($P < 0.05$). Ethanol extract of *Pedalium murex* fruits possessed significant activity for prevention of renal calculi.[18]

2. **Vaya Rajkumar et al., (2016)** had studied antiulcer activity of fresh juice of the leaves of *Pedalium murex.* The results obtained from the study showed that fresh juice of the leaves of *Pedalium murex* possessed antiulcer effect on ethanol induced ulcers. In ethanol and stress induced model, there was a decrease in ulcer index, total acidity, total volume of gastric secretion, total protein and an increase in glutathione content and pH of gastric secretion when compared with control. In the study famotidine was used as a standard. Therefore the ethanol extract of leaves of *Pedalium murex* was regarded as a favourable antiulcerogen.[19]

3. **Prabhakaran D et al., (2016)** had investigated antimicrobial activity of *Pedalium murex* (Flowers). The purpose of the study was to examine the antimicrobial effect of the sample isolated from the ethyl acetate fraction of flowers of *Pedalium murex.* This compound was shown to possess antimicrobial activity against bacteria and fungi. Six bacterial strains were *Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus substilis, Lacto bacillus* and two fungal strains *Curvularia lunata and Candida albicans* using disc diffusion method. The antibacterial activity of the compound isolated from ethyl acetate fraction was almost comparable with the standard solvent control Chloramphenicol. The antifungal activity was almost comparable with the standard solvent control Fluconazole. From this study, it was concluded that *Pedalium murex* (flowers)exposed antimicrobial activity against various human pathogenic bacteria.[20]

4. **Anandalakshmi K et al., (2015)** had studied characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. In the study, an aqueous extract of fresh leaves of *Pedalium murex* was used for the synthesis of silver (Ag) nanoparticles. Different biological methods gained recognition for the production of silver nanoparticles (AgNPs) due to their multiple applications. The use of plants in the green synthesis of nanoparticles emerged as a cost-effective and eco-friendly approach. Characterization of nanoparticles were done using different methods, which included ultraviolet–visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), field emission scanning electron microscope (FE-SEM), energy dispersive X-ray analysis (EDAX), fluorescence emission spectroscopy, transmission electron microscope (TEM), dynamic light scattering (DLS), zeta potential and anti-bacterial activity. UV–visible spectrum of the aqueous medium contained silver nanoparticles which showed absorption peak at around 430 nm. Fourier transform infrared spectra had shown that the biomolecule compounds were responsible for the reduction and capping material of silver nanoparticles. XRD study showed that the particles were crystalline in nature, with a face-centered cubic (fcc) structure. The size and stability were detected using DLS and zeta potential analysis. The anti-bacterial activity of AgNPs against generally found bacteria was assessed to find their potential use in silver containing antibacterial product.[21]

5. **Ajit N Solanki et al., (2015)** had described antihepatotoxic effect of ethanol extract of *Pedalium murex* fruits. The fruit powder of *P. murex* was extracted with 20% ethanol. Acute oral toxicity study of ethanol extract of fruits of *P. murex* was performed on female Swiss albino mice. Antihepatotoxic effect of ethanolic extract of fruits of *P. murex* was assessed in ethanol induced hepatotoxic rats. LD_{50} value of extract was found more than 5 gm/kg on the basis of acute toxicity study. Administration of ethanol (3.76 gm/kg, bid, po) for 25 days produced significant changes in biochemical and histological (damage to hepatocytes) parameters of liver as well as endogenous antioxidant enzymes (SOD, GSH), reflecting liver damage. Pre-treatment with ethanol extract of fruits of *P. murex* (200 and 400 mg/kg, bid, p.o) 1 hr before ethanol administration for

25 days significantly improved the biochemical, histological changes and endogenous antioxidant enzyme levels induced by ethanol. Results suggested that the ethanolic extract of fruits of *P. murex* protected the livers of rats against ethanol induced hepatic damage.[22]

6. **Muhammad Imran et al., (2015)** had reviewed phytochemical and pharmacological potentials of *Pedalium murex* Linn and its traditional medicinal uses. The aim of the study was to assess the pharmacological and phytochemical aspects of the *Pedalium murex* Linn and its traditional medicinal uses from different parts of the plant. Flavonoids, phenolic compounds, glycosides, carbohydrates, reducing sugars, phytosterols, tannins, triterpenoids, alkaloids, xanthoproteins, aromatic oil, stable oil, saponins and resins are the main phytochemical groups that was found in different chemical extracts of *P. murex*. Pharmacological activities of *P. murex* proved its importance for medicinal uses.[23]

7. **Dhivya Met al., (2015)** had identified antioxidant activity and immune modulatory activity of *Pedalium murex*. The study was carried out for the characterization, physical, chemical, microbial and immunological strength of the development of immune system for human era. The phytochemical compounds were analyzed. Twelve male Wister albino rats (200-220gm) two months of age were used as experimental animals and were divided into four groups. In this animal model studies assessment of immune modulatory activity was carried out by various hematological and serological tests like determination of phagocytic activity and spleen weight. The study presented that different doses (100, 200 mg/kg b.w/day) of the ethanolic extract of the fruits showed significant activity and increased phagocytic response and spleen weight. The results were compared with control and standard drug (septilin).[24]

8. **Abirami P and Rajendran A, (2015)** had investigated the evaluation of anti dermatophytic activity of *Pedalium murex* Linn. In the investigation, different solvent extracts of *Pedalium murex* Linn were screened against two dermatophytes viz., *Trichophyton rubrum* and *Microsporum gypseum.* The prominent zone of inhibition was observed in methanol and petroleum ether

extract of *Pedalium murex* against in *Microsporum gypseum* at 200 µg/ml concentration. The distilled water and methanol extracts of *Pedalium murex* showed significant activity against *Trichophyton rubrum*. The standard drugs griseofulvin, fluconazole and ketoconazole were used as the positive control and zones of inhibition were from 10 to 20 mm.[25]

9. **Vedhi C et al., (2015)** had reported biosynthesis of gold nanoparticles using cold and hot water extract of *Pedalium murex* Linn leaf. Biosynthesis of gold nanoparticles was done using cold and hot water extract of *Pedalium murex* Linn leaf. Prepared gold nanoparticle was analyzed by UV-visible spectroscopy, cyclic voltammetric, XRD, SEM and TEM. UV studies showed well-built surface plasmon resonance absorption peak at 540 nm. The band gap energy of 2.95 eV and 2.90 eV was achieved for gold nanoparticles prepared by cold and hot water extract respectively. The cyclic voltammetric behaviour of both types of nanoparticles was studied at different pH. The XRD spectra for deposited thin film samples confirmed the crystalline nature and highly stable gold nanoparticles. SEM image showed that the nanoparticles were semi-spherical and their sizes were controlled within the range of 180 nm to 200 nm. Hexagonal, triangular, and spherical nanoparticles were seen in the transmission electron micrographs for both types of particles. The selected-area electron diffraction patterns revealed that the sample is semi crystalline (002) and (111) phase.[26]

10. **Shanmuga Sundaram R et al., (2014)** had reported evaluation of potential central protective role of ethanol extract of *Pedalium murex* Linn in acute and chronic unpredictable stress induced models in SD rats. Animals were divided into five groups each for AS and CUS models. The animals were subjected to AS (immobility-induced stress) and CUS paradigms (immobility + forced swimming + dark phase) for 10 days followed by a battery of behavioural and biochemical analyses. The changes in food and water intake, body weight and general behaviour were measured for 4 weeks. In addition, the effect of EEPM on the antioxidant enzyme systems [superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH)] in whole brain of animals, *in vitro* antioxidant and free radical scavenging activities were also screened. AS and CUS induced

anxiety, depression and impairment of cognition and memory. Pre-treatment with EEPM (200 mg/kg and 400 mg/kg p.o.) for 30 days, significantly reduced stress-induced anxiety and related mood disorders. The normalization of SOD, CAT and GSH levels further substantiated the protective role of EEPM. The herb was found to be effective in preventing CUS than the AS model.[27]

11. **Thangadurai Chitra et al., (2013)** had revealed laboratory and field efficacy of *Pedalium murex* and predatory copepod, *Mesocyclops longisetus* on rural malaria vector, *Anopheles culicifacies*. To test the potentiality of the leaf extract of *Pedalium murex* and predatory copepod *Mesocyclops longisetus* (*M. longisetus*) in individual and combination in controlling the rural malarial vector, *Anopheles culicifacies* (*An. culicifacies*) in laboratory and field studies. Predator survival test showed that the methanol extract of *Pedalium murex* is non-toxic to the predatory copepod, *M. longisetus*. Experiments were also conducted to evaluate the efficacy of methanolic extract of *Pedalium murex* and *M. longisetus* in the direct breeding sites (paddy fields) of *An. culicifacies*. Reduction in larval density was very high and sustained for a long time in combined treatment of *Pedalium murex* and *M. longisetus*.[28]

12. **Siva V et al., (2012)** had determined evaluation of antipyretic activity of *Pedalium murex* against brewer's yeast-induced pyrexia in rats. The aqueous and ethanolic extracts of *Pedalium murex* (Pedaliaceae) was investigated for antipyretic activity in rats using brewer's yeast-induced pyrexia models. brewer's yeast (15%) was used to induce pyrexia in rats. Both the extract (200 and 400 mg/kg b.w p.o) produced a significant ($p<0.05$) dose dependent inhibition of temperature elevation compared with the standard drug paracetamol (150mg/kg b.w). At doses of 200 mg/kg b.w, the aqueous extract significantly ($P<0.001$) decreased yeast induced pyrexia in rats. These results indicated that leaf extracts of *Pedalium murex* possessed potent antipyretic effects in the management of fever.[29]

13. **Sharma V et al., (2012)** had analyzed a comparative study of ethanol extracts of *Pedalium murex* Linn fruits and sildenafil citrate on sexual behaviours and serum testosterone level in male rats during and after treatment. Findings

provided experimental *in vivo* and *in vitro* evidence that the ethanolic extract of *Pedalium murex* fruits possessed aphrodisiac property.[30]

14. **Patel DK et al., (2012)** had reported aphrodisiac activity of ethanolic extract of *Pedalium murex* Linn fruit. The study represented an interesting case report for a very good aphrodisiac activity observed during an oral glucose tolerance test performed while evaluating the antidiabetic potential of *Pedalium murex* Linn fruit. Pregnancy was observed in the treated groups after 20-25 days of treatment in females which resulted in birth of pubs ranging up to ten in some females (more significant in case of 500 mg/kg p.o.). The observation also showed a significant increase in weights of pubs along with a normalbehaviour pattern. The increased pregnancy rate in the drug treated groups was due to the healthy viable sperm and enhancement of sexual desire of the rats.[31]

15. **Muruganantham Sermakkani et al., (2011)** had explored evaluation of phytochemical and antibacterial activity of *Pedalium murex* Linn root. In the study, petroleum ether, chloroform, acetone and methanol extract of *Pedalium murex* Linn root was subjected to preliminary phytochemical studies and antibacterial activity of certain human pathogenic microorganisms. The extracts indicated the presence of flavonoids, glycosides, steroids, phenols, alkaloids and tannins. Maximum antibacterial activity was observed in methanolic extract against gram positive bacteria, *Streptococcus pyogenes* and *Enterococcus faecalis* than the gram negative bacteria.[32]

16. **Kevalia J et al., (2011)** had observed identification of fruits of *Tribulus terrestris* Linn and *Pedalium murex* Linn: a pharmacognostical approach. *Gokshura* is a well-known ayurvedic drug that is used in many preparations. Botanically it is identified as *Tribulus terrestris* Linn especially the roots and fruits of the plant. But instead the fruits of another plant *Pedalium murex* Linn was commonly used and the drug was frequently substituted. Pharmacognostical study had been carried out to identify the distinguishing features both morphological and microscopic of the fruits of *Tribulus terrestris* Linn and *Pedalium murex* Linn. This knowledge helped to reduce the problem of substitution of the genuine drug.[33]

17. **Patel DK et al., (2011)** had outlined *Pedalium murex* Linn: an overview of its phytopharmacological aspects. Different parts of the plant were used to treat various ailments like cough, cold and as an antiseptic. Phytochemically the plant is popular for the presence of a considerable amount of diosgenin and vanillin which are regarded as an important source and useful starting materials for synthesizing steroidal contraceptive drugs and isatinalkaloids. Other phytochemicals reported in the plant included quercetin, ursolic acid, caffeic acid, amino acids (glycine, histidine, tyrosine, threonine, aspartic acid and glutamic acid) and various classes of fatty acids (triacontanoic acid, nonacosane, tritriacontane, tetratriacontanyl and heptatriacontan-4-one). Pharmacologically, the plant had been investigated for antiulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, antioxidant, antimicrobial and insecticidal activities. From all these reports it was concluded that the plant was found to have a better profile with potential natural source for the treatment of various range of either acute or chronic disease.[34]

18. **Thamizhmozhi M et al., (2011)** had summarized phytochemical and pharmacognostical studies on *Pedalium murex* Linn. The entire plant of *Pedalium Murex* Linn was subjected to various pharmacognostical evaluations like morphological, microscopical and powder analysis. Results have revealed clearly that the entire plant is genuine. The phytochemical constituents of leaves of *Pedalium murex* Linn have been worked out. The dry powder of the entire plant was successfully extracted with total petroleum ether extract, alcohol extract, chloroform extract and aqueous extract. All the extracts were subjected to preliminary phytochemical screening. It showed the presence of carbohydrates, glycosides, alkaloids, steroids and flavonoids.[35]

19. **Jalaram H et al., (2011)** had elucidated *in vitro* antioxidant activity of aqueous fruit extract of *Pedalium* murex. To understand the mechanisms of pharmacological actions, the *in vitro* antioxidant activity of aqueous extract of fruits of *Pedalium murex* was investigated for DPPH scavenging activity and superoxide scavenging activity. Percentage inhibition of free radicals was measured. The antioxidant property may be related to the phenolic acids and

micronutrients present in the extract. Results clearly indicate that *Pedalium murex* is effective free radical scavenger.[36]

20. **Srinivas P et al., (2011)** had evaluated antioxidant activity of *Pedalium murex* fruits in carbon tetrachloride induced hepatopathy in rats. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GRD) in CCl_4-intoxicated rats and its retrieval towards near normalcy in CCl4 + MEC administered rats revealed the efficacy of methanol extract of fruits of *Pedalium murex* (MEC) in combating oxidative stress due to hepatic damage. Elevated level of glutathione transferase (GTS) observed in hepatotoxic rats too showed signs of returning towards normalcy in MEC co-administered animals, thus corroborating the antioxidant efficacy of MEC.[37]

21. **Patel DK et al., (2011)** had estimated *Pedalium murex* L. (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. Here the antioxidant activities of *Pedalium murex* were evaluated using six *in vitro* assays, namely total antioxidant assay, DPPH assay, reducing power, nitric oxide scavenging, hydrogen peroxide scavenging and deoxyribose scavenging assays and total phenol contents were also investigated.[38]

22. **Banji D et al., (2010)** had composed scrutinizing the aqueous extract of leaves of *Pedalium murex* for the antiulcer activity in rats. The antiulcer efficacy of the aqueous extract of leaves of *Pedalium murex* on ethanol induced gastric lesions was investigated. This has been substantiated by ascertaining the content of total acid, acid volume, total protein, ulcer index and glutathione. Ulceration was induced in 36 hrs fasted rats by the administration of 80% ethanol (1 ml/kg) orally. The reference standard (famotidine, 3 mg/kg) and aqueous extract of leaves of *Pedalium murex* in doses of 50, 100, 200 mg/kg was given to different groups, one hr before the administration of ethanol. Marked gastric mucosal lesions were observed with ethanol. A perceptible elevation in ulcer index, total acidity, acid volume, total protein and diminution of glutathione was observed. Pre-treatment with aqueous extract of leaves of *Pedalium murex* particularly at a

dose of 200 mg/kg in a single schedule and 100 mg/kg for 15 and 30 days treatment annihilated these alterations and elevated the level of glutathione. Therefore the aqueous extract of leaves of *Pedalium murex* was regarded as a favourable anti-ulcerogen which could be attributed to its content of flavonoids and mucilage.[39]

23. **Mukundh N et al., (2008)** had assessed antihyperlipidemic activity of *Pedalium murex* (Linn.) fruits on high fat diet fed rats.The main objective of the study was to investigate about the antihyperlipidemic potential of the ethanolic extract from the fruits of *Pedalium murex* at doses of 200 and 400mg/kg p.o. in high fat diet fed rats. Biochemical parameters like serum total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG) levels were compared with animals concurrently treated with reference standards gemfibrozil and atorvastatin. The ethanolic extract showed a significant decrease in triglycerides ($p<0.01$), LDL ($p<0.001$), VLDL ($p<0.01$), cholesterol ($p<0.001$) and a significant increase in HDL ($P<0.05$) Levels at the tested doses.[40]

24. **Sahayaraj K et al., (2008)** had pronounced insecticidal and antifeedant effect of *Pedalium murex* Linn. root and on *Spodoptera litura* (fab.) (lepidoptera: noctuidae). *Pedalium murex* reduced the food consumption index, growth rate, approximate digestability, eficiency of conversion of ingested food, efficiency of conversion of digested food of *S. litura* indicating the antifeedant activity of this plant. Qualitative analysis of *Pedalium murex* root extract revealed that it contains phytochemical such as steroids, terpenoids, phenolics, saponines, tannins and flavanoids. Phenol, 2-(5, 6-dimethyl pyrazinyl) methyl (molecular weight 214); O-Terphenyl-13C (molecular weight 230) and 3,3A, 4,9B-Tetrahydro- 2H-Furo (3,2-C)(1) Benzopyran (molecular weight 206) were identified from the ethanol root extract of *Pedalium murex* by using GC-MS. *Pedalium murex* impact was more than the neem based bio pesticideneem gold. Hence this plant was explored as biopesticidal plant in the near future.[41]

25. **Bhakuni RS et al., (1992)** had computed flavonoids and other constituents from *Pedalium murex*. Two new compounds isolated from the fruits of *Pedalium*

murex were characterized as 2′,4′,5′-trihydroxy- 5,7-dimethoxyflavone and triacontanyldotriacontanoate by physico-chemical methods. Luteolin, rubusic acid, nonacosane, tritriacontane, triacontanoic acid, tritriacontanoic acid and sitosterol-β-d-glucoside had also been isolated and identified.[42]

26. **Shukla YN et al., (1983)** had worked on heptatriacontan&4&one, tetra tri acontanyl octa cosanoate and other constituents from *Pedalium murex* Linn. Two new compounds isolated from the fruits of *Pedalium murex* were characterized as heptatriacontan-4-one and tetra tri acontanyl octa cosanoate by spectral studies. Pentatriacontane, sitosterol, hexatriacontanoic acid, hentriacontanoic acid, ursolic acid and vanillin had also been isolated and identified.[43]

4. AIM AND PLAN OF WORK

As know that everything in this world change time by time, since thousands of year the era was of ayurveda or herbal origin drug. But last few years it was replaced by allopathic system of medicine, which was fastly accepted worldwide, but latter due to its lot of adverse effect, again men step down on Ayurveda because of its better therapeutic result and safety profile and now the people are more believing in natural origin drug.

Countless drugs have entered in the International Pharmacopoeia through the study of Ethno pharmacology and traditional medicines. For ayurveda and other traditional medicines newer guidelines of standardization, manufacture and quality control are required. Employing a unique holistic approach, ayurvedic medicines are usually customized to an individual constitution, Traditional knowledge, driven drug development can follow a reverse pharmacology path and reduce time and cost of development. Powerful search engine and most importantly, will greatly facilitate international, focused and safe natural product research to rediscover the drug discovery process.

Looking to the scope of herbal drug and increasing demand especially in case of diseases like liver disorders, hypertension, diabetes, cancer, diarrhoea, arthritis and skin diseases etc. Hence, it is planned to study a plant like *Pedalium murex* Linn which is having a variety of traditional uses. This plant is selected for present study based on its easy availability, degree of research work which is not done. The literature survey revealed that some amount of pharmacological work has been carried out on *Pedalium murex* Linn. Many works had been done previously in the flowers and seeds of *Pedalium murex* Linn. But the leaves of the plant are also having many traditional uses. So in order to explore the activities in the leaves of *Pedalium murex* Linn. I planned to go for the following studies.

PLAN OF WORK

The work was planned as under mentioned

PHYTOCHEMICAL STUDIES

- Collection and authentication of plant material
- Extraction of the plant material.
- Preliminary phytochemical screening of the extract.
- *In vitro* free radical scavenging activity.
- Thin layer chromatography of the extract.
- Isolation of plant constituent by column chromatography.
- Characterization of isolated compounds by IR, NMR, Mass spectroscopy.

PHARMACOLOGICAL STUDIES

- *In vitro* anticancer activity

  1. Cell treatment procedure

  2. MTT Assay.

5. MATERIALS AND METHODS

Phase I: PHYTOCHEMICAL STUDIES

Collection and Authentication of Plant

The leaves of *Pedalium murex* Linn. was collected from surrounding areas of Komarapalayam, Namakkal District, Tamilnadu, India.

The plant was authenticated by Dr. G.V.S. Murthy, Scientist F, Botanical survey of India, Coimbatore, Tamilnadu (No.BSI/SRC/5/23/2012-13/Tech/1934) and the specimen was preserved in Pharmacognosy lab, JKKN College of Pharmacy.

Extraction Procedure

The leaves of *Pedalium murex* Linn.were precisely washed with tap water and dried underneath dimness, in room temperature for one week. Then they were crushed into powder and stored in room temperature. The pulverized materials were passed through sieve no. 40 and 80; the crushed materials of identical size present between those two sieves were collected and packed in an airtight container for supplementary use. About 2 kg of shaded and dried plant leaves of *Pedalium murex* Linn. was extracted in soxhlet apparatus successively with n-hexane, chloroform, ethyl acetate and 90% v/v ethanol. Each extract was evaporated using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant leaves. The consistency and colour of the extract were noted. All the solvents used for this work were of analytical grade.

Preliminary Phytochemical Analysis

All the four extracts of *Pedalium murex* Linn. Leaves (*P.murex*) were subjected to qualitative tests for the identification of various plant constituents.[44-47]

1) Test for alkaloids

a) **Dragendroff's test: -** 1 ml of the extract was added with 1 ml of dragendroff's reagent (potassium bismuth iodide solution). An orange red precipitate specified the presence of alkaloids.

b) **Mayer's test: -** 1 ml of the extract was added with 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow coloured precipitate designated the presence of alkaloids.

c) **Hager's test: -** 1 ml of the extract was added with 3 ml of Hager's reagent (saturated aqueous solution of picric acid), yellow coloured precipitate indicated the presence of alkaloids.

d) **Wagner's test: -** 1 ml of the extract was added with 2 ml of Wagner's reagent (Iodide in Potassium Iodide), formation of reddish brown precipitate suggested the presence of alkaloids.

2. Test for Saponins

a) **Foam test: -**The extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 min lengthwise. A 1cm layer of foam indicated the presence of Saponins.

3. Test for glycosides

a) **Legal's test: -** The extract was dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation from pinkish red to red colour showed the presence of glycosides.

b) **Keller-Killiani test: -** The ethanol extract of 0.5 ml of strong solution of lead acetate was added and filtered. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was separated in a porcelain dish and the solvent was removed by gentle evaporation. The cool residue was dissolved in 3 ml of glacial acetic acid containing 2 drops of ferric chloride solution. This solution was carefully transferred to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer was formed at the junction of the two

liquids and the upper layer slowly became bluish green, darkening upon standing.

c) **Borntrager's test: -** A few ml of dilute sulphuric acid was added to 1 ml of the extract solution. The filtrate was boiled, filtered and extracted with chloroform and the chloroform layer was treated with 1 ml of ammonia. The formation of red colour of the ammonical layer showed the presence of anthraquinone glycosides.

4) Test for carbohydrates

a) **Molisch's test: -** 2 ml of the extract was added with 1 ml of α- napthol solution and concentrated sulphuric acid along the sides of the test tube. Reddish violet colour at the junction of the two liquids indicated the presence of carbohydrates.

b) **Fehling's test:-**1 ml of the extract was added with equal quantities of Fehling's solution A and B and upon heating formation of a brick red precipitate indicated the presence of reducing sugars.

c) **Benedict's test: -** 1 ml of extract was added with 5 ml of Benedict's reagent and boiled for 2 min and cooled. Formation of red precipitate showed the presence of sugars.

5) Test for tannins & phenolics

a) **Gelatin Test: -** 1 ml of extract was added with 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate indicated the presence of tannins.

b) **Ferric chloride test:-**1 ml of extract was added with 1 ml ferric chloride solution, formation of dark blue or greenish black product showed the presence of tannins.

c) **Lead acetate test: -** A little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

6) Test for flavonoids

a) **Shinoda's test:-** To the extract solution few fragments of magnesium ribbon and concentrated HCL was added drop wise which gave cherry red colour appearance after few min. It showed the presence of flavonoids.

b) **Alkaline reagent test: -** The extract was treated with sodium hydroxide, formation of yellow colour indicated the presence of flavonoids.

c) The extract was treated with concentrated sulphuric acid, formation of yellow or orange colour indicated the presence of flavonoids.

7) Test for steroids

a) **Libermann-Burchard's test: -** Chloroform solution, 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid along the sides of the test tube was added to 2 ml of extract. Appearance of bluish-green colour showed the presence of steroids.

b) **Salkowsky's test: -** The extract was dissolved in chloroform solution and 2 ml of concentrated sulphuric acid was added. Chloroform layer appeared red color which indicated the presence of steroids.

8) Test for Proteins

(a) **Ninhydrin test:-**1 ml of the extract was treated with 3 drops of 5% ninhydrin solution in boiling water bath for 10 min. The appearance of purplish or bluish colour indicated the presence of proteins, peptides or amino acid.

(B) **Millon's test: -** 1 ml of the extract was treated with millon's reagent (mercuric nitrate in HNO_3). A white precipitate turned to brick red indicated the presence of proteins.

9) Test for Triterpenoids

Knoller's test: - 2 or 3 granules of tin metal was dissolved in 2 ml of thionyl chloride solution. Then 1 ml of the extract was added into the test tube and warmed, the formation of pink colour indicated the presence of Triterpenoids.

10) Test for fixed oils and fats

(a) Stain test: - A small quantity of extract was pressed between two filter papers. The stain on the filter paper indicated the presence of fixed oils.

(b) **Saponification test: -** A few drops of 0.5 N of alcoholic potassium hydroxide was added to small quantity of various extracts along with a drop of phenolphthalein separately and was heated on water bath for 1 to 2 hrs. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

11) Test for Gums and Mucilage

10 ml of extract was slowly added to 25 ml of absolute alcohol with constant stirring, the precipitate was filtered and dried in air. The precipitate, for its swelling property, indicated the presence of carbohydrates.

IN VITRO FREE RADICAL SCAVENGING ACTIVITY

1. Total antioxidant activity

Total antioxidant activity of *P. murex* extracts was determined according to the thiocyanate method.[48] For the stock solution, 20 mg *P.murex* extracts were dissolved in 20 ml water. Then the solution, which contained different amount of stock *P.murex* extracts solution or standard samples (12.5, 25, 50, 100, 200 µg) in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 ml of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37°C in the dark. At intervals during incubation, each solution was stirred for 3 min. 0.1 ml of the incubation solution; 0.1 ml $FeCl_3$ and 0.1 ml thiocyanate were transferred to the test tube which contained 4.7 ml ethanol. Then the solution was incubated for 5 min. Finally, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (8500 II, Bio-CromGmbh, Zurich, Switzerland). During the linoleic acid oxidation, peroxides were formed and these compounds oxidized Fe^{2+} to Fe^{3+}. Fe^{3+} ions formed complex with SCN^-, which has a maximum absorbance at 500 nm. Hence higher absorbance values indicated higher linoleic acid oxidation. The solutions without the addition of *P.murex* extracts or standards were used as blank samples. Five millilitres of linoleic acid emulsion consisted of 17.5 gm Tween-20, 15.5ml linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 ml control was composed of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 M, pH 7.0). All data about total antioxidant activity was the average of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

Percent inhibition = $[A_0 - A_1/A_0] \times 100$

Where A_0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample of *Pedalium murex.*

2. DPPH radical scavenging activity

1 mmol solution of DPPH radical solution in methanol was prepared, and then 1ml of this solution was mixed with different concentrations of *P. murex* extracts, the mixture was then vortexed vigorously and left for 30 min at room temperature in the dark and the absorbance was measured at 517 nm with a spectrophotometer and was calculated.[49-51]

DPPH Scavenging activity % = [(Control Absorbance – Extract Absorbance) / Control Absorbance] X 100.

For control 1.0 ml of methanol was added to 1ml of 1mmol solution of DPPH radical solution**.**

Thin Layer Chromatography

Thin layer chromatography is a procedure for analytical adsorption chromatography which was first introduced by Stahl (1958) who was mainly responsible for bringing out standard equipment for preparing thin layers. It is an important analytical tool for qualitative and quantitative analysis of a number of natural products and for separation and estimation of different components.

The principle of separation is adsorption. One or more compounds were spotted on a thin layer of adsorbent coated on TLC plate. The mobile phase flowed through because of capillary action (against gravitational force). The component moved according to their affinity towards the stationary phase. The component with lesser affinity towards the stationary phase traveled faster and vice versa which led to the separation of components.

The information provided by a finished chromatography included the “migrating behaviour” of the separated substances. It was given in the form of R_f value (relative to front).

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$R_f$value usually lies in the range of 0.1 – 1.[52,53]

Procedure

The silica gel G (60-120 mesh) was utilized for the preparation of TLC plates. Silica gel G was mixed with sufficient quantity of water and triturated well to make slurry. The prepared slurry was spread on the meticulously cleaned and scratch free glass plates of definite dimension with the help of TLC spreader. The thickness of the absorbent was adjusted to 2mm throughout the plate. Then the prepared plates were allowed to set for 15-30 min. The activated plates were stored in a vacuum desiccator for future use. Also prior to the use, the plates were once again activated.

The saturation of atmosphere of TLC chamber by mobile phase, toluene: ethyl acetate: formic acid (5:4:1) before the start of the experiment was almost important to avoid the flawed results due to tailing effect. The sample EEPM was spotted on the plate 2 cm away from the bottom. The plate was then developed in the chamber by allowing the plate to run up to $\frac{3}{4}^{th}$distance of the plate. The plate was then removed, dried up to room temperature and sprayed with suitable spray reagent or kept in iodine chamber for identification of spots.

Column Chromatography

The column chromatographic technique is widely used for separation, isolation and purification of the natural products. The principle underlying the separation of the compounds is adsorption at the solid liquid interface. The solid support and the interaction between adsorbent and component must be reversible. The adsorbent was washed with fresh solvent the various components were moved down the column until they were arranged in order of their affinity towards the adsorbent. Those with least affinity moved down the column at a faster rate and were eluted from the end of the column before those with greatest affinity for the adsorbent. By changing the polarity of the mobile phase, the separation was achieved by column chromatography. Characterization of the isolated compounds was carried out by analytical techniques, like IR, NMR and Mass spectroscopy.

Procedure for Preparation of Column

The silica gel 60-120 mesh was made into slurry with selected solvent system, the silica gel was previously activated by heating in hot air oven at100°C for 1hr.

1. The bottom of the column was plugged by cotton and then the silica gel slurry was poured into the column which was filled with solvent system, toluene: ethyl acetate: formic acid (50:4:10) up to 40 cm height, after that it was set aside for 10 min and allowed to settle.

2. The ethanol extract of *Pedalium murex* Linn was mixed with small amount of silica gel and wetted with solvent system and the solvent was allowed to evaporate to set the dry residue.

3. Then the dry residue was charged on column with the help of solvent system, after that cotton was placed over it, in order to avoid the disturbance of the top layer of the adsorbent fresh mobile phase was added to the column.

4. The column was eluted with the selected solvent system by isocratic method and the fractions were collected in clean 100 ml beaker up to 25 ml and the speed of the drops was 20 drops/min.

Each collected fraction was tested for the presence of various constituents by TLC. The number of types of constituent and similar fractions was pooled together.[54-56]

Spectral Characterization

1. FT-IR Spectroscopy

Isolated compounds were analyzed by IR spectral studies by KBr pellet technique. In this method, the drug and KBr was mixed at the ratio of 1:100. Then this mixture was pressed into a pellet. The FT-IR spectra were recorded for isolated compounds, using KBr pellet method in the region of 4000 - 400cm^{-1}.

2. NMR Spectroscopy

NMR is used to elucidate the structure of an unknown compound, there are three pieces of information which should be considered, the position of resonance of the peak (or chemical shift), the number of hydrogen atoms causing the signal (integration) and the number of peaks constituting the signal (multiplicity). NMR data is used to solve the structure but it is equally acceptable to use MS or IR data to solve the unknown.

3. Mass Spectroscopy

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. The MS principle consist of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios.[57,58]

Phase II: PHARMACOLOGICAL STUDIES

IN VITRO ANTICANCER ACTIVITY

The human breast cancer cell line (MCF-7) and cervical cancer cell lines (HeLa) were obtained from National Centre for Cell Science (NCCS), Pune. The (MCF-7) cells were grown in Dulbecco's modified eagles medium (DMEM) and HeLa cells were grown in Eagles minimum essential medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37ºC, 5% CO_2, 95% air and 100% relative humidity. Maintained cultures were passaged weekly, and the culture medium was changed twice a week.

CELL TREATMENT PROCEDURE

The monolayer cells were detached with trypsin – ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium with 5% FBS to give final density of $1 x 10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37ºC, 5% CO_2, 95% air and 100% relative humidity.

After 24 hr the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 10, 20, 50, 100, 200 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37ºC, 5% CO_2, 95% air and 100% relative humidity for 48 hr. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT ASSAY

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48 hr of incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4hr. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) X 100.

Nonlinear regression graph was plotted between percent Cell inhibition was determined using Microsoft Excel software.[59, 60]

6. RESULTS AND DISCUSSION

The leaves of *Pedalium murex* Linn belonging to family Pedaliaceae has been investigated in a sytematic way covering phytochemical characterization and pharmacological studies. Litrerature survey revealed that not much work is done on this plant. Therefore, it was thought worthwhile to carry out the phytochemical characterization and pharmacological studies on this plant.

RESULTS

1. Phytochemical studies

Extraction of *Pedalium murex* Linn Leaves

Dried crushed leaves of *Pedalium murex* Linn was extracted with n-hexane, chloroform, ethyl acetate and ethanol (90% v/v) continuously with soxhlet apparatus and the results were tabulated in Table No. 2.

Table No. 2. Data showing the extractive values of leaves of *Pedalium murex* Linn.

S.No	Extract	Colour/ Physical nature	Percentage yield (% w/w)
1	n-hexane	Green/ Waxy Semisolid	4.90
2	Chloroform	Green/ Semisolid	6.20
3	Ethyl acetate	Brownish Green/Solid	4.53
4	Ethanol (90% v/v)	Brownish Green/ Solid	6.38

Preliminary Phytochemical Screening of *Pedalium murex* Linn Leaves

The extracts of *Pedalium murex* Linn leaves were subjected to qualitative phytochemical screening to identify the phytoconstituents present and the results were expressed in Table No. 3.

Table No. 3. Preliminary Phytochemical Screening of *Pedalium murex* Linn. Leaves

S. No.	Chemical Test	n- hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol (90% v/v) extract
1.	**Alkaloids**				
a.	Mayer's Test	-	-	+	-
b.	Dragendroff's Test	-	-	+	-
c.	Wagner's Test	-	-	+	-
d.	Hager's Test	-	-	+	-
2.	**Carbohydrates**				
a.	Molisch's Test	-	-	+	+
b.	Fehlings Test	-	-	+	+
c.	Barfoed's Test	-	-	+	+
d.	Benedict's Test	-	-	+	+
3.	**Glycosides**				
a.	Legal's Test	-	+	-	+
b.	Keller-Killiani Test	-	+	-	+
c.	Borntrager's Test	-	+	-	+
4.	**Fixed Oil & Fat**				
a.	Stain Test	+	-	-	-
b.	Saponification Test	+	-	-	-
5.	**Tanins & Phenolics**				
a.	Ferric Chloride Test	-	-	-	+
b.	Lead Acetate Test	-	+	-	+
c.	Gelatin Solution Test	-	+	-	+
6.	**Steroids**				
a.	Salkowsky's Test	+	-	-	+
b.	Libermann Burchard's Test	+	-	-	-
7.	**Saponins**		-	-	
a.	Foam Test	-	-	+	+
8.	**Proteins**				
a.	Million's Test	-	-	+	-
b.	Ninhydrin Test	-	-	+	-
9.	**Flavonoids**		-	-	
a.	Aqueous NaOH Test	-	-	-	+
b.	Conc.Sulphuric Acid Test	-	-	-	+
c.	Shinoda Test	-	-	-	+
10.	**Gum & Mucilage**	+	-	-	-
11.	**Triterpenoids**				
a.	Knoller's Test	-	-	-	+

+ Present **-** Absent

In vitro Free Radical Scavenging Activities

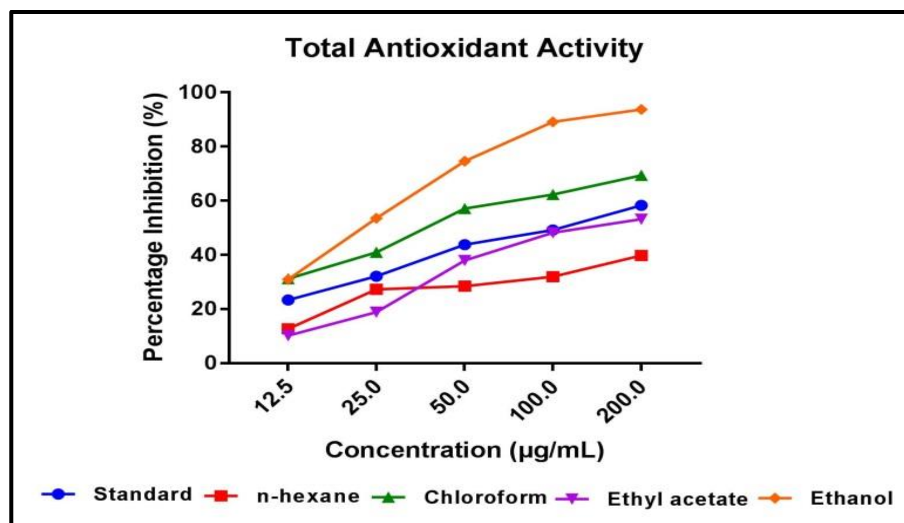
1. Total Antioxidant Activity of *Pedalium murex* Linn Leaves

The total antioxidant capacity of ethanol extract was found to be higher (93.73% at 200 µg/ml) when compared to other extracts used. The IC_{50} values of total antioxidant capacity of ascorbic acid (Standard), n-hexane, chloroform, ethyl acetate and ethanol extracts were found to be 129.75 µg/ml, >200 µg/ml, 65.41 µg/ml, 154.73 µg/ml and 12.74 µg/ml respectively. Amongst, ethanol (90 % v/v) and chloroform extracts exhibited significant dose dependent antioxidant activity was mentioned in Table No. 4 and Fig No.3

Table No. 4. Total Antioxidant Activity of *Pedalium murex* Linn Leaves

S. No.	Concentration (µg/ml)	Standard (Ascorbic acid)	n-hexane	Chloroform	Ethyl acetate	Ethanol (90 % v/v)
		% inhibition	% inhibition	% inhibition	% Inhibition	% inhibition
1	12.5	23.35	12.62	31.21	10.23	30.99
2	25	32.13	27.33	40.92	18.86	53.55
3	50	43.78	28.42	57.15	37.97	74.63
4	100	49.24	31.97	62.31	48.24	89.15
5	200	58.32	39.75	69.36	53.23	93.73
6	IC_{50} value	129.75	>200	65.41	154.73	12.74

Fig No. 3. Total antioxidant activity of *Pedalium murex* Linn. Leaves



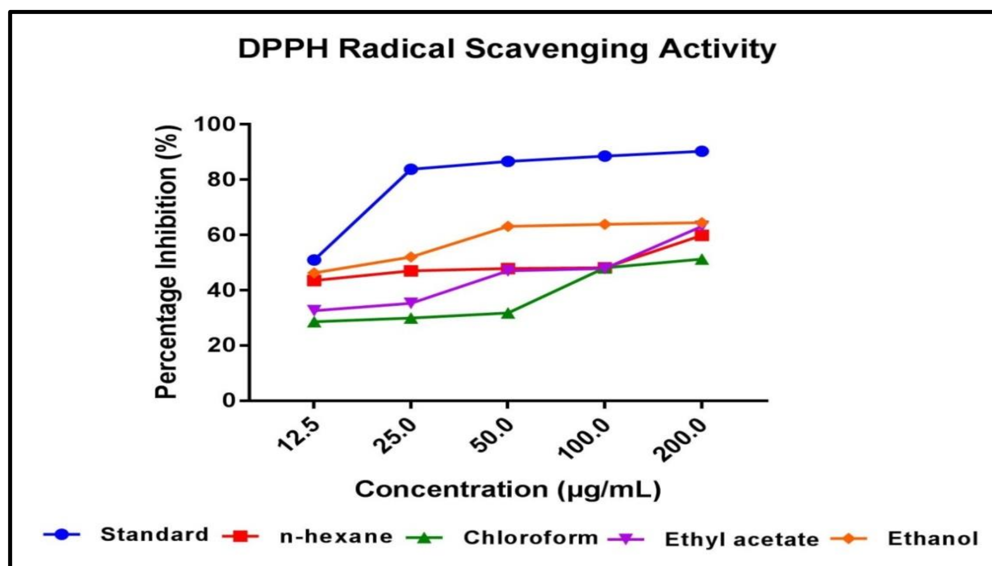
2. DPPH Radical Scavenging Activity of *Pedalium murex* Linn Leaves

The DPPH radical is considered to be a model for lipophilic radical chain reaction initiated by the lipid auto oxidation. Among the extracts used in the study, ethanol extract of *Pedalium murex* Linn leaves exhibited a significant dose dependent DPPH radical scavenging activity. Percentage of scavenging activity or percentage inhibition was calculated by linear regression method. The IC_{50} values of ascorbic acid and ethanol extract was found to be <12.5µg/ml and 21.91 µg/ml respectively were mentioned in Table No. 5 and Fig No.4.

Table No. 5. DPPH Radical Scavenging Activity of *Pedalium murex* Linn Leaves

S. No.	Concentration (µg/ml)	Standard (Ascorbic acid)	n-hexane	Chloroform	Ethyl acetate	Ethanol (90 % v/v)
		% inhibition	% inhibition	% inhibition	% Inhibition	% inhibition
1	12.5	50.95	43.58	28.60	32.62	46.25
2	25	83.75	47.05	29.94	35.29	52.03
3	50	86.62	47.86	31.81	47.05	63.10
4	100	88.53	48.12	48.12	47.86	63.90
5	200	90.28	59.89	51.33	63.10	64.43
6	IC_{50} value	<12.5	115.92	158.36	114.05	21.91

Fig No. 4. DPPH Radical Scavenging Activity of *Pedalium murex* Linn Leaves



On the basis of preliminary phytochemical studies and *in vitro* free radiacl scavenging activities, ethanol extract has been selected for the isolation.

Thin Layer Chromatography (TLC)

EEPM was subjected to thin layer chromatography on silica gel G which had shown good resolution of solutes system like ethyl acetate: formic acid: water (50:4:10). The different spot developments in each system were identified by means of corresponding detecting agent and R_f values were calculated and showed in Table No. 6 and Fig No. 5.

Fig No. 5. Thin Layer Chromatography of EEPM

Table No. 6. Thin Layer Chromatography of EEPM

Solvent system	No. of spots	Spray reagent	R_f Values
Ethyl acetate : Chloroform (6:4)	1	Iodine vapours	0.60
n-butanol: Acetic acid: Water (3:1:1)	1	Iodine vapours	0.67
n-butanol: Acetic acid: Water (4:1:5)	1	Iodine vapours	0.63
Ethyl acetate: Formic acid: Water (50:4:10)	2	Iodine vapours	0.65 0.74

Column Chromatography

On the basis of phytochemical screening and TLC study, isolation of active constituents of EEPM was done by column chromatography through isocratic elution technique with the help of solvent system ethyl acetate: formic acid: water (50:4:10) was shown in Table No. 7 and Fig No. 6.

Fig No. 6. Column Chromatography of EEPM

Table No. 7. Column Chromatography of EEPM

Fraction No.	Nature of residue	Analysis by TLC	Colour of the spot	R_f Value
1-4	No residue	---	---	---
5-8	No residue	---	---	---
9-12	Light green	---	---	---
13-16	Green	----	---	---
17-20	Green	---	---	---
21-24	Greenish yellow	2	Yellow Greenish brown	0.65 0.74
25-28	Light yellow	2 spots with tailing effect	Yellow Brown	0.78 0.84
29-32	No residue	----	----	---
33-36	Yellow	1	Brown	0.53
37-40	No residue	----	----	---

The isolated compounds from the Fraction 21-24 obtained by column chromatography was named as EEPM1 and EEPM 2.

Analysis of the isolated compounds

Thin Layer Chromatography of the Isolated Compound EEPM 1 & EEPM 2

The isolated compounds EEPM 1 & EEPM 2 obtained from column chromatography was analysed by chemical test, thin layer chromatography using the solvent system ethyl acetate: formic acid: water (50:4:10) and detected by iodine vapours. EEPM 1 showed the presence of one yellow colour spot with R_f value of 0.65 & EEPM 2 showed the presence of one brown colour spot with R_f value of 0.74 was shown in Fig No. 7 & 8.

Characterization of EEPM 1

Table No. 8. Chemical tests for EEPM 1

Chemical Tests	EEPM 1
Salkowsky's Test	Positive
Libermann Burchard's Test	Positive

Table No. 9. Physical Characters of EEPM 1

Parameters	EEPM 1
Yield	46 mg
Physical state and color	Light yellow crystalline solid
Solubility	Water, Acetone and DMSO
Melting point	140ºC
TLC solvent system	Ethyl acetate: Formic acid: Water (50:4:10)
R_f value	0.65

Characterization of EEPM 2

Table No. 10. Chemical tests for EEPM 2

Chemical Tests	EEPM 2
Knoller's Test	Positive

Table No. 11. Physical Characters of EEPM 2

Parameters	EEPM 2
Yield	32 mg
Physical state and color	Light yellow crystalline solid
Solubility	DMSO, Methanol
Melting point	186ºC
TLC solvent system	Ethyl acetate: Formic acid: Water (50:4:10)
R_f value	0.74

Fig No. 7. Thin Layer Chromatography of EEPM 1

Fig No. 8. Thin Layer Chromatography of EEPM 2

Both the isolated compounds from the leaves of *Pedalium murex* Linn was tested and it showed the positive test for steroids and triterpenoids tests. The physical characters of both the compounds were compared and the results are tabulated above.

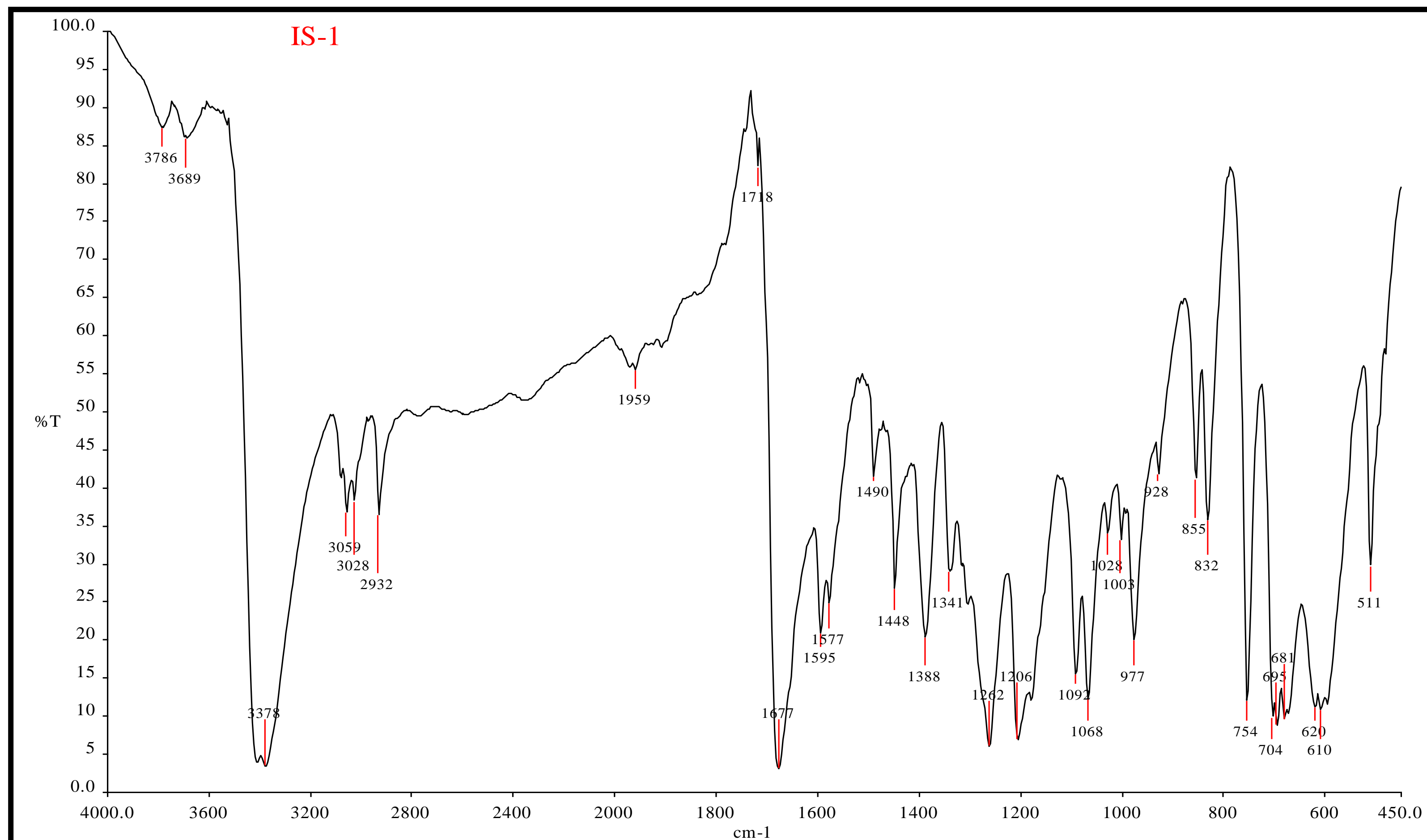


Fig. 9. IR Spectrum of isolated compound EEPM 1

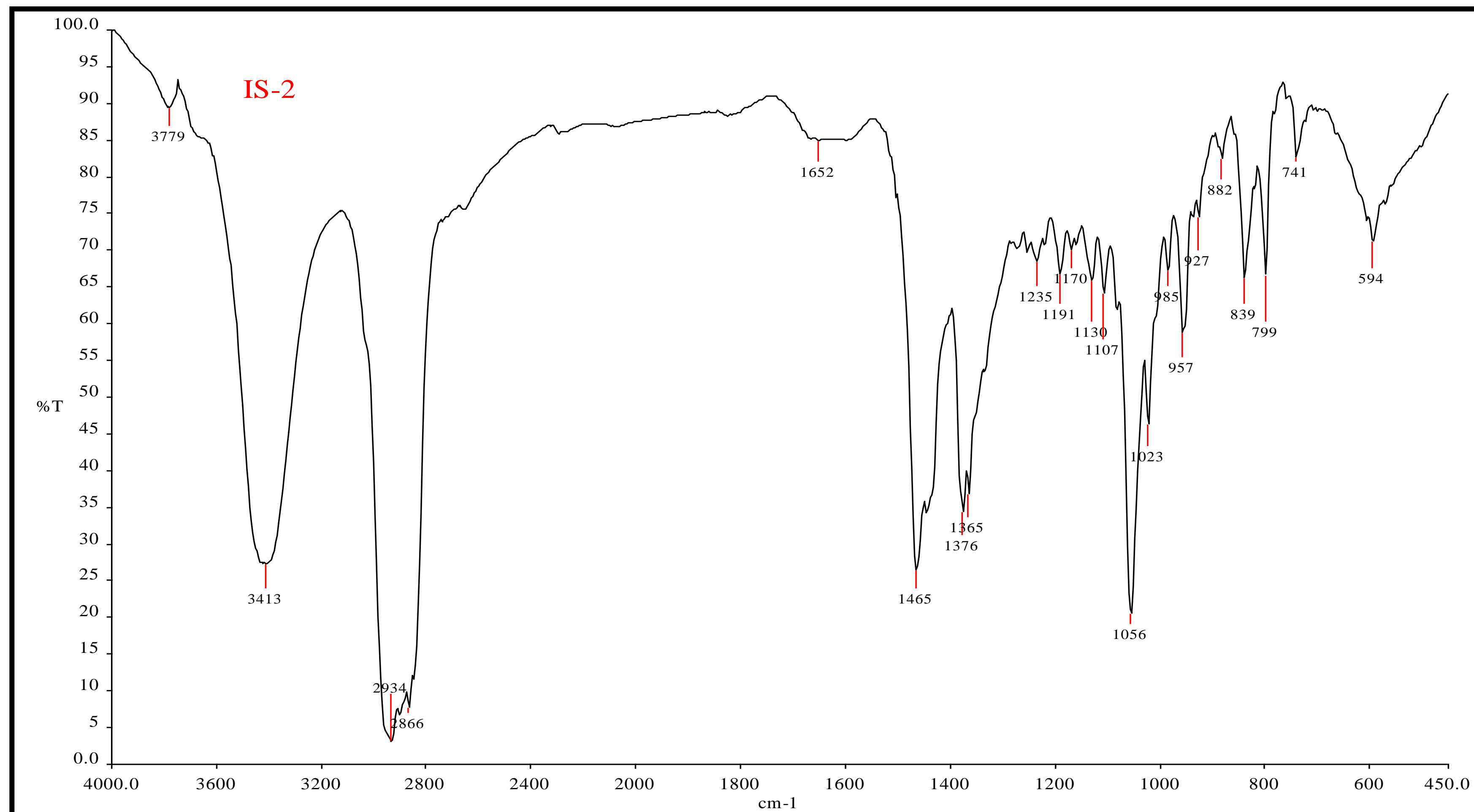


Fig. 10. IR Spectrum of isolated compound EEPM 2

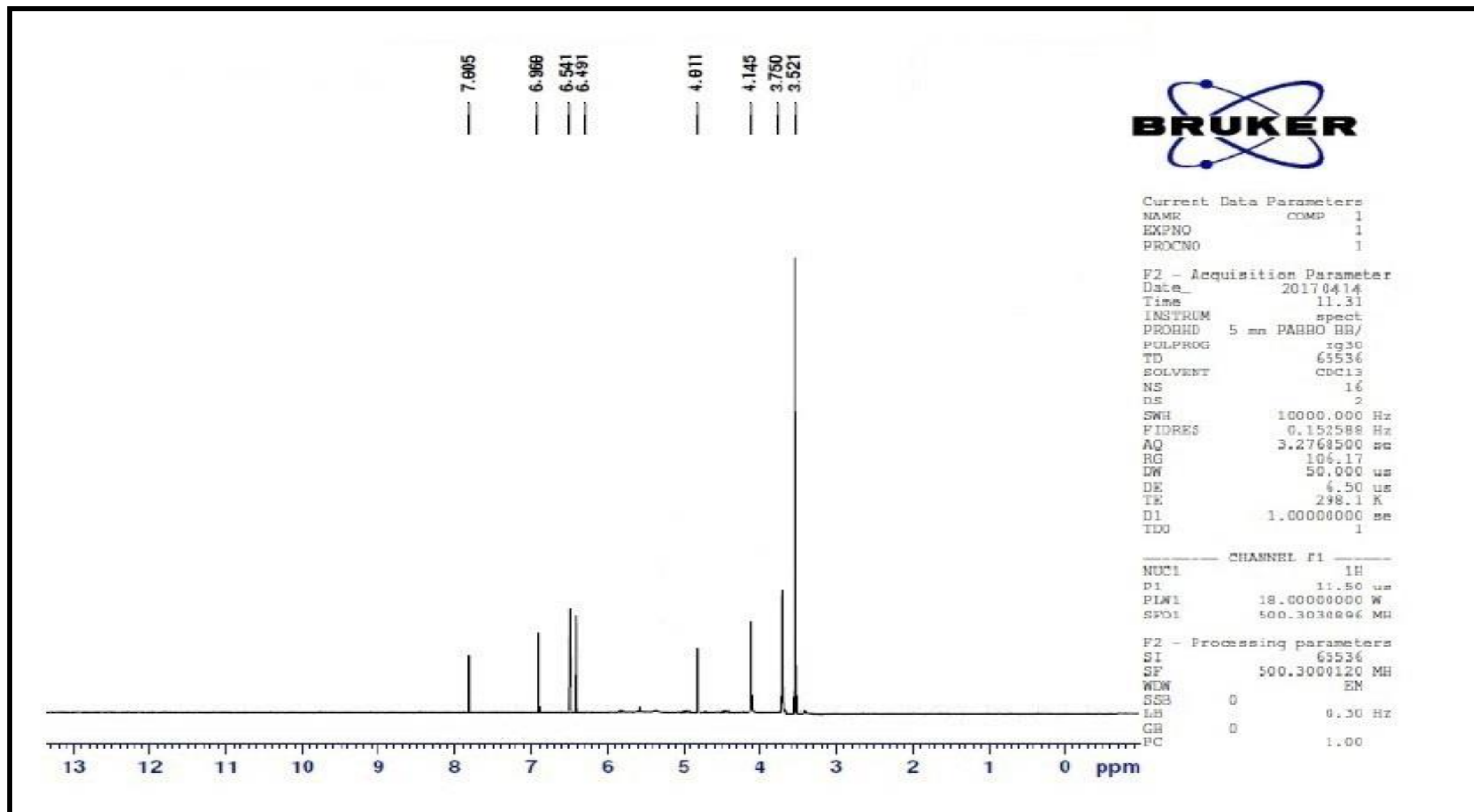


Fig. 11. ^{1}H NMR Spectrum of isolated compound EEPM 1

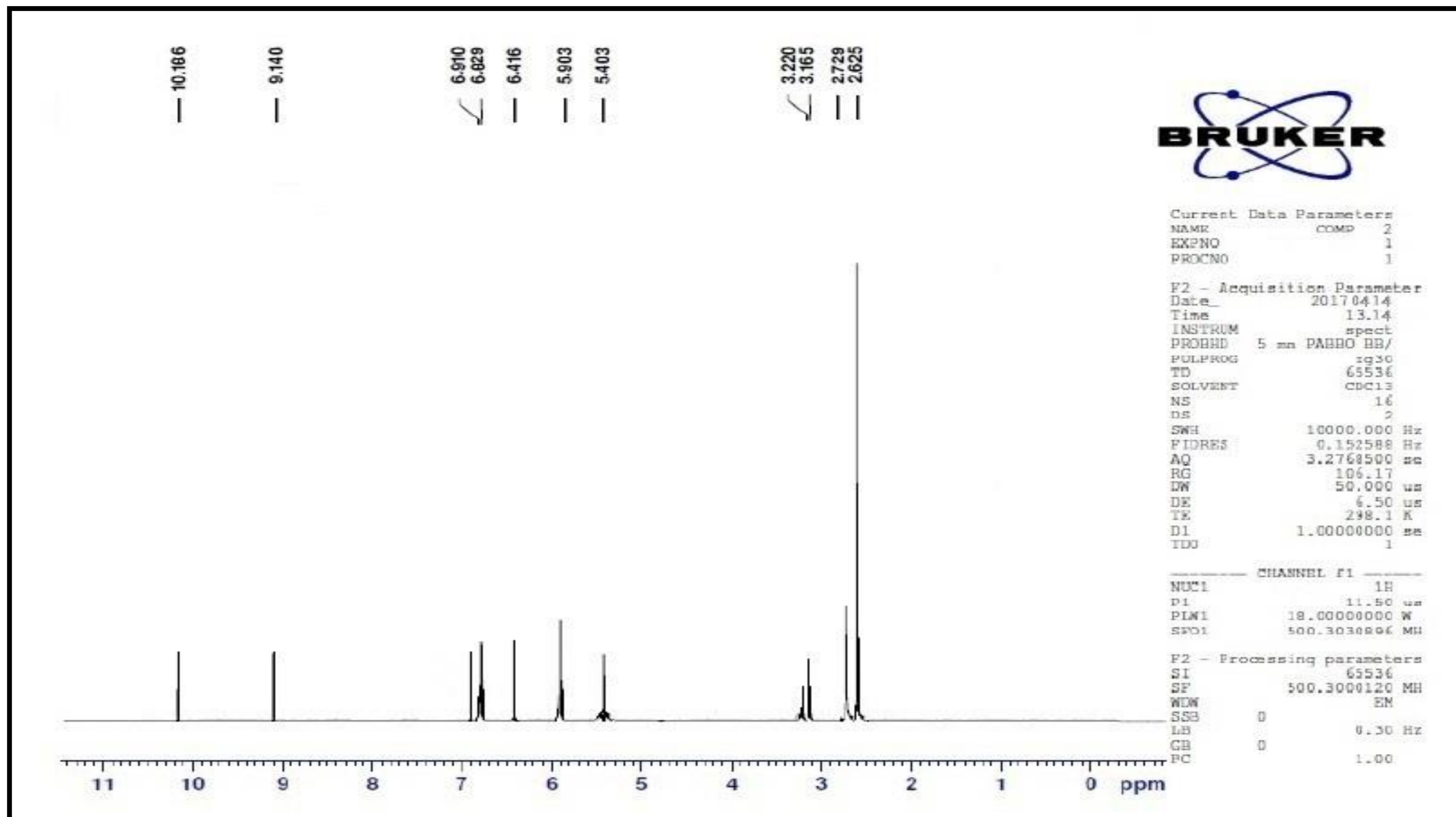


Fig. 12. ^{1}H NMR Spectrum of isolated compound EEPM 2

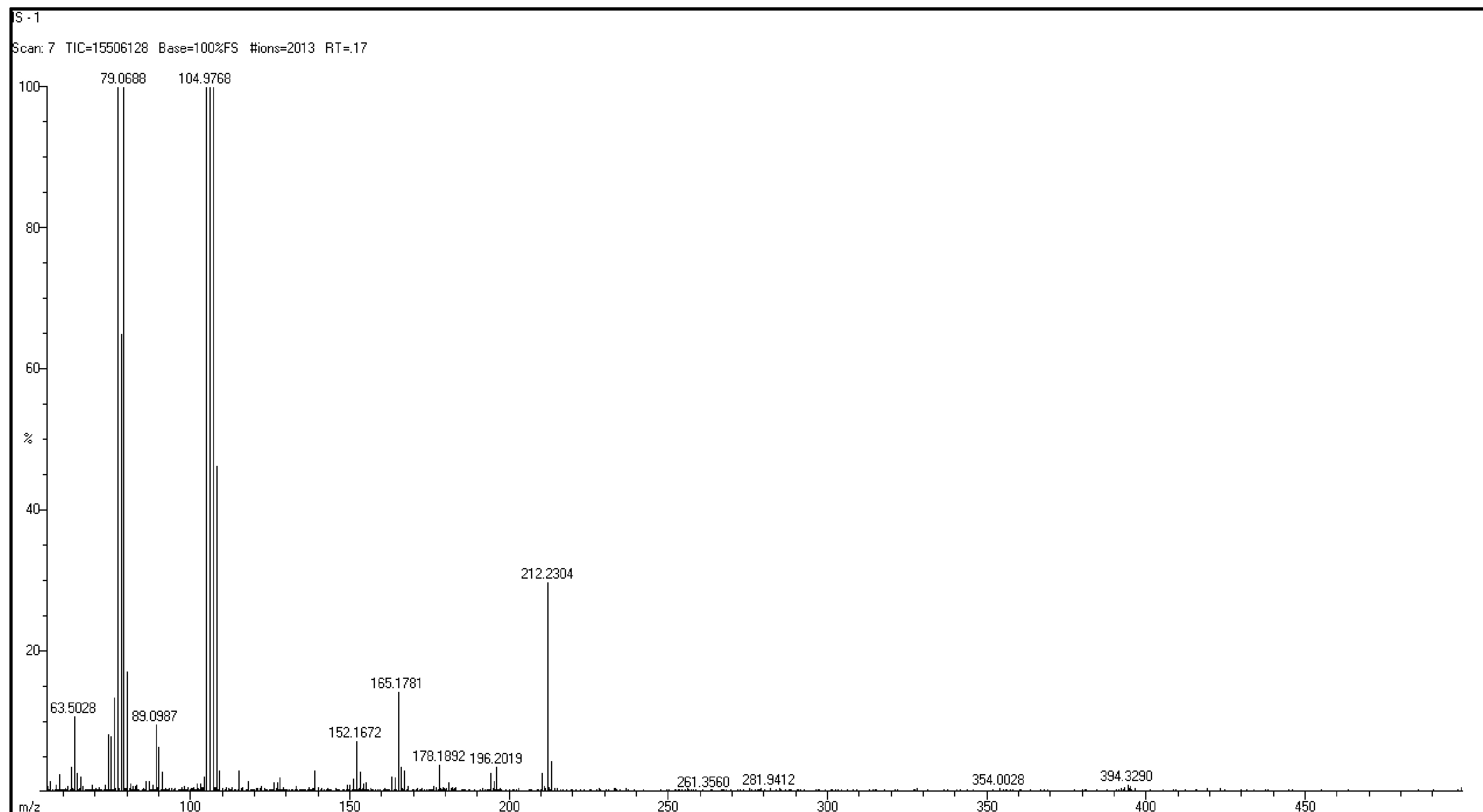


Fig. 13. Mass Spectrum of isolated compound EEPM 1

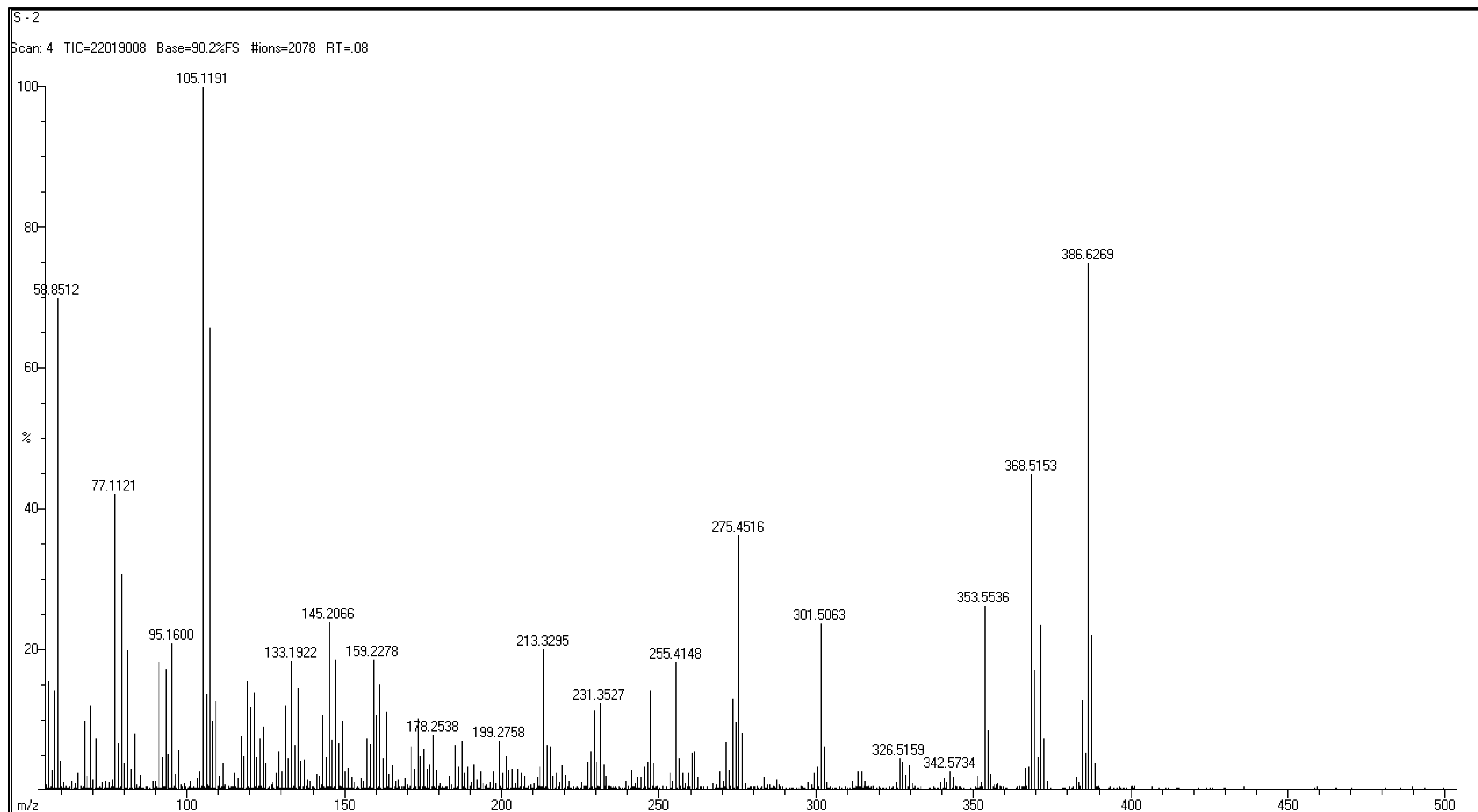


Fig. 14. Mass Spectrum of isolated compound EEPM 2

The qualitative analysis of the ethanol extract of *Pedalium murex* Linn was summarized in Table No.3. Preliminary phytochemical screening revealed that n-hexane extract contains the compounds includes fixed oils and fats, steroids, gum & mucilage. Chloroform extract contains glycosides, tannins, phenolic compounds. Ethyl acetate extract contains alkaloids, carbohydrates, proteins, saponins. Ethanol extract contains flavonoids, glycosides, tannins, carbohydrates, phenolic compounds, triterpenoids and saponins. The TLC study was carried out and summarized in Table No. 6. From the TLC study of the ethanol extract of *Pedalium murex* Linn, the presence of two spots were observed as maximum number of spots with ethyl acetate: formic acid: water (50:4:10) using iodine vapours as detecting agent. The R_f values of the spots were calculated and found to be 0.65, 0.74 respectively. Column chromatography of the ethanol extract of *Pedalium murex* Linn was summarized in Table No.7. Phytochemical analysis of the isolated compound EEPM 1 gave positive result for sterols and EEPM 2 has given positive result for terpenoids.

IR spectra of the isolated compound EEPM 1 was carried out and data showed the presence of C-H stretching at 2932 cm^{-1}, O-H stretching at 3689cm^{-1}, C=C stretching at 1677 cm^{-1}, aromatic carbonyl (C=O) stretching at 1577 cm^{-1}, C=O stretching at 1718 cm^{-1}.

IR spectra of the isolated compound EEPM 2 was carried out and data showed the presence of phenolic OH at 3413 cm^{-1}, C-H methylene stretch at 2934 cm^{-1}, quinone functional group at 1652 cm^{-1}, O-H bending at 1465 cm^{-1}, alcoholic OH out of plane bend at 594 cm^{-1}.

Protocol of the H-1 NMR Prediction: EEPM 1

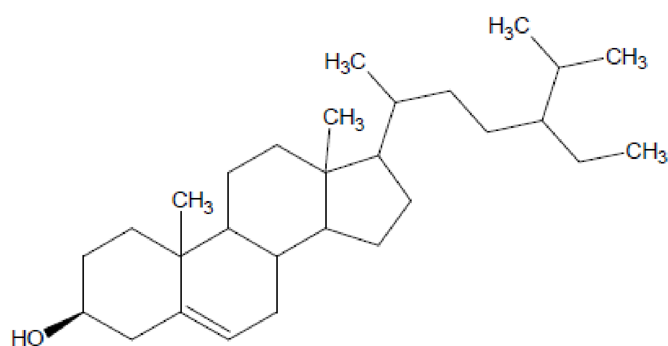
^{1}H-NMR (MeOD) (δppm): 1.13-2.32 (m, 20H, Cyclohexane-methylene), 1.35-1.60 (s, 4H, Cyclopentane-methylene), 1.40-3.25 (8H, Methine), 3.45 (m, 1H OH alcohol), 5.21 (m, 1H Cyclohexene-ethylene), 0.96-1.29 (m, 12H methyl).

Protocol of the H-1 NMR Prediction: EEPM 2

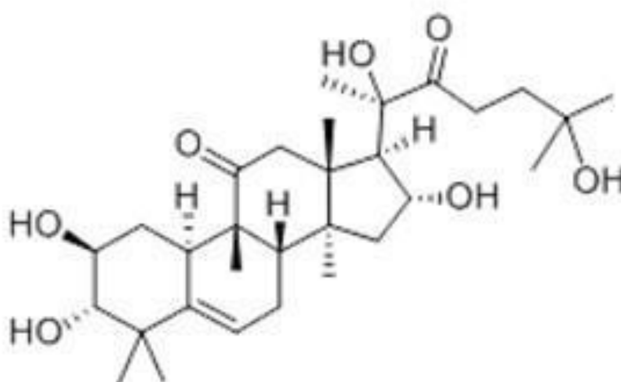
^{1}H-NMR (MeOD) (δppm): 1.13-2.32 (m, 10H, Cyclohexane-methylene), 1.35-1.60 (s, 2H, Cyclopentane-methylene), 1.40-2.25 (3H, Methine), 2.82-5.83 (m, 5H OH alcohol), 5.37 (m, 1H Cyclohexene-ethylene), 0.91-1.86 (m, 24H methyl).

The mass spectrum of EEPM 1 showed the m/z 394.32 suggesting approximate molecular weight as 395 and the spectrum gave the fragment ion peaks at 212.23, 165.17, 104.97, 79.06 and of EEPM 2 showed the m/z 386.62, 368.51, 353.55, 275.45, 105.11 suggesting approximate molecular weight as 387. The phytochemical screening and spectral datas of the isolated compounds suggested that EEPM 1 structural similarities with Stigmast-5-en-3 beta-ol EEPM 2 may be (10α)-2β, 3α, 16α, 20, 25-Pentahydroxy-9β-methyl-19-norlanost-5-ene-11, 22-dione.

Structure of isolated compound EEPM 1(Stigmast-5-en-3 beta-ol)



Structure of isolated compound EEPM 2 ((10α)-2β, 3α, 16α, 20, 25-Pentahydroxy-9β-methyl-19-norlanost-5-ene-11, 22-dione)



PHARMACOLOGICAL SCREENING

In vitro Anticancer Activity

The anticancer activity of all four extracts of *Pedalium murex* Linn was studied on various cell lines such as human breast cancer cell lines (MCF-7) and cervical cancer cell lines (HeLa) using MTT assay method. The EEPM at doses of 10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml produced a significant anticancer activity against MCF-7 and HeLa cancer cell lines.

Table No. 12. % Cell Inhibition of EEPM in MCF-7 Cell Line

Concentration (µg/ml)	% Cell Inhibition			
	n-hexane	Chloroform	Ethyl acetate	Ethanol
10	10.23	17.23	15.05	32.54
20	22.17	38.01	19.37	40.31
50	28.96	45.18	36.34	54.15
100	38.45	49.21	52.93	63.03
200	43.56	57.05	65.32	78.27

Table No. 13. % Cell Inhibition of EEPM in HeLa Cell Line

Concentration (µg/ml)	% Cell Inhibition			
	n-hexane	Chloroform	Ethyl acetate	Ethanol
10	21.67	17.11	10.23	45.23
20	35.23	43.67	28.12	53.34
50	48.17	51.2	35.31	65.22
100	57.45	59.19	47.06	77.52
200	71.03	84.13	63.87	95.32

Fig. 15. % Cell Inhibition of EEPM in MCF-7 Cell Line

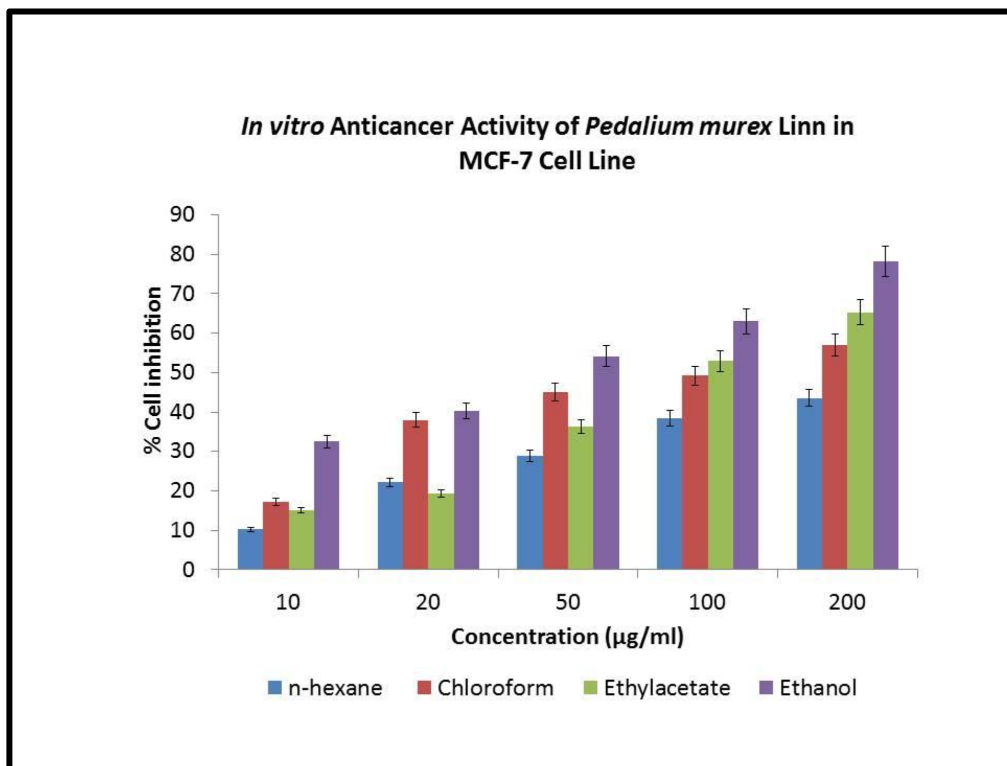
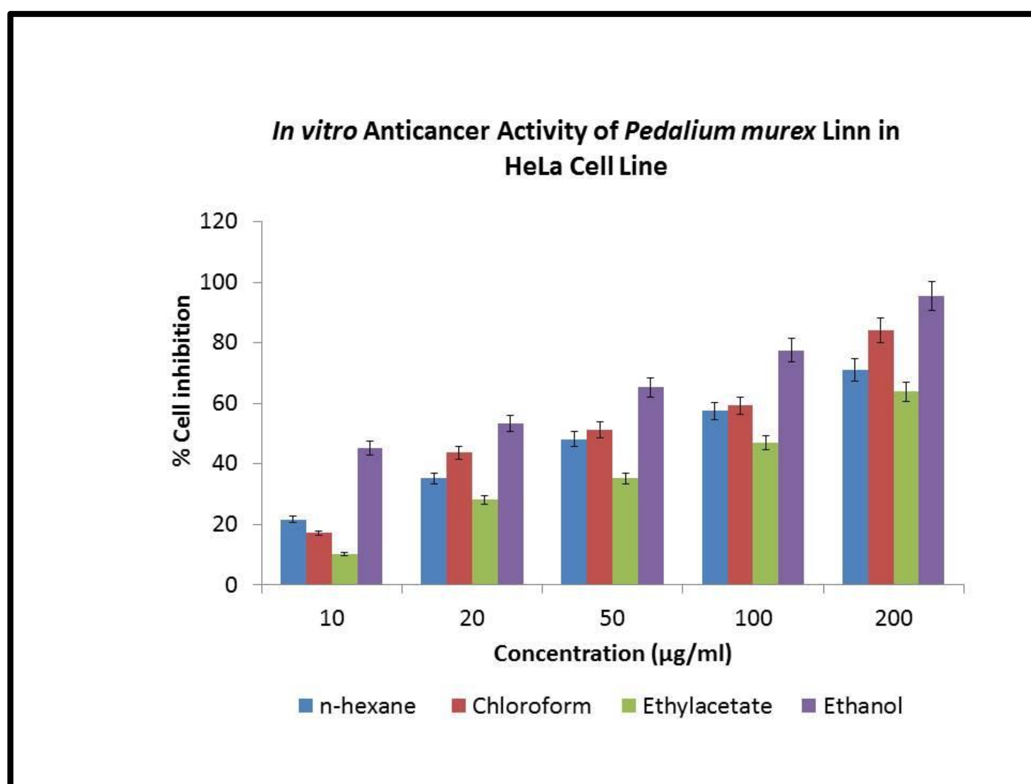


Fig. 16. % Cell Inhibition of EEPM in HeLa Cell Line



The pharmacological screening of the four extracts of *Pedalium murex* Linn were performed as per standard procedures. The data of percentage cell inhibition by four extracts of *Pedalium murex* Linn at different concentrations were presented in Table No. 12 and 13 and fig No. 15 and 16. The results indicated that ethanol extract of *Pedalium murex* Linn showed significant inhibition on both cell lines when compared to other extracts.

7. SUMMARY AND CONCLUSION

In the thesis entitled Phytochemical and Pharmacological Evaluation of *Pedalium murex* Linn Leaves (Family: Pedaliaceae) has been studied to compare four different extracts such as n-hexane, chloroform, ethyl acetate and ethanol (90% v/v) and given the detailed report on phytochemical and pharmacological studies. The leaves of *Pedalium murex* Linn were selected for present project on the basis of ethanobotanical information.

Literature survey revealed that not much work has done in this plant. So I felt it worthwhile to validate scientifically, the folkclaim for its therapeutic activity. The detailed preliminary phytochemical investigations proved its appropriate identification and rationalized its use as a drug of therapeutic importance. This plant have so many phytoconstituents like flavonoids, alkaloids, saponins, tannins and so on. The phytoconstituents are found to possess many pharmacological activities like antioxidant, anticancer, antiinflammatory, antimicrobial activities. So it was planned to isolate active constituents from ethanol extract of *Pedalium murex* Linn leaves.

The present study concluded that the phytoconstituents were isolated from the leaves of ethanol extract of *Pedalium murex* Linn and characterized systematically with IR, H^1 NMR and Mass spectroscopy. The spectral datas of the isolated compounds suggested that EEPM 1 showed the structural similarities with Stigmast-5-en-3 beta-ol and EEPM 2 may be (10α)-2β, 3α, 16α, 20, 25-Pentahydroxy-9β-methyl-19-norlanost-5-ene-11, 22-dione.

All the four extracts were screened for MCF-7 and HeLa cell lines by MTT assay method. In both the cell lines, ethanol extract produced more significant *in vitro* anticancer activity than other extracts. It showed that triterpenoids and steroids were present in extract might be responsible for the anticancer activities.

A few suggestions can be made to improve the results in the future work. First of all, the pharmacological activity has to perform with isolated compounds which will give better result than the extract because other phytoconstituents of the extract may also responsible for the anticancer activity. Further studies are necessary to asses the *in vivo* potential of active constituents in a variety of animal models and other unknown pharmacological activities also need to be explored.

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