ISOLATION, GC-MS ANALYSIS AND ANTI-CANCER ACTIVITY OF HYDRO-ALCOHOLIC EXTARCT OF PONGAMIA PINNATA (Linn.) Pierre

Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI-32.

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

MASTER OF PHARMACY

Submitted by

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(Reg. No. 261715356)

UNDER THE GUIDANCE OF

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

ANAND NAGAR, KRISHNAN KOIL -626 126

TAMILNADU

NOVEMBER-2019

CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **"ISOLATION, GC-MS ANALYSIS AND ANTI-CANCER ACTIVITY OF HYDRO-ALCOHOLIC EXTARCT OF PONGAMIA PINNATA (Linn.) Pierre"** submitted by **Reg. No: 261715356** to the **Tamil Nadu Dr. M.G.R. Medical University, Chennai** for the partial fulfilment of the requirement for the Degree of Master of Pharmacy in Pharmaceutical Chemistry. This research work was carried out in the Department of Pharmaceutical Chemistry under the guidance and supervision of **Dr. R. Rajapandi, M.Pharm., Ph.D., Professor,** Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil - 626 126.

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EVALUATION CERTIFICATE

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INDEX

INTRODUCTION

In the 21st century, with the increased efficacy in pharmacological effects of medicinal plants, herbal medicine has been considered as a promising future medicine for the management of health care. Recently, there has been a swing in universal trend from synthetic to herbal medicine, which is claimed as "Return to Nature." [1] Overall, now a days, the demand for plant-based medicines, health products, food supplements, and cosmetics is being amassed in both developing and developed countries.

The reason behind it is the growing recognition that the natural products are nontoxic, have less side effects, and are easily available at affordable prices [2]. Medicinal plants are considered as rich sources of phytochemical ingredients which play a vital role for the development of new drugs. People have been using plants as a medicine without scientific knowledge and proper guidance for thousand years ago. It has been scientifically established that every part of plants have medicinal properties including roots, stems, leafs, flowers, fruits, and seeds.

However, it has also been witnessed that some plants are not safe for consumption as being toxic and show adverse effects in the body [3]. Therefore, to develop drug from the phytocompounds, the bioactive extract should be standardized on the basis of active compound and should also undergo limited safety studies. In recent years, there has been a resurgence of interest to rediscover medicinal plants as a source of potential drug candidate.

Herbal medicine: Definition

Traditional medicine refers to health practices and approaches which are based on knowledge and beliefs incorporating plants as medicines, spiritual therapies, and physical therapy; either applied singularly or in combination to treat, diagnose, and prevent illnesses or maintain well-being.

In developed countries, adaptations of traditional medicine are termed complementary and alternative medicine [4]. Whereas, herbal medicine or phytomedicine is the use of merely plants for medicinal and therapeutic purpose for curing of diseases and improve human health.

World Health Organization (WHO) has defined herbal medicines as finished labelled medicinal product that contain an active ingredient, aerial, or underground parts of the plant or other plant material or combinations [5] [6].

Fig. 1

At pharmacodynamics scale herbal medicines are classified as

(1) Herbal drugs with proven efficacies with known active compounds and doses,

(2) Herbal drugs with expected efficacies and active compound need to be standardized, and

(3) Herbal drugs with uncertain efficacies but documented history of its traditional use [6].

Plants being used as food or raw material in traditional medicine are more likely to yield pharmacologically active compounds. Plants are also rich dietary sources of biomolecules, vitamins, and minerals which are crucial for maintaining the healthy body [7].

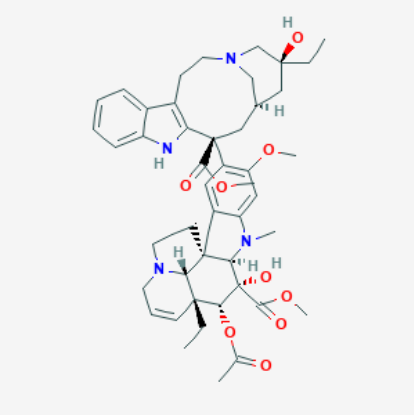
Herbal medicine received a worldwide boost when the WHO exhilarated developing countries to use traditional plant medicine to accomplish needs unmet by modern systems [8]. WHO has reported that 4 billion people (80% of the world's population) use herbal medicines for one or other aspect of primary health care [9].

The pharmacological effects of plants are indebted into the presence of metabolites, which are organic compounds and classified into primary and secondary metabolites. Primary metabolites such as glucose, starch, polysaccharide, protein, lipids, and nucleic acids are beneficial for growth and development of the human body. Whereas, plants produce secondary metabolites including alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins,

volatile oils, etc. to protect plants against microbial infections or invasions by pests. The therapeutic efficacy of plants is because of these secondary metabolites and these are actually termed as "phytocompounds." Which are pharmacologically active ingredients and are exploited as drugs because of their therapeutic properties [10]. The use of such compounds has reduced the risk of many human diseases including cardiovascular diseases, hepato-renal diseases, diabetes, cancers, and neurodegenerative disorders. Additionally, plants are bestowed with several other pharmacological characters such as antioxidant, antiviral, antimicrobial, and anti-parasitic for human use [11] [12].

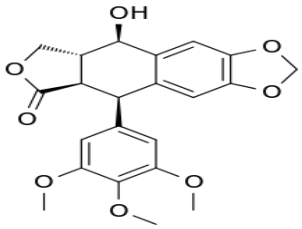
Medicinal plants play a vital role for the development of new drugs. According to WHO, nearly 25% of the modern medicines have been derived from plants being used in traditional medicine. Many others are synthetic analogs fabricated on model compounds isolated from plants. And now WHO has recognized herbal medicine as a crucial components for primary health care [13].

Plant-based drugs have contributed revolutionarily to modern therapeutics. Like, vinblastine from the *Catharanthus rosesus* is successfully used in treating Hodgkin's, choriocarcinoma, non-Hodgkin's lymphomas, leukaemia in children, testicular, and neck cancer [14].



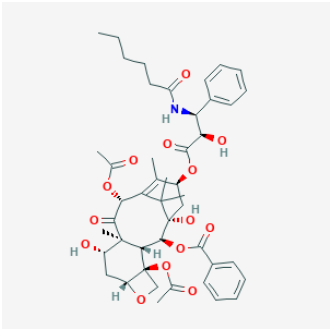
Vinblastine

Podophyllotoxin, isolated from *Phodophyllum emodi*, is efficaciously used against testicular, lung cancer, and lymphomas.



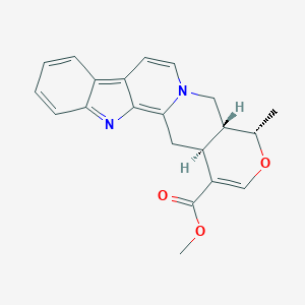
Podophyllotoxin

Taxol isolated from *Taxus brevifolius* is used for the treatment of metastatic ovarian cancer and lung cancer.



Taxol

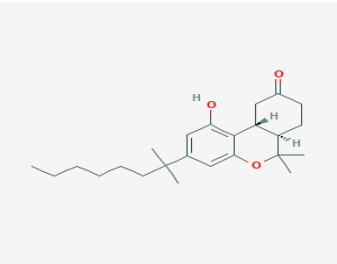
Moreover, in 1953, a compound named serpentine isolated from the root of *Rauwolfia serpentina* is a noteworthy discovery in the treatment of hypertension and reducing the blood pressure [15].



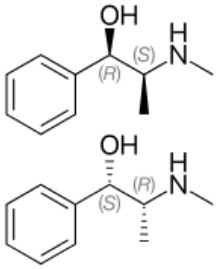
Serpentine

It has been reported that during 1950 to 1970 about 100 new drugs based on plants were introduced in the US pharmaceutical industry including deserpidine, reseinnamine, reserpine,

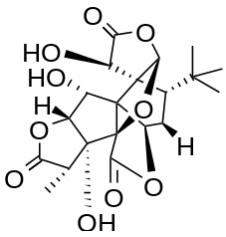
vinblastine, and vincristine. From 1971 to 1990 new drugs isolated from plants such as ectoposide, E-guggulsterone, teniposide, nabilone, plaunotol, Z-guggulsterone, lectinan, artemisinin, and ginkgolides. From 1991 to 1995, some more drugs of plant origin including paciltaxel, toptecan, gomishin, and irinotecan find their place in pharmaceutical industries [16]. Moreover, many researchers in recent decades have recognized several other chemical compounds derived from plant sources including quinine, digoxin, aspirin, ephedrine, atropine, and colchicine [17] [18].



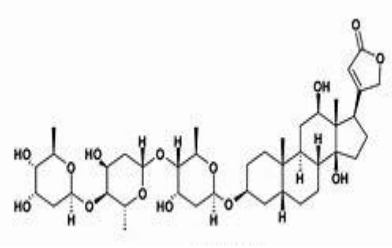
Nabilone



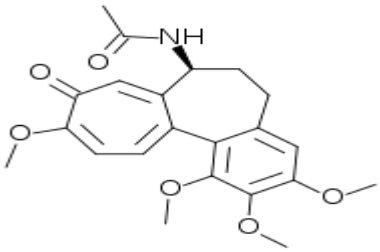
Ephedrine



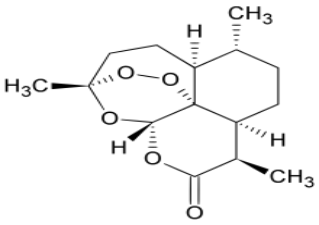
Ginkgolides



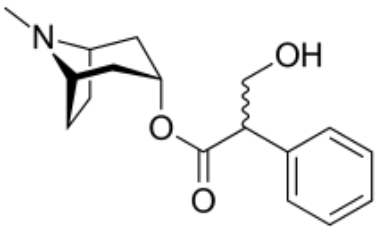
Digoxin



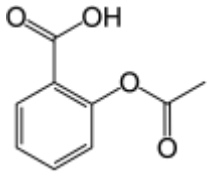
Colchicine



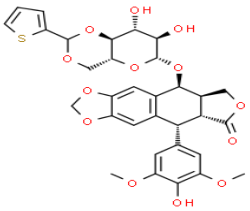
Artemisinin



Atropine



Aspirin



Teniposide

Worldwide it is expected that 80% of the population uses herbs, and in the developing countries rates could be as high as 95% [19].

Local ethnomedical preparations are scientifically evaluated and disseminated properly, people will be better aware and satisfied regarding efficacious drug treatment and improved health status [4] [20] [21]. Plants remain a potential source of therapeutic agents, and also serving as raw material base for the extraction of semisynthetic chemical compounds such as cosmetics, perfumes, and food industries [21] [22].

Moreover, when trials are conducted, due to the regulations and classifications defined by modern medical system which are suitable for conventional chemotherapeutic agents; it is not applicable to phytodrugs [23] [24].

WHO has also issued operational guidelines regarding regulatory requirements needed to support clinical trials of herbal products [5].

Standardization of herbal medicines is often a very challenging due to the presence of complex and diverse secondary metabolites. Additionally, the therapeutic actions depend fundamentally on age, geographical location, and parts of the plant species used [25]. The variability in phytochemical constituents in herbal products from the same plant species leads to intense differences in pharmacological activity. Also, the timing of harvesting process and incidents of adulterations with microorganisms affects in attaining the absolute standards of herbal medicines globally [26].

As evidenced by enormous publications of scientific research papers, there is an increased interest among pharmacologist, microbiologist, biochemist, botanist, and natural product chemists, to explore medicinal plants for newer phytochemicals leading to discovery of drugs for the treatment of several ailments [27] [28]. Most of these research workouts cover the areas of isolation, purification, bio-analytical methodology, and characterization of the bioactive principles of phytocompounds. Furthermore, research efforts in herbal medicine are aiming to elucidate their molecular structures, and establishing their mechanism of action and probable toxicological properties [29].

The safety apprehensions of consuming certain herbal medicine has also been assessed and recognized using *in-vitro* and *in-vivo* systems [30].

Many scientific proofs from randomized clinical trials have provided favourable outcomes toward the use of most of the herbal preparations [31]. Furthermore, the Omic techniques have helped in understanding of mechanism of action of herbal bioactive principles, which has flagged the way for the modernization and standardization of several herbal

medicine [32]. It is worthwhile to note that novel approaches and current insights into herbal medicine research have made a great impact on herbal remedies to compete enough in the mainstream biomedical science.

ANTIOXIDANTS [33]_[34]

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

All these are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage.

Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others.

Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.

Most of the oxidants produced by cells occur as:

• A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.

• Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.

• Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load.

ANTIOXIDANT PROTECTION

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals [35].

These components include:

• Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.

• Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalase free radical quenching reactions.

• Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.

• Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

CANCER

Cancers are a family of diseases that involve abnormal growth of the cells which spreads to other parts of the body [36]. These abnormal cells form malignant growths which called neoplasm. The disease was first named cancer by the Greek physician Hippocrates, Father of Medicine, who applied Greek words “carcinoma” and “Karakinos” to describe a tumor [37].

Cancer was named about the type of tissue from which they arise [38]. Tumor resulting from epithelia are called “carcinomas.” In both genders, cancers of the lung, colon, and rectum are the most significant problem. Breast cancer is common in women and prostate cancer in men. Breast cancers are not quite as prevalent as these “major four” diseases. They include carcinomas of the bladder, stomach, liver, kidney, pancreas, esophagus, and cervix and ovary in women. Epidemiology of cancers is most natural skin cancer. They are rarely deadly, with the important exception of melanoma. Testicular cancer is the most frequent cancer affecting young adult males [39].

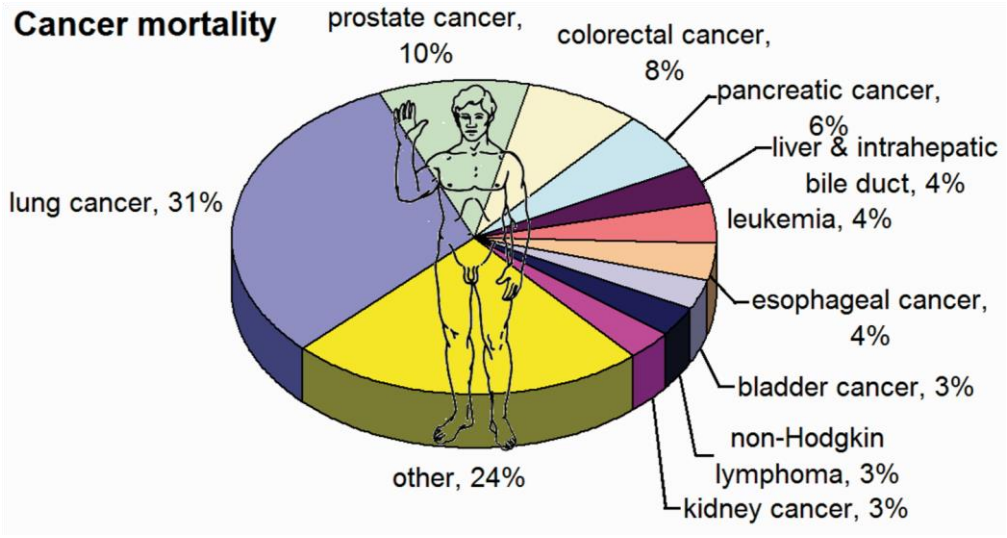


Fig. 2: Cancer mortality in males

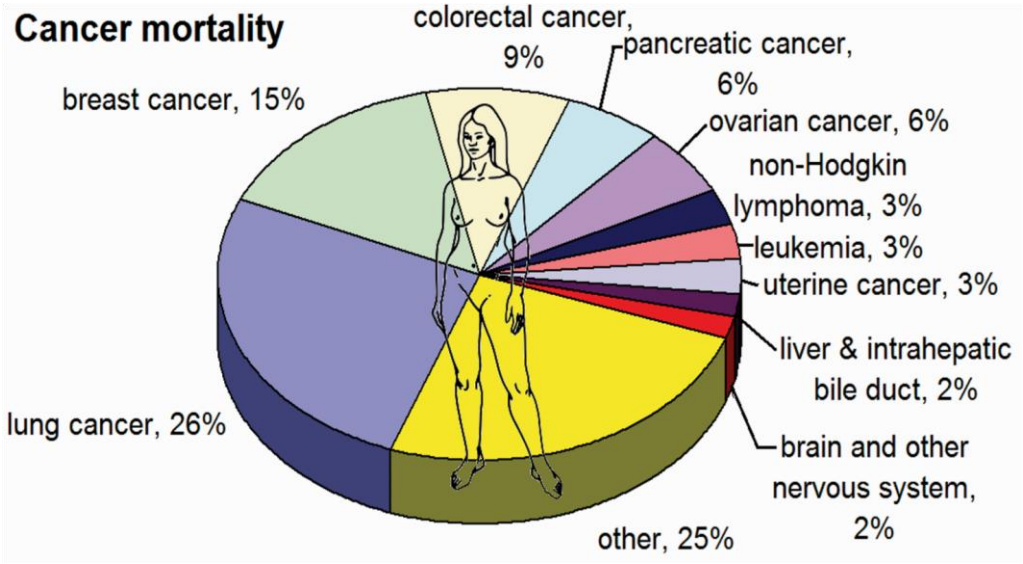


Fig. 3: Cancer mortality in females

Causes of cancer

The majority of cancers are due to environmental factors. The main reason of cancer are related to the environmental, lifestyle or behavioural exposures. The ecological factors that contribute to cancer death include chemicals in tobacco smoke, radiation, such as ultraviolet rays from the sun, obesity, stress, lack of exercise and environmental pollutants. Exposure to substances linked to specific types of cancer such as exogenous chemical, physical, or natural carcinogens [40].

Classification of human carcinogen

a. Chemical carcinogens: Nickel, cadmium, arsenic, nitrosamines, trichloroethylene, arylamines, benzopyrene, aflatoxins, and reactive oxygen species.

b. Physical carcinogens: Ultraviolet irradiation (specifically UVB), ionizing radiation.

c. Biological carcinogens: Human papillomavirus, Hepatitis virus B, Helicobacter pylori, Epstein–Barr Virus, etc.

d. Endogenous processes: DNA replication, metabolic reactions, and chronic inflammation.

Cancer by genetic changes

Changes in genes cause disease. The mutation in the different types of a gene often are associated with different forms of cancer. These altered or mutated genes can be broadly classified into three groups, such as proto-oncogenes, tumor suppressor genes, and DNA repair genes.

1. Proto-oncogenes genes involved in healthy cell growth and division. Alteration in these genes may become cancer-causing genes.

2. Tumor suppressor genes involved in controlling cell growth and division.

3. DNA repair genes participate in repairing damaged DNA. Mutation in these genes develops additional variation in other genes. These mutations may cause the cells become cancerous [40] [41].

Characteristics of cancer and cancer cells

Human diseases share several essential features:

• Increased cell proliferation (often autonomous)

• Insufficient apoptosis

• Altered cell and tissue differentiation

• Altered metabolism

• Genomic instability

• Immortalization (growth beyond replicative senectitude)

• Conquering into different tissue layers and other tissues

• Metastasis to local lymph nodes and distant tissues.

Classification of cancer

Cancer is classified regarding the site of origin of the malignant cells; the histology or cell lysis (called grading); and the extent of disease (called staging) [42].

1. **Site of cancer origin**

This classification describes the tissues in which the cancer cells begin to develop. Following are the examples of the location of tumorgenesis categorization.

• Adenocarcinoma (prostate cancer) - originates in gland cells.

• Blastoma (embryonal carcinosarcoma) - arises in fetal tissues.

• Carcinoma (cancer) - originates in epithelial tissue.

• Myeloblastic Leukemia - occurs in tissues which generate cells of blood.

• Lymphoma (malignant neoplastic disease) - occurs in tissue.

• Myeloma - a tumor of the bone marrow composed of cells normally found in bone marrow.

• Sarcoma - originates in connective tissue such as bone, cartilage, and muscle [42].

2. **Grading**

The degree of malignancy of a tumor is estimated by grading systems. The abnormal behaviour of the cells determines the grade of cancer. Increasing abnormality of cells increases the degree, from 1 to 4. The most general scheme is G grading, which ranks from G0 to G4.

• G0 denotes normal differentiation and no cellular atypia.

• G4 denotes cellular morphology entirely different from the normal tissue.

• G1, G2, and G3 grades are defined well-differentiated, moderately and poorly differentiated [42].

3. Histological classification

Cancer is classified histologically by the location of the tumor. Histological typing of tumours performed by evaluating their morphology. A tumor is histologically classified from surgical specimens. Biological markers improved tumor classification by histopathological classification. For haematological classification, genetic science techniques are used [43].

4. Staging classification

The extension of a tumor is defined by "staging." Two types of stages were described as follows:

A. Clinical stage:

Before surgery, a clinical stage is defined by visual examination, pulsation, and various imaging techniques. These methods use ultrasound, X-rays, computed tomography, and magnetic resonance. "c" prefix denotes it.

B. Pathological stage:

After surgery performed, a more precise examination of the tumor can be made by inspection of the tumor site and by histopathological investigation of the specimen. The stage defined pathological stages, and "p" prefix denotes it.

Mostly used and systematic staging system is the tumor, node, and metastasis system.

Cancer is classified by tumor size (T), the degree of node development (N), and distant metastasis (M), while others remain in use for specific cancers [43].

PREVENTION OF CANCER

Healthy behaviors can go a long way toward improving health and lowering risk of many cancers as well as heart disease, stroke, diabetes, and osteoporosis. So take control of health, and encourage family to do the same.

- Maintain a healthy weight
- Exercise regularly
- Don't smoke
- Eat a healthy diet
- Drink alcohol only in moderation, if at all

- Protect yourself from the sun and avoid tanning beds
- Protect against Sexually Transmitted Infections
- Get screening tests

Maintain a healthy weight

Keeping weight in check is often easier said than done, but a few simple tips can help. First off, if you're overweight, focus on not gaining any more weight. This by itself can improve health. Then, when ready, try to take off some extra pounds for an even greater health boost.

Tips - Fit physical activity and movement into life each day. Limit time in front of the TV and computer. Eat a diet rich in fruits, vegetables, and whole grains. Choose smaller portions, and eat more slowly.

Exercise regularly

Few things are as good for as regular physical activity. While it can be hard to find the time, it's important to fit in at least 30 minutes of activity every day. More is even better, but any amount is better than none.

Tips - Choose activities to enjoy. Many things count as exercise, like walking, gardening, and dancing. Make exercise a habit by setting aside the same time for it each day try going to the gym each day at lunchtime or taking a walk regularly after dinner. Stay motivated by exercising with someone. Play active games with kids regularly, and go on family walks and bike rides when the weather allows.

Don't smoke

If you smoke, quitting is absolutely the best thing you can do for your health. Yes, it's hard, but it's also far from impossible. Over 1,000 Americans stop for good every day.

Tips - Keep trying! It often takes 6 or 7 tries before you quit for good. Talking to a doctor can double your chances of success. When appropriate, talk to kids about the dangers of smoking and chewing tobacco.

Eat a healthy diet

The basics of healthy eating are pretty simple. They should focus on fruits, vegetables, and whole grains, and keep red meat and processed meat to a minimum. It's also important to cut back on bad fats (saturated and trans fats), and choose healthy fats (polyunsaturated and monounsaturated fats) more often. Taking a multivitamin with folate every day is a great nutrition insurance policy.

Tips - Make fruits and vegetables a part of every meal. Put fruit on your cereal. Eat vegetables as a snack. Choose chicken, fish, or beans, instead of red meat. Choose whole-grain cereal, brown rice, and whole- wheat bread over more refined choices. Choose dishes made with olive or canola oil, which are high in healthy fats. Cut back on fast food and store-bought snacks (like cookies), which are high in bad fats. Buy a 100% DV multivitamin that contains folate.

Drink alcohol only in moderate, if at all

Moderate drinking is good for the heart, but it can also increase the risk of cancer. If don't drink, don't feel that you need to start. If you already drink moderately (less than 1 drink a day for women, less than 2 drinks a day for men), there's probably no reason to stop. People who drink more, though, should cut back.

Tips - Choose non-alcoholic beverages at meals and parties. Avoid occasions centered around alcohol. Talk to a health care professional if you feel you have a problem with alcohol. When appropriate, discuss the dangers of drug and alcohol abuse with children. A health care professional or school counsellor can help.

Protect yourself from the sun and avoid tanning beds

While the warm sun is certainly inviting, too much exposure to it can lead to skin cancer, including serious melanoma. And tanning beds can be just as harmful. Skin damage starts early in childhood, so it's especially important to protect children.

Tips - Steer clear of direct sunlight between 10:00 a.m. and 4:00 p.m. (peak burning hours). It's the best way to protect yourself. - Use hats, long-sleeve shirts, and sunscreens with SPF30 or higher. Don't use sun lamps or tanning booths. Protect kid's first and set a good example by always wearing sunscreen and the right clothing.

Protect against Sexually Transmitted Infections

Among other problems, sexually transmitted infections — like human papillomavirus (HPV) — are linked to a number of different cancers. Protecting yourself from these infections can lower your risk. Getting girls and boys vaccinated against HPV will lower their cancer risk later in life.

Tips - Get boys and girls vaccinated against HPV at 11 or 12 years old. Older kids can also be vaccinated. Talk to a health care provider. - Aside from not having sex, the best protection is to be in a committed, monogamous relationship with someone who does not have a sexually transmitted infection. For all other situations, be sure to always use a condom and follow other safe sex practices. Never rely on your partner to have a condom. Always be prepared. When appropriate, discuss with children the importance of abstinence and safe sex.

Get screening tests

There are a number of important screening tests that can help protect against cancer. Some of these tests find cancer early when they are most treatable, while others can actually help keep cancer from developing in the first place. For colorectal cancer alone, regular screening could save over 30,000 lives each year. That's three times the number of people killed by drunk drivers in the United States in all of 2014. Talk to a health care professional about which tests you should have and when. Cancers that should be tested for regularly: - Colon and rectal cancer - Cervical cancer - Breast cancer - Lung cancer (in current or past heavy smokers) [44].

TREATMENT OF CANCER

There are many types of cancer treatment. The types of treatment that you receive will depend on the type of cancer you have and how advanced it is. Some people with cancer will have only one treatment. But most people have a combination of treatments, such as surgery with chemotherapy and/or radiation therapy. When you need treatment for cancer, you have a lot to learn and think about. It is normal to feel overwhelmed and confused. But, talking with your doctor and learning about the types of treatment you may have can help you feel more in control.

- Surgery
- Radiation therapy
- Chemotherapy
- Immunotherapy to treat cancer
- Targeted therapy
- Hormone therapy
- Stem cell transplant
- Precision medicine

Surgery

When used to treat cancer, surgery is a procedure in which a surgeon removes cancer from your body. Learn the different ways that surgery is used against cancer and what you can expect before, during, and after surgery.

Radiation therapy

Radiation therapy is a type of cancer treatment that uses high doses of radiation to kill cancer cells and shrink tumours. Learn about the types of radiation, why side effects happen, which ones you might have, and more.

Chemotherapy

Chemotherapy is a type of cancer treatment that uses drugs to kill cancer cells. Learn how chemotherapy works against cancer, why it causes side effects, and how it is used with other cancer treatments.

Immunotherapy to treat cancer

Immunotherapy is a type of treatment that helps your immune system Fight cancer. Get information about the types of immunotherapy and what you can expect during treatment.

Targeted therapy

Targeted therapy is a type of cancer treatment that targets the changes in cancer cells that help them grow, divide, and spread. Learn how targeted therapy works against cancer and about common side effects that may occur.

Hormone therapy

Hormone therapy is a treatment that slows or stops the growth of breast and prostate cancers that use hormones to grow. Learn about the types of hormone therapy and side effects that may happen.

Stem cell transplant

Stem cell transplants are procedures that restore blood-forming stem cells in cancer patients who have had theirs destroyed by very high doses of chemotherapy or radiation therapy. Learn about the types of transplants, side effects that may occur, and how stem cell transplants are used in cancer treatment.

Precision medicine

Precision medicine helps doctors select treatments that are most likely to help patients based on a genetic understanding of their disease. Learn about the role precision medicine plays in cancer treatment, including how genetic changes in a person's cancer are identified and used to select treatments. [45].

PEOPLE WITH CANCER USE HERBAL MEDICINE

Nowadays, cancer considered as one of the most prevalent diseases in the world, and its mortality is increasing. It is necessary to investigate new strategies to prevent and treat disease. Therefore, developing a new approach is one of the primary objectives of immune-pharmacological studies to improve cancer treatment results [46]. Nowadays, herbal medicines have played a significant role in controlling cancer symptoms and treatments with minimizing side effects [47]. Many people with cancer choose to take herbal medicines because of no side effects on healthy cells. They usually take them while having their regular cancer treatment [50].

Fig. 4: Indian medicinal herbs render cancer chemopreventive and therapeutic effects.

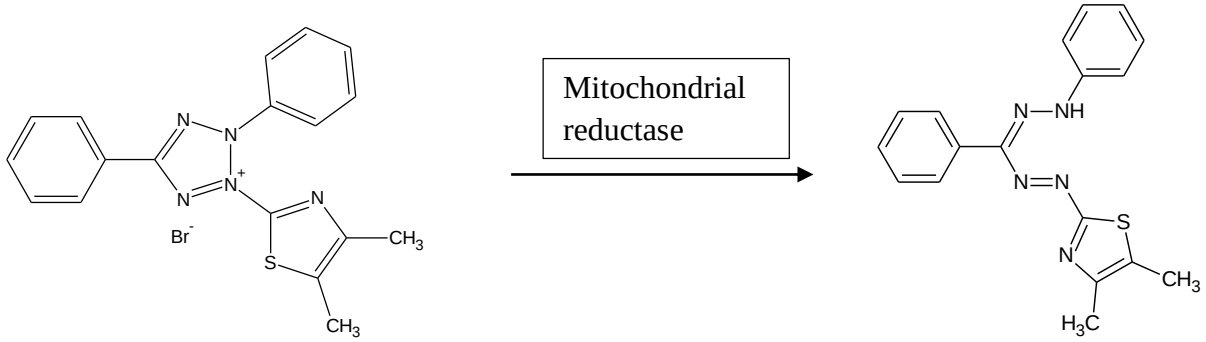
Medicinal plant constituents include vinca alkaloids (vinblastine and vincristine), taxanes (paclitaxel and docetaxel), podophyllotoxin, and its derivatives (topotecan and irinotecan). Camptothecins have clinically used as plant-derived anticancer agents [48] [49].

There are many reasons for using herbal medicine:

i. To treat the side effects of cancer treatment including tiredness and nausea
ii. To kill cancer cells
iii. To boost the immune system (the body's natural defence again illness)
iv. To improve quality of life and well-being
v. To relieve symptoms when all other therapies have failed [50].

MTT Method [103-104]

The *in-vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.



(3-[4, 5- dimethylthiazol-2-yl] - 2, 5- 2- diphenyltetrazolium bromide)

MTT

(2E, 4Z) - (4, 5- dimethylthiazol-yl) - 3, 5- diphenylformazan

Formazan

LITERATURE REVIEW

Literature review of plant

Deepthi VJ *et al* [51] **2016** they have attempt to compare on the preliminary phytochemical and pharmacognostic parameters of leaves of Karanja with that of the established standardization in API.

Ahmat N *et al* [52] **2013** this review discusses the current knowledge of traditional uses, phytochemistry, biological activities, and toxicity of this species in order to reveal its therapeutic and gaps requiring future research opportunities.

Savita Sangwan *et al* [53] **2010** have briefly reviews the botany, distribution, ecology, uses of the plant and as a source of biodiesel. This is an attempt to compile and document information on different aspect of *Pongamia pinnata (*Linn.*)* Pierre and its potential use as a source of biodiesel.

Arote SR *et al* [54] **2010** they have aimed to compile up to date and comprehensive information of *Pongamia pinnata* (Linn.) Pierre with special emphasis on its phytochemistry, various scientifically documented pharmacological activities, traditional and folk lmedicine uses along with its role in biofuel industry.

Chopade VV *et al* [55] **2008** have briefly discuss about Phytochemical constituents, Traditional uses and Pharmacological properties of *Pongamia pinnata* (Linn.) Pierre.

Literature review of plant related activity

Peng-Fei Tu *et al* [56] **2018** have performed phytochemical study on the roots of *Pongamia pinnata* (Linn.) Pierre yielded 52 flavonoids, including four previously undescribed flavone and four previously undescribed chalcone derivatives. The structures of the isolated compounds were determined on the basis of the 1D, 2D NMR, and mass spectroscopic data. The absolute configurations of the compounds were assigned via the specific rotation, Mosher's method, as well as the electronic circular dichroism (ECD) spectra. All the isolates were evaluated for their inhibitory effects on NO production in LPS-stimulated BV-2 microglial cells. Ten compounds showed significant inhibitory effects against NO production, comparable to the positive control, dexamethasone.

Deepak Dwivedi *et al* [57] **2017** have investigated wound healing, antimicrobial and antioxidant activity of methanolic extract of *Pongamia pinnata* (Linn.) Pierre leaf.

Ill- Min Chung *et al* [58] **2017** have performed to develop an easy and eco-friendly method for the synthesis of Ag-NPs using extracts from the medicinal plant, *Millettia pinnata* flower extract and investigate the effects of Ag-NPs on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), antibacterial and cytotoxicity activity.

Maidul Hossain *et al* [59] **2016** have performed the green synthesis of silver nanoparticles using ethanolic extract of *Pongamia pinnata* (Linn.) Pierre seed and Characterization, antibacterial property, and spectroscopic investigation of interaction with human serum albumin.

Rajeshkumar S [60] **2016** have performed the synthesis and characterization of silver nanoparticles using aqueous fresh bark of *Pongamia pinnata* (Linn.) Pierre and investigate its antibacterial activity against gram positive and gram negative pathogens.

Rajeshkumar S *et al* [61] **2015** have performed the hepatoprotective activity of aqueous and ethanol extract of *Pongamia pinnata* (Linn.) Pierre leaves against acetaminophen-induced liver damage in albino rats.

Gang Chen *et al* [62] **2015** have performed the phytochemical research of dry stem of *Pongamia pinnata* (Linn.) Pierre identified a new chlorinated flavonoid, 2', 6'- dichloro-3', 5'- dimethoxy [2", 3": 7, 8] furano flavone together with 29 known compounds and the anti-neuroinflammatory activities were evaluated.

Sundrarajan M *et al* [63] **2015** have synthesised the highly stable and hexagonal phase ZnO nanoparticles using *Pongamia pinnata* (Linn.) Pierre leaves aqueous extract and were characterized by XRD, UV–vis, DLS, SEM, TEM and FT-IR spectroscopy. They were perform the antibacterial activity against gram positive and gram negative bacteria.

Geetha D *et al* [64] **2015** (Ecotoxicology and Environmental safety) they were performed the comparative study of antioxidant activity of chemically synthesized silver nanoparticles and biosynthesized *Pongamia pinnata* (Linn.) Pierre aqueous leaf extract mediated silver nanoparticles. These biosynthesized nanoparticles were characterized with the help of UV–vis Spectroscopy, Photoluminescence (PL) and Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Atomic force microscopy (AFM), Dynamic light scattering (DLS) and Zeta Potential (ZP).

Rupesh K Gautam *et al* [65] **2013** have carried out to compare the anti-arthritic activity of ethanolic extract of seeds of *Pongamia pinnata* (Linn.) Pierre (EEPP) and methanolic extract of rind of *Punica granatum* (Linn.) Pierre (MEPG) by in-vitro techniques. The results of both models exhibited that EEPP, MEPG and standard drug (diclofenac sodium) showed concentration dependent inhibition of protein (egg albumin) denaturation as well as stabilization towards HRBC membrane. By comparing the present findings, it can be concluded that MEPG has more potent anti-arthritic activity than EEPP.

Khumanthem Deepak Singh *et al* [66] **2013** have performed phytochemical screening and gut motility activity of methanolic extract of bark of *Pongamia pinnata* (Linn.) Pierre was evaluated in Wistar Albino Rats of either sex (150-200g) for Gut motility activity at a dose of 200 mg/kg and 400 mg/kg (p.o). The extract reduced the laxative activity as well as distance travelled by the charcoal meal. The presence of anthraquinone glycosides in the plant extract is responsible for the gut motility effect. Thus from the study and literature, it can be concluded that *Pongamia pinnata* (Linn.) Pierre have potent gut motility activity.

Divya Singh *et al* [67] **2013** have investigated the anti-arthritic and anti-inflammatory activity of hydroalcoholic extract of *Pongamia pinnata* (Linn.) Pierre seed. The anti-arthritic and anti-inflammatory activity of *Pongamia pinnata* (Linn.) Pierre hydro-alcoholic extract was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) in vitro methods.

Guna Ranjan Kolli *et al* [68] **2013** have evaluated the mosquito larvicidal activity of methanol and hydroalcohol extracts of *Pongamia pinnata* (Linn.) Pierre bark part against three mosquito vectors.

Gehan J Panagoda *et al* [69] **2013** have investigated for *in vitro* anti-candidal activity of ethanolic extracts of bark, roots and leaves of *Pongamia pinnata* (Linn.) Pierre on different candidal species.

Dayanand CD *et al* [70] **2013** have performed the antibacterial activity of the ethanolic and methanolic seed extracts of *Pongamia pinnata* (Linn.) Pierre

Behera Saiprasanna *et al* [71] **2012** have examined the phytoconstituents and cardioprotective activity of *Pongamia pinnata* (Linn.) Pierre (PP) leaf extract (hydro-alcoholic) in experimentally induced myocardial infarction in Wistar Albino rats.

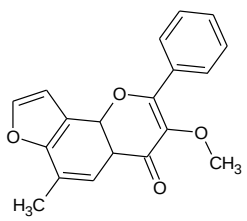
Kotoky J *et al* [72] **2012** have investigate the *in vitro* antifungal activity of *Ranunculus sceleratus* (R. sceleratus) and *Pongamia pinnata* (Linn.) Pierre (P. pinnata), collected from North East India. Chloroform, Methanol and Water extracts of the plants were evaluated for anti-ringworm activity against five strains of dermatophytes viz., *Trichophyton rubrum* (T. rubrum), *Trichophyton mentagrophytes* (T. mentagrophytes), *Trichophyton tonsurans* (T. tonsurans), *Microsporum gypseum* (M. gypseum) and *Microsporum fulvum* (M. fulvum).

Anuradha R *et al* [73] **2012** have performed the hepatoprotective activity of methanolic extract of *Pongamia pinnata* (Linn.) Pierre flowers in rats with lead acetate induced hepatotoxicity. Administering 160 mg/kg b.wt/day of lead acetate for 90 days to male albino rats resulted in significantly elevated levels of ALT, AST, GGT, ALP, Bilirubin and protein were also observed on lead acetate administration as compared with those of the experimental control rats.

Subhash L Bodhankar *et al* [74] **2012** have evaluated the analgesic and anti-inflammatory activity of alcoholic extract of *Pongamia pinnata* (Linn.) Pierre stem bark (PPSBAE) in laboratory animals.

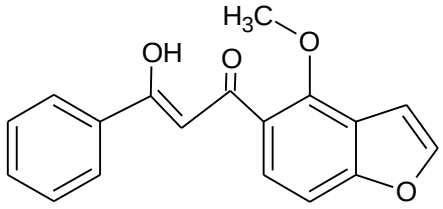
Sinha BK *et al* [75]**2012** have evaluated the insecticidal activity of *Pongamia pinnata* (Linn.) Pierre seed extracts as an insecticide against American bollworm helicoverpa armigera (hubner).

Akhilesh K Tamrakar *et al* [76] **2011** have identified anti-hyperglycaemic molecule, karanjin, isolated from the fruits of *Pongamia pinnata* (Linn.) Pierre were investigated on glucose uptake and GLUT4 translocation in skeletal muscle cells.



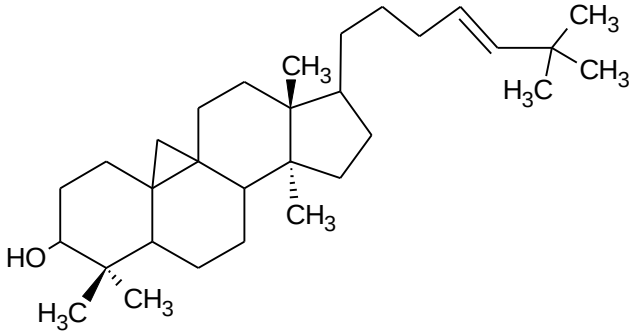
Karanjin

Akhilesh K Tamrakar *et al* [77] **2011** have investigated the effect of pongamol, an identified lead molecule from the fruits of *Pongamia pinnata* (Linn.) Pierre, on glucose uptake and GLUT4 translocation in skeletal muscle cells.



Pongamol

Sachin L Badole *et al* [78] **2011** have evaluated the protective effect of cycloart-23-ene-3β, 25-diol (B2) on vital organs in streptozotocin- nicotinamide induced diabetic mice.



Cycloart-23-ene-3β, 25-diol (B2)

Mohamed M Essa *et al* [79] **2010** have studied for its protective effect of ethanolic extract of *Pongamia pinnata* (Linn.) Pierre leaf during ammonium chloride induced hyperammonemia in Wistar rats.

Mahendra A Giri *et al* [80] **2010** have designed to investigate the antiulcer effect of hydroalcoholic extract of leaves of *Pongamia pinnata* (Linn.) Pierre (HLEPP) using different models of gastric ulceration in rats. Hydroalcoholic extract of leaves of *Pongamia pinnata* (Linn.) Pierre (HLEPP) was subjected to preliminary phytochemical screening. Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including aspirin or ethanol or indomethacin or pylorus-ligated technique. Gastric tissue was also examined histologically. HLEPP was administered in the dose of 400 mg/kg orally in all experiments; dose was calculated on the basis of acute toxicity study.

Anbu Jeba Sunilson J *et al* [81] **2010** have performed anthelmintic activity of the various doses of aqueous extract of *Pongamia pinnata* (Linn.) Pierre leaves was evaluated on earthworms (*Pheretima posthuma*) and the results were compared with standard drug, albendazole (10 mg/mL).

Masami Ishibashi *et al* [82] **2010** have performed the activity to overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistance, and performed the bioassay-guided fractionation of a semi mangrove, *Pongamia pinnata* (Linn.) Pierre, collected from Bangladesh, and isolated a new compound, (2S)-(2", 3":7, 8)-furanoflavanone, along with six known flavonoids. Two of the compounds significantly overcame TRAIL-resistance in human gastric adenocarcinoma (AGS) cell lines.

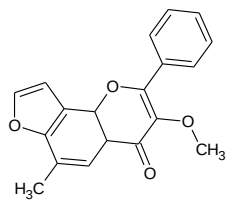
Manoj Kumar Sagar *et al* [83] **2010** has evaluated the anti-inflammatory and analgesic potential of the methanolic extract of *Pongamia pinnata* (Linn.) Pierre stem bark (PSBE) in different experimental animal models.

Priyank A Shenoy *et al* [84] **2010** have evaluated the photoabsorptive property of different extracts (aqueous, methanol and acetone) of the leaves of *Pongamia pinnata* (Linn.) Pierre, Fabaceae, in the ultraviolet region (200–400 nm) and its comparison with a well-established standard sunscreen drug, p-aminobenzoic acid (PABA).

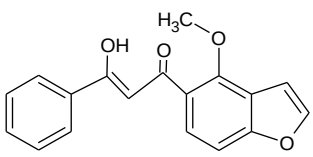
Ashish Manigauha *et al* [85] **2009** have investigated the anticonvulsant efficacy of the 70% ethanolic leaf extract of *Pongamia pinnata* (Linn.) Pierre using maximal electroshock-induced seizure (MES) in mice.

Dahikar SB *et al* [86] **2009** have investigated the antibacterial activity of various extract (Petroleum ether, chloroform, ethyl acetate and methanol) of leaves of *Pongamia pinnata* (Linn.) Pierre.

Arvind K Srivastava *et al* [87] **2008** have identified pongamol and karanjin as lead compounds with antihyperglycemic activity from *Pongamia pinnata* (Linn.) Pierre fruits.

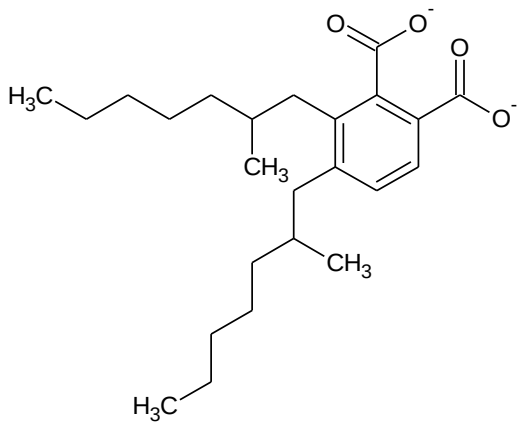


Karanjin



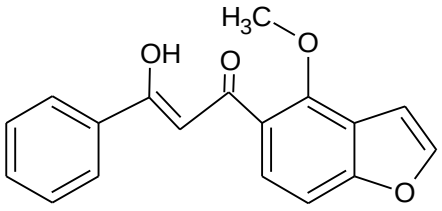
Pongamol

Ramasamy P *et al* [88] **2007** have performed antiviral activity of bis (2-methylheptyl) phthalate isolated from the ethanolic extract of *Pongamia pinnata* (Linn.) Pierre leaves against White Spot Syndrome Virus of *Penaeus monodon* Fabricius.



Bis (2-methylheptyl) phthalate

Alam Khan *et al* [89] **2007** have performed the sub-acute toxicity study of pongamol isolated from *Pongamia pinnata* (Linn.) Pierre on long Evan's rats. The studies included the gross observation such as changes in body weight, haematological profiles (total count of red blood cell, white blood cell and platelet, differential count of white blood cell, erythrocyte sedimentation rate and haemoglobin percentage), biochemical parameters of blood (serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum alkaline phosphatase, serum bilirubin, creatinine and urea) and histopathology of the liver, kidney, heart and lung of both control and experimental groups of rats.



Pongamol

Manoharan S *et al* [90] **2006** have evaluated the anti-hyperglycaemic and anti-lipidperoxidative effect of ethanolic extract of *Pongamia pinnata* (Linn.) Pierre (Leguminosae) flowers (PpEt) in normal rats and alloxan induced diabetic rats. Hyperglycemia, elevated lipid peroxidation [thiobarbituric acid reactive substances

(TBARS)] and disturbed non-enzymatic [Vitamin E, Vitamin C and glutathione] and enzymatic antioxidants status were noticed in alloxan induced diabetic rats.

Perumal Subramanian *et al* [91] **2005** investigate the antihyperammonemic efficacy of the ethanolic extract of *Pongamia pinnata* (Linn.) Pierre leaf (PPEt), on blood ammonia, plasma urea, uric acid, non-protein nitrogen and serum creatinine in control and ammonium chloride induced hyperammonemic rats.

Annie Shirwaikar *et al* [92] **2003** have designed to study the protective effect of ethanolic extract of *Pongamia pinnata* (Linn.) Pierre flowers against cisplatin and gentamicin induced renal injury in rats.

Literature review of gas chromatography- mass spectroscopy

Prasanth G K, Krishnaiah, [93] **2014** they have aimed to carry out the phytochemical screening and GC-MS analysis of the leaves of *Pongamia pinnata* (Linn.) Pierre. Phytochemical screening of aqueous and ethanolic extracts of leaves revealed that the presence of alkaloids, carbohydrates, reducing sugars, etc. GC-MS analysis of the ethanolic extract indicated the presence of many constituents in the leaves of *Pongamia pinnata* (Linn.) Pierre.

Valentin Bhimba *et al* [94] **2010** have evaluated the antibacterial activity of foliar extracts of *Rhizophora mucronata* (R. mucronata) against pathogens belonging to human origin and to identify the compound hitherto unprecedented in nature by GC-MS analysis. The results reveals that UV visible and GS-MS analysis suggested the active principle compound were a mixture of squalene (19.19%), n-Hexadecanoic acid (6.59%), phytol (4.74%), 2-cyclohexane-1- one, 4-hydroxy-3,5, (4.20%) and oleic acid (2.88%).

Literature review of FT-IR (Fourier Transform- Infra Red)

Sasidharan S *et al* [95] **2011** have focused on the analytical methodologies, which include the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations. The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as HPLC and, TLC as well as non- chromatographic techniques such as immunoassay and Fourier Transform Infra-Red (FTIR) are discussed.

Subramanian Arulkumar *et al* [96] **2010** have synthesized the gold nanoparticles using the plant extract *Mucuna pruriens* and have achieved rapid formation of gold nanoparticles in a short duration. The nanoparticles were examined using UV-Visible Spectroscopy, FT – IR, Transmission Electron Microscopy (TEM), and X-ray diffraction (XRD) spectrum of the gold nano-particles analyses.

Literature review of ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry

Veena Sharma, Pracheta Janmeda, [97] **2017** they executed extraction, isolation, and identification of flavonoid from *Euphorbia neriifolia.* The isolated compound was subjected to characterization done by IR, ^{1}H-NMR, and MS.

Bulama J S *et al* [98] **2015** they carry out isolation of Beta- Sitosterol from ethyl acetate extract of root bark of *Terminalia glaucescens.* The isolated compound was further subjected to IR, UV, ^{1}H-NMR, ^{13}C-NMR and elucidate the compound.

Ketekhaye S D *et al* [99] **2012,** have perform the recovery of karanjin from karanja seed oil (*Pongamia pinnata* seed oil) by Liquid- Liquid extraction with methanol. The extract was purified by HPLC. The structure of the compound was elucidated by MS and NMR spectral analysis.

Sindhu Kanya T C *et al* [100] **2010** have perform the recovery of karanjin from karanja seed oil (*Pongamia pinnata* seed oil) by Liquid- Liquid extraction with methanol. The extract was purified by HPLC. The structure of the compound was elucidated by MS and NMR spectral analysis.

Literature review of Anti-cancer activity (Lung Cancer)

Yan Chen *et al* [101] **2013** have studied the anti-lung cancer activity through enhancement of immunomodulation and induction of cell apoptosis of total triterpenes extracted from *Ganoderma luncidum.*

Kandasamy Sivakumar *et al* [102] **2019** have workout the antioxidant, anti-lung cancer and anti-bacterial activities of *Toxicodendron vernicifluum.*

Gonzalez R J, Tarloff J B [103] **2001** have evaluated the hepatic subcellular fractions for Alamar blue and MTT reductase activity.

Hattori N *et al* [104] **2003** they carried out enhanced microbial biomass assay using mutant luciferase resistant to benzalkonium chloride.

Crouch, S P M *et al* [105]**1993** they designed to measure cell proliferation and cytotoxicity by using ATP bioluminescence.

Kangas L *et al* [106] **1984** have perform bioluminescence of cellular ATP: A new method for evaluating cytotoxic agents in vitro.

AIM OF THE WORK

From the detailed literature survey we revealed that *Pongamia pinnata* (Linn.) Pierre under the Fabaceae family having enormous pharmacological activity against various ailments. Moreover the plant has been widely used for local healers, physician, ethnic peoples for especially non-communicable disease like cancer, Type II diabetes and CVS disorder. But the scientific proof of the above activity is unclear and no documentation so for. In this content, the present study is an attempt to carry out the phytochemical evaluation of hydroalcoholic leaf extract of *Pongamia pinnata* (Linn.) Pierre and also isolate the active constituents and study the characterisation of isolated compound. It also aimed to evaluate the antioxidant and anticancer activity of above elucidated compound as well as extract.

PLAN OF WORK

- To collect and authenticate the leaves of the plant of *Pongamia pinnata* (Linn.) Pierre.
- To evaluate the physiochemical properties of the shade dried powdered leaves of *Pongamia pinnata* (Linn.) Pierre.
- To extract the dried powdered leaves of *Pongamia pinnata* (Linn.) Pierre by cold maceration (Double maceration method) using hydro-ethanol (Ethanol 70%: Water 30%) and its preliminary phytochemical evaluation was done.
- To fractionate the hydro-alcoholic extract and perform the GC-MS analysis of Fractions.
- To isolate active constituents from the extract of desirable solvents by column chromatographic and thin layer chromatographic techniques.
- To characterize the isolated chemical compound by spectroscopic techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass Spectroscopy studies.
- To evaluate *in-vitro* antioxidant and anti-cancer activities of the leaf extract as well as fractionated compound.

PLANT PROFILE [53, 54, 107, 108]

Pongamia pinnata is a marsh growing Indian tree. It grows in humid environment all over India. *Pongamia pinnata* the only species of the genus pongamia is a semi-mangrove tree distributed along the pacific coast from Southeast Asia to northern Australia.

The 'Pongam Tree' is being cultivated in a large number of gardens and along the countless roads in India and is becoming the one of the most admired city trees. It grows wild in the coastal forests throughout India and beside the streams and rivers.

The tree is named as 'Pongamia pinnata' in science. The name 'Pongamia' has derived from the Tamil name, 'pinnata' that refers to the 'Pinnate leaves'.

Different parts of this plant have been widely used ass traditional medicines to treat a broad spectrum of diseases and wounds.

TAXONOMICAL CLASSIFICATION

Kingdom - Plantae

Subkingdom - Tracheobionta

Superdivision - Spermatophyta

Division - Magnoliophyta

Class - Magnoliopsida

Subclass – Rosidae

Order - Fabales

Family – Fabaceae

Genus – Pongamia

Species – Pinnata

SYNONYMS

Derris indica (Lam.) Bennett

Millettia novo-guineensis Kane. and Hat.

Pongamia glabra Vent.

Pongamia pinnata Merr**.**

BOTANICAL NAME

Pongamia pinnata (Linn.) Pierre

VERNACULAR NAMES

Sanskrit: Ghrtakarauja, Karanjaka, Naktahva, Naktamala

Bengali: Dahara karanja, Karanja, Natakaranja

Assamese: Korach

Kannada: Honge, Hulagilu

Marathi: Karanja

Guajarati: Kanaji, Kanajo

Punjabi: Karanj

Telugu: Ganuga, Kanugu

Hindi: Karuaini, Dithouri

Oriya: Karanja

Tamil: Pungai, Pongana

Urdu: Karanj

Malayalam: Pungu, Ungu, Unu, Avittal

GEOGRAPHICAL DISTRIBUTION

It is widely distributed throughout tropical Asia and the Seychelles Islands, South Eastern Asia, Australia, India and locally distributed throughout the State of Maharashtra (India) along the banks of rivers; very common near the sea-coast in tidal and beach-forests in Konkan; along Deccan rivers.

Fig. 5

Fig. 6

Fig. 7

BOTANICAL DESCRIPTION OF PONGAMIA PINNATA

Plant type:

Medium-sized, evergreen, perennial and deciduous tree

Height: 35 to 40 feet

Growth rate: Fast

Texture: Medium

Chromosome number: 22

Growing requirements:

Light requirement: tree grows in full sun.

Soil tolerances: clay; loam; sandy; slightly alkaline; acidic; well-drained.

Drought tolerance: high

Aerosol salt tolerance: moderate

Winter interest: no special winter

(a) Leaf:

Alternate, odd pinnately compound, 2 to 4 inches, evergreen, hairless.

(b) Flower:

Lavender, pink; white, 2- 4 together, short-stalked, pea shaped, 15-18mm long.

(c) Pods:

3-6cm long and 2-3cm wide, smooth, brown, thick-walled, hard, indehiscent, 1-2 seeded.

(d) Seed:

Compressed ovoid or elliptical, been-like, 10-15cm long, dark brown, oily.

(e) Root:

Taproot is thick and long, lateral roots are numerous and well developed.

(f) Bark:

Thin grey to greyish brown and yellow on the inside.

All parts of the plant are toxic and will induce nausea and vomiting if eaten.

TRADITIONAL USES

The fruits and sprouts are used in folk remedies for abdominal tumours in India, the seeds for keloid tumours in Sri Lanka and a powder derived from the plant for tumours in Vietnam. In Sanskritic India, seeds were used for skin ailments. Today, the oil is used as a liniment for rheumatism. Leaves are active against Micrococcus; their juice is used for cold, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy. Roots are used for cleaning gums, teeth, and ulcers. Bark is used internally for bleeding piles. Juices from the plant as well as the oil are antiseptic. It is said to be an excellent remedy for itch, herpes and pityriasis versicolor. Powdered seeds are valued as a febrifuge, tonic and in bronchitis and whooping cough. Flowers are used for diabetes. Bark has been used for beriberi. Juice of the root is used for cleansing foul ulcers and closing stulous sores. Young shoots have been recommended for rheumatism. The oil is known to have value in folk medicine for the treatment of rheumatism, as well as human and animal skin diseases. It is effective in enhancing the pigmentation of skin affected by leucoderma or scabies.

USES

Root

General use:

Root is used as fish poison

Medicinal use:

Juice of roots with coconut milk and lime water used for treatment of gonorrhoea - Used for cleaning gums, teeth and ulcers. Roots are bitter anti-helminthic and used in vaginal and skin diseases. Juice of the root is used for cleansing foul ulcers and closing fistulous sores.

Stem

General use:

Used for stove top fuels, poles and ornamental carvings. Ash of wood used for dyeing. Cabinet making, cart wheels, posts. Agricultural implements, tool handles and combs.

Medicinal use:

Aqueous extracts of stem bark exhibit significant CNS sedative and antipyretic activity.

Leaf

General use:

Used as cattle fodder. Used in stored grains to repel insects. Used as manure for rice and sugarcane field.

Medicinal use:

Juice of leaves is used for cold, cough, diarrhoea, dyspepsia, flatulence, gonorrhoea, leprosy. Leaves are anti-helminthic, digestive and laxative used for inflammations, piles and wounds. As an infusion to relieve rheumatism. As an extract to treat itches and herpes.

Fruit

General use:

Fruits are edible.

Medicinal use:

Fruits used for abdominal tumours. Useful in ailments of female genital tract, leprosy, tumour, piles, ulcers and upward moving of the wind in the abdomen.

Seed

General use:

After oil extraction has been used as "green manure" as it is rich in protein and nitrogen. Used as insecticides.

Medicinal use:

Used for keloid tumours. Used in hypertension, skin ailments and rheumatic arthritis. Seed powder valued as a febrifuge, tonic and in bronchitis and whooping cough. Useful in inflammations, pectoral diseases, chronic fevers, haemorrhoids and anaemia.

Oil

General use:

Used as fuel for cooking and lamps, as a lubricant, water-paint binder, pesticide and in soap-making, candles and tanning industries. Used as lipids for commercial processes. Used in cosmetics.

Medicinal use:

Oil is styptic, anthelmintic, and good in leprosy, piles, ulcers, chronic fever and in liver pain. Useful in rheumatism arthritis scabies and whooping cough. Mixture of oil and zinc oxide used for eczema.

Bark

General use:

String and rope can be made from the bark fibre. Used for paper pulp.

Medicinal value:

For bleeding piles, for beriberi, reduce swelling of the spleen. Useful in mental disorder, cough and cold.

Flower

General use:

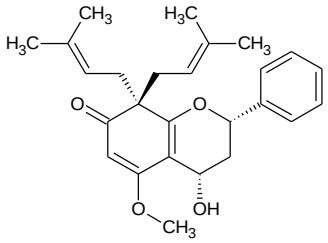
Good sources of pollen for honey bees. Flowers are edible.

Medicinal value:

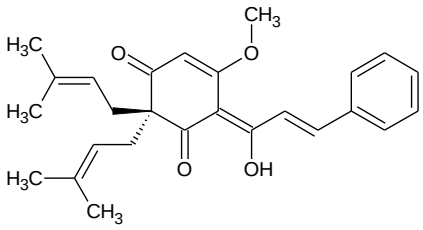
Useful to quench dipsia in diabetes, for alleviating vata and kapha and for bleeding piles.

PHYTOCHEMISTRY

From the stem bark of Pongamia pinnata two phenylated flavonoid derivatives with a modified ring A, pongaflavanol and tunicatachalcone was isolated.



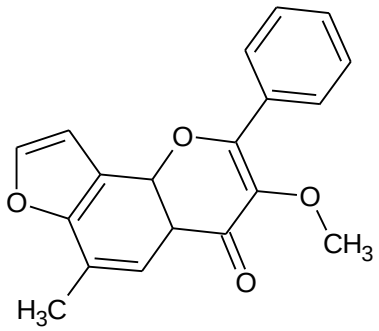
Pongaflavanol



Tunicatachalcone

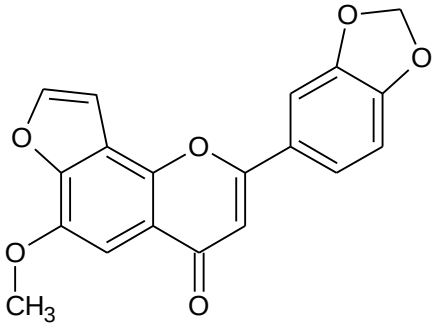
Pongaflavanol was a new compound and its structure was elucidated on the basis of spectroscopic data interpretation. Pongaflavone represented the first example of a naturally occurring prenylated flavan-4-ol with a modified ring A, while compound 2, tunicatachalcone was isolated for the first time from Pongamia pinnata.

Karanja (Pongamia pinnata) seed oil contains karanjin, a bioactive molecule with important biological attributes.

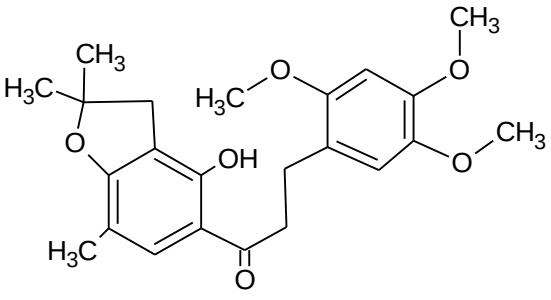


Karanjin

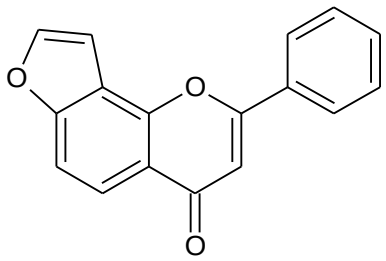
Karangin, pongamol, pongagalabrone and pongapin, pinnatin and kanjone have been isolated and characterized from seeds. Immature seeds contain a flavone derivative 'pongol'. The other flavonoid isolated from the seeds includes 'Glybanchalcone, isopongachromene'. The leaves and stem of the plant consist of several flavone and chalcone derivatives such as Pongone, Galbone, Pongalabol, pongagallone A and B. The structures of Karangin, isopongachromene, Glybanchalcone and pongal.



Isopongachromene



Glybanchalcone



Pongol

The metabolites, beta-sitosteryl acetate and galactoside, stigma sterol, its galactoside and sucrose are being reported for the first time from this plant. The saturated and unsaturated fatty acids (two monoenoic, one dienoic and two trienoic) were present in exactly the same amount. Oleic acid occurred in highest amount (44.24%), stearic (29.64%) and palmitic (18.58%) acids were the next in quantity. Hiragonic and octadecatrienoic acids were present in trace amounts (0.88%).

Their structures were elucidated with the help of physiochemical methods and spectroscopic techniques.

MATERIALS AND METHODS

1. MATERIALS

1.1. Plant materials

Table 1. Plant material

S. No	Plant name	Parts used	Family	Collection period	Place of collection
1.	*Pongamia pinnata*	Leaf	Fabaceae	February 2019	Virudhunagar

1.1. General chemicals and reagents

Unless stated, general chemicals and reagents were purchased from Sigma, Cadila. Fisher chemicals were of analytical grade or equivalent.

General chemicals used

Table 2. The general chemicals and reagents used for the present study and their source

S.No	Chemical Name	Supplier
1.	(3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)	Sd.fine chemicals, Mumbai, India
2.	(2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid))	Sd.fine chemicals, Mumbai, India
3.	Acetic acid	Sd.fine chemicals, Mumbai, India.
4.	Acetic anhydride	Sd.fine chemicals, Mumbai, India.
5.	Acetone	Nice Chemicals, Kerala, India.
6.	Acetyl acetone	Sd.fine chemicals, Mumbai, India.
7.	Ammonium molybdate	Sd.fine chemicals, Mumbai, India.
8.	Ascorbic acid	Sd.fine chemicals, Mumbai, India.
9.	Chloroform (AR)	Sd.fine chemicals, Mumbai, India.
10.	Dimethyl Sulphoxide	Sd.fine chemicals, Mumbai, India.

11.	Dipotassium monohydrogen phosphate	Sd.fine chemicals, Mumbai, India.
12.	DL-α-Tocopherol acetate	Sd.fine chemicals, Mumbai, India.
13.	DPPH	Sigma Chemicals, U.S.A
14.	EDTA	Sd.fine Chemicals, Mumbai, India
15.	Ethanol (AR)	Nice Chemicals, Kerala, India.
16.	Ethyl acetate (AR)	Sd.fine chemicals, Mumbai, India.
17.	Formalin	Sigma Chemicals, U.S.A
18.	Hydrogen peroxide	Sd.fine chemicals, Mumbai, India.
19.	Liquid paraffin	Sigma Chemicals, U.S.A
20.	Methanol (AR)	Sd.fine chemicals, Mumbai, India.
21.	Nitroblue terazolium dye	Sigma Chemicals, U.S.A
22.	Paraffin wax	Sigma Chemicals, U.S.A
23.	Petroleum ether (AR)	Sd.fine chemicals, Mumbai, India.
24.	Potassium dihydrogen phosphate	Sd.fine chemicals, Mumbai, India.
25.	Potassium persulphate	Sd.fine chemicals, Mumbai, India.
26.	Riboflavin	Sd.fine chemicals, Mumbai, India.
27.	Sodium hydroxide	Sd.fine chemicals, Mumbai, India.
28.	Microcrystalline cellulose	Sd.fine chemicals, Mumbai, India.
26.	Empty capsules shells	Sd.fine chemicals, Mumbai, India.

1.1.1. Reagents and solutions were used

1.2.1. Instruments used for the study

Table 3. Instruments used for the present study and their source

S.No	Instruments	Supplier
1.	^{13}C-NMR spectrophotometer	Bruker, USA.
2.	^{1}H-NMR spectrophotometer	Bruker, USA.
3.	Auto analyser	Shimadzu, Japan.
4.	Eliza micro plate reader- Model 550	Bio Rad Laboratories Inc., California, USA.
5.	Heating mandle	Concord Instruments Pvt. Ltd., cochin, India.

6.	Homogeniser	Remi Ltd., India.
7.	Incubator	Perfit, India.
8.	IR Infra spectrophotometer	Nicolet 170SX.
9.	Mass spectrophotometer	Shimadzu, Japan.
10.	Metabolic Cage	Techniplast, Italy.
11.	Microscope	Nickon, Japan
12.	Rotary evaporator	Perfit, India.
13.	Ultra centrifuge	Remi Ltd., India.
14.	UV-Visible spectrophotometer UV 1601	Shimadzu CO. Japan.
15.	Water bath	Discovery scientific, India.
16.	Weighing balance	Shimadzu, Japan.

2. METHODS

2.1. Collection and identification of *Solanum trilobatum* (Linn.)

The leaves of *Pongamia pinnata* (Linn.) Pierre was collected from Virudhunagar, Virudhunagar District of Tamil Nadu, India. The plant specimen were identified and authenticated taxonomically by Dr. D. Stephen, M.Sc., Ph.D., Professor, Department of Botany, The American College, Madurai – 625002.

The leaf of *Pongamia pinnata* (Linn.) Pierre were dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The plant powdered materials were put away in a hermetically sealed holder.

2.2. Physicochemical analysis of plant powder from *Pongamia pinnata* (Linn.) Pierre [111-119]

2.2.1. Foreign matter:

Foreign matter is material consisting of any or all of the following:

(i) Parts of medicinal plant material or material other than those named with the limits specified for the plant material concerned.

(ii) Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned.

(iii) Mineral admixtures not adhering to the medicinal plant materials such as soil, stones, sand, and dust

(iv) Moulds, insect or other animal contamination.

Procedure

Weigh accurately about 100 gm. of original sample and spread it out in a thin layer. Inspect the sample with the unaided eye or with the use of a 6X lens and separate the foreign organic matter manually as completely as possible. Weigh and determine the percentage of foreign organic matter from the weight of the drug taken and noted in the table no. 5. Use the maximum quantity of sample for coarse or bulky drugs.

2.2.2. Determination of ash

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The ash of any organic material is composed of inorganic (metallic salts and silica) can be detected by ash value. There are four different methods were adopted for determining ash value.

1. Total ash
2. Acid insoluble ash
3. Water soluble ash
4. Sulphated ash

Determination of Total ash

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "Physiological ash" which is derived from the plant tissues itself and "Non physiological ash" which is the residue of the extraneous matter (e. g) sand and soil adhering to the plant surfaces.

Procedure

About 2gm of the ground air dried material was placed in a silica crucible, and ignited in an electrical burner at 85˚C for 15 minutes, until it white which indicates the absence of carbon, cooled in a desiccators and weighed, the percentage of total ash was calculated with reference to air dried drug. The procedure was repeated thrice and average was calculated and noted in the table no. 6.

Determination of acid insoluble ash

This method is designed to measure the amount of silica present, especially as sand and siliceous earth.

Procedure

The total ash was taken in a silica crucible, 25ml of hydrochloric acid was added, covered with a watch glass, boiled for 5 minutes. The watch glass was rinsed with 5ml of hot water and added this liquid to the silica crucible. The insoluble matter was collected in a Whatmann filter paper, washed with hot water, ignited in desiccator and weighed. The percentage of acid insoluble ash calculated with reference to air dried drug. The procedure was repeated thrice and average was calculated and noted in the table no. 6.

Determination of Water soluble ash

The total ash was taken in a silica crucible to this add 25ml of water and boiled for 5 minutes. The insoluble matters was collected in a Whatmann filter paper, washed with hot water, ignited in a silica crucible at 85 ˚C for 15 minutes. The percentage of water soluble ash was calculated in mg per gm. of dried material. The procedure was repeated thrice and average was calculated and noted in table no. 6.

2.2.3. Determination of moisture content (loss on drying)

About 2gm of air dried material was taken in a previously dried china dish. This was placed in a hot air oven and heated at 100˚C for 1 hour. Then it was cooled to room temperature and weighed. The percentage of moisture content was calculated with reference to air dried drug material. The procedure was repeated thrice and average was calculated and noted in the table no. 6.

2.2.4. Determination of extractive values

This method is used to determine the amount of active constituents present in a given amount of medicinal plant material when extracted with solvent. The extractive value is used for evaluating the crude drugs which are not readily estimated by other means for example, any of crude drug have certain range of their extractive value indicates the addition of exhausted or unwanted material with original drug or incorrect processing of the drug during the process of drying, storage etc.

Water soluble extractive

Weighed accurately about 2gm of the air dried coarsely powder drug was macerated with 100ml of water in a closed flask for 24hrs. It was shaken frequently for first 6hrs and allowed to stand for 18hrs, thereafter, filter rapidly taking precautions against loss of water, evaporated to dryness in a watch glass then dried at 105˚C and weighed, the percentage of extractive value was calculated with reference to the air dried drug. The procedure was repeated thrice and average was calculated and noted in table no. 7.

Alcohol soluble extractive

Weighed accurately about 2gm of the air dried coarsely powder drug was macerated with 100ml of ethanol in a closed flask for 24hrs. It was shaken frequently for first 6hrs and allowed to stand for 18hrs, thereafter, filter rapidly taking precautions against loss of ethanol, evaporated to dryness in a watch glass then dried at 105˚C and weighed, the percentage of extractive value was calculated with reference to the air dried drug. The procedure was repeated thrice and average was calculated and noted in table no. 7.

This experiment was repeated with various solvents (Petroleum ether, ethyl acetate, chloroform) using plant material and average was calculated and noted in table no. 7.

2.3. Preparation of extract from *Pongamia pinnata*

Cold- Maceration method

The shade-dried leaf powder was extracted with sufficient amount of hydro-ethanol (30:70) solvent for first maceration for 48 hours with occasional shaking. Strain the liquid and press the marc.

Macerate again for 24 hours with the remaining hydro-ethanol (30:70) solvent required for second maceration with occasional shaking. Strain the liquid and press the marc.

Mix the liquids obtained from two macerations, after completion of the extraction, the Hydro-alcoholic was filtered and concentrated at 50˚C on water bath.

Then the concentrated Hydro-alcoholic extract was mixed with small quantity of water. This extract was fractionated with Chloroform: Methanol (95: 5) (85: 5). Then the small portion of fraction were subjected to GC-MS analysis.

2.4. Phytochemical analysis of hydro-alcoholic extracts of *Pongamia pinnata* [116-121]

The Hydro-alcoholic extracts of *Pongamia pinnata* was subjected to qualitative analysis to test for the identification of various phytochemical constituents like carbohydrates, glycosides, flavonoids, phytosterols, triterpenoids and tannins etc.,

PROCEDURE:

Qualitative phytochemical screening of *Pongamia pinnata* extracts:

The Hydro-alcoholic extracts of *Pongamia pinnata* are subjected to chemical tests for the identification of various active constituents as described below:

Test for alkaloids

The small quantity of the extracts were taken separately and stirred with few drops of dilute hydrochloric acid and filtered. The filtrate were tested for alkaloids.

- **Mayer's test**

  Take small amount of filtrate in a test tube and two drops of Mayer's reagent (Potassium Mercuric Iodide) are added along the sides of the test tube. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

- **Wagner's test**

  Take small amount of filtrate in a test tube and add few drops of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

- **Dragendroff's test**

  Take small quantity of filtrate in a test tube and add few drops of Dragendroff's test (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

- **Hager's test**

  Take small quantity of filtrate in a test tube and add a small amount of Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Test for carbohydrates

A small quantity of extracts was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to various tests to detect the presence of carbohydrates.

- **Molisch's test**

  Take small quantity of filtrate was treated with 2-3 drops of 1% of alcoholic α- napthol solution and 2ml of concentrated sulphuric acid (Molisch's reagent) was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids indicates the presence of carbohydrates.

- **Fehling's test**

  Take small portion of filtrate, equal parts of Fehling's solution A and B was added and kept in boiling for few minutes. Formation of red precipitate indicates the presence of reducing sugar.

- **Barfoed's test**

  An equal volumes of filtrate and Barfoed's reagent are mixed and kept in boiling for few minutes. Formation of red precipitate indicates the presence of reducing sugar.

- **Benedict's test**

  A small volume of filtrate, 5ml of Benedict's reagent was added and kept in boiling for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

Test for glycosides

- **Borntrager's test**

The extract was mixed with dilute sulphuric acid and filtered. 2 ml of filtrate is mixed with 3ml of chloroform and the chloroform layer was separated and add 10% ammonia. Appearance of pink colour solution indicates the presence of glycosides.

- **Modified Borntrager's Test**

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

- **Legal's test**

The plant extract is dissolved in pyridine and add sodium nitroprusside. Then the solution is made alkaline using 10% sodium hydroxide and pink colour solution proves the presence of glycosides.

- **Baljet test**

To the extract, sodium picrate solution was added. Appearance of yellow to orange colour indicates the presence of glycosides.

Test for saponins

- **Froth test**

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

- **Foam Test:**

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

- **Haemolysis test**

The extract of the plant was separated over a glass slide to form a thin film layer on which a drop of human blood was placed and spreaded over extract layer. After 30 minutes the slide was examine under microscope for changing the structure and shape

of the red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis.

Test for proteins and amino acids

The plant extract is dissolved in 10 ml of distilled water and the filtrate is used for the following tests.

- **Millon's test**

  A few drops of Millon's reagent is added to 2 ml of filtrate. The white precipitate proves the presence of proteins.

- **Biuret test**

  One drop of 2% copper sulphate solution is added to 2 ml of filtrate. Then 1 ml of 95% ethanol is added following by excess of potassium hydroxide pellets. Pink colour in ethanolic layer indicates the presence of proteins

- **Ninhydrin test**

  Two drops of ninhydrin solution are added to 2 ml of the filtrate and purple colour proves the presence of amino acids.

- **Xanthoproteic test**

  The plant extract is treated with few drops of conc. Nitric acid. The formation of yellow colour indicates the presence of proteins.

Test for flavonoids

- **Alkaline reagent test**

  A small amount of the extract is treated with few drops of sodium hydroxide and if the intense yellow colour solution becomes colourless on addition of dilute acid proves the presence of flavonoids.

- **Lead acetate test**

  Extract is treated with few drops of lead acetate solution and the formation of yellow colour solution indicates the presence of flavonoids.

- **Magnesium and hydrochloric acid reduction**

A small amount of extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and few drops of concentrated hydrochloric acid is added. Any pink to crimson colour development indicates the presence of flavanol glycosides.

Test for phytosterols

- **Libermann-Burchard's test**

A small amount of the extract (50 mg) is dissolved in chloroform and add 2 ml of acetic anhydride and few drops of concentrated sulphuric acid is added. Appearance of red, then blue and finally bluish green colour shows the presence of steroids.

- **Salkowski's test**

A small portion of extract was treated with chloroform and the filtrate of that is treated with few drops of acetic anhydride. Then the solution is boiled and cooled and the formation of brown ring at the junction indicates the presence of phytosterols.

Test for phenols

The plant extract is treated with few drops of ferric chloride solution and the formation of bluish black colour proves the presence of phenols.

Test for tannins

A few drops of 1% gelatin solution containing sodium chloride is added to the plant extract. The formation of white precipitate indicates the presence of tannins.

Test for diterpenes

- **Copper acetate test**

The plant extract is dissolved in water and 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for triterpenoids

- **Libermann-Burchard's test**

A small amount of the extract (50 mg) is dissolved in chloroform and add 2 ml of acetic anhydride and few drops of concentrated sulphuric acid is added. Appearance of wine red colour shows the presence of triterpenoids.

2.5. GC-MS Analysis [93-94]

GC-MS analysis of fraction of A (Chloroform: Methanol, 85:15) and B (Chloroform: Methanol, 95:5) from Hydro-alcoholic extract of *Pongamia pinnata* was analysed by using the equipment on Jeol spectrometer (Model: Accu TOF GCV). One microliter of each sample was injected into the gas chromatograph (GC) with mass selective detector (MS). GC-MS analysis were carried out using a HP7650 gas chromatograph coupled to a HP5973 mass spectrometer (Electron Multiplier Potential 2KV, filament current 0.35Ma, electron energy 70eV, and the spectra were recorded every 1 s over the range m/z 80-800) using a splitless injection mode. A 15m, 0.32 mm, ID 5% cross-linked phenylmethyl siloxane capillary column (HP-5MS) with a 0.25 mm film thickness was used for separation. Helium was used as a carrier gas at a constant flow of 1.1ml. An isothermal hold at 50˚C was kept for 2 min, followed by a heating gradient of 10˚C to 32˚C, with the final temperature held at for 10 min. The injection port temperature was 220˚C. The MS interface temperature was 300˚C.

2.6. Isolation and identification [119, 123, 124-126]

Chromatography

Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase. Currently, there are many techniques are used. The column chromatography is a simple techniques.

The separation of components in column chromatography involves principle of adsorption i.e., the components of the mixture have different affinity towards adsorbent material hence, they gets adsorbed and migrates at different rate. So it is possible to isolate single component by adjusting the solvent system i.e., by increasing or decreasing the polarity of solvent system.

Sample preparation

The hydro-alcoholic was dissolved in small amount of benzene and mixed thoroughly with silica gel and dried to have free flowing nature. This mixture was taken for column study.

Preparation of column

Method: Wet Packing

The adsorbent material, silica gel was mixed with benzene and poured gently from the top of the column to a desire length then the same solvent was run through the column for 2-3 times to prevent air entrapment and the solvent used was maintained up to 10cm above the column bed.

The sample mixture was poured from the top of the column with the aid of funnel. The column was allowed to keep overnight, undisturbed. In the next day column was eluted with different solvents with gradually increasing polarity by changing the solvent (benzene, chloroform, ethyl acetate, and ethanol). The flow rate of solvent system was adjusted between 16-20 drops per minute. Each fraction was collected to maximum of 100ml and it was evaporated at low temperature. Then identify by TLC and chemical tests.

Table 4. Various fractions of column chromatography (Hydro-alcoholic extract)

S. No	Solvents	Ratio (%)	Nature of residue
1.	Benzene	100	Light green
2.	Benzene: Chloroform	90 : 10	Green residue
3. *	**Benzene: Chloroform**	**80 : 20**	Colourless
4.	Benzene: Chloroform	70 : 30	Green residue
5.	Benzene: Chloroform	50 : 50	Light green
6.	Benzene: Chloroform	30 : 70	Dark brown
7.	Chloroform	100	Green residue
8.	Chloroform: Ethyl acetate	70 : 30	Light green
9.	Chloroform: Ethyl acetate	50 : 50	Brownish residue
10.	Chloroform: Ethyl acetate	30 : 70	Reddish brown
11.	Ethyl acetate	100	Light green
12. *	**Ethyl acetate: Ethanol**	**90 : 10**	Colourless
13.	Ethyl acetate: Ethanol	80 : 20	Brownish residue
14.	Ethyl acetate: Ethanol	70 : 30	Reddish brown
15.	Ethyl acetate: Ethanol	50 : 50	Reddish brown
16.	Ethyl acetate: Ethanol	30 : 70	Light brown
17.	Ethanol	100	Brown residue

*= Taken for further studies

2.7. In-vitro antioxidant activity of *Pongamia pinnata* [122]

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH radical scavenging assay of A, B and E extract were performed according to the modified method described by Perumal *et al*., 2018 [122]. In brief, 0.135 mM DPPH was prepared in methanol. Different concentration of extract (5, 10, 20, 40, 80, 160 and 320 µg/ml) was mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexes thoroughly and kept at room temperature for 30 min. The Absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability of plant extract to scavenge DPPH radical and control was calculated from the following formula:

$$\% \text{ DPPH inhibition} = [(\text{OD of control} - \text{OD of test})/ (\text{OD of control})] \times 100$$

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay

ABTS radical scavenging assay of A, B, and E sample were performed according to the modified method of Perumal *et al.*, 2018 [122]. The ABTS (7 mM, 25 ml in deionized water) stock solution was prepared with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). Different concentration of standard and test sample (5, 10, 20, 40, 80, 160 and 320 µg/ml) were mixed with the ABTS working solution (2.0 ml) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Abs was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect was calculated by the equation:

$$\text{ABTS radical scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the control; A_1 is the test

Hydrogen Peroxide Scavenging Capacity

The ability of the plant extracts to scavenge hydrogen peroxide was determined according to the method of Perumal *et al.*, 2018 [122]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of the plant extract and standard compound alpha-tocopheral were calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(AC – AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards.

E= Hydro-alcoholic extract (Ethanol: Water, 70:30)

A= Fraction of A (Chloroform: Methanol, 95:5)

B= Fraction of B (Chloroform: Methanol, 85:15)

2.8. In-vitro anticancer activity of *Pongamia pinnata* [103-106]

Requirements:

1. MTT reagent (the solution is filtered through a 0.2 μm filter and stored at 2–8 °C for frequent use or frozen for extended periods)
2. DMSO
3. CO_2 incubator
4. Micro Plate reader
5. Inverted microscope
6. Refrigerated centrifuge

Preparation of test solutions

For Anticancer studies, serial two fold dilutions (3.125-100μg/ml) were prepared from this for carrying out anticancer studies.

Cell lines and culture medium

A549 cell lines (Lung cancer) were procured from NCCS, stock cells was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) in an humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 h at 37ºC, 5% CO_2 incubator.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100μl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100μl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100μl of

MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37^{o}C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

IC_{50} Value:

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve.

The direct Microscopic Observations of Drug Treated Images of Cell lines by Inverted Biological Microscope were enclosed with the report.

E= Hydro-alcoholic extract (Ethanol: Water, 70: 30)

A= Fraction of A (Chloroform: Methanol, 95:5)

B= Fraction of B (Chloroform: Methanol, 85:15)

RESULTS AND DISCUSSION

1. Physicochemical parameters

The collected, shade dried leaf powder of *Pongamia pinnata* (Linn.) Pierre was taken out for physiochemical analysis (Foreign organic matter, Ash value and Extractive value).

Table 5: Foreign organic matter of *Pongamia pinnata*

Sample	% Foreign organic matter
Pongamia pinnata	0.029± 0.001

Table 6: Ash Values of *Pongamia pinnata*

Physical Constants	Experiment			
	I (%)	II (%)	III (%)	Average (%)
Total ash	7	6.5	6	6.50±0.408
Water soluble ash	2	2.45	1.8	2.083±0.272
Acid insoluble ash	0.8	1	0.65	0.82±0.143
Sulphated ash	2.5	2.65	2.1	2.42±0.232
Loss on drying	5.5	7.5	7	6.67±0.849

Table 7: Extractive Values of *Pongamia pinnata*

Solvents	Experiments			Average (w/w) (%)
	I (%)	II (%)	III (%)	
Petroleum ether	3.5	4.25	3.4	3.71±0.379
Ethyl acetate	6	5.5	6.05	5.85±0.248
Chloroform	5	4.95	5.1	5.02±0.062
Ethanol	6	6.15	5.95	6.03±0.084
Water	21.5	21.05	21.75	21.43±0.289

2. Preparation of extract and evaluation of phytochemical screening of *Pongamia pinnata* Linn.

The dried leaf powder was extracted with hydro ethanol (Ethanol 70: Water 30) using maceration process (Double maceration method). The preliminary phytochemical evaluation of Hydro-alcoholic leaf extract of *Pongamia pinnata* (Linn.) Pierre shows the presence of alkaloids, carbohydrates, glycosides, phytosterols, sterols, flavonoids, tannins, phenolic compounds and triterpenoids.

Table 8: Phytochemical screening of Hydro-alcoholic leaf extract of *Pongamia pinnata* extracts

S. No	Extract	Components											
		Alkaloids	**Carbohydrates**	**Glycosides**	**Phytosterols**	**Sterols**	**Flavonoids**	**Tannins**	**Phenolic compounds**	**Protein**	**Amino acids**	**Saponins**	**Triterpenoids**
1.	Hydro alcoholic Extract	+	+	+	+	+	+	+	+	-	-	-	+

Note:

(+ Indicates presence of constituents)

(- Indicates absence of constituents)

3. GC-MS Analysis

From GC-MS analysis 15 active components were detected from fraction of A (Chloroform: Methanol, 85:15) and another 15 components were detected from fraction of B (Chloroform: Methanol, 95:5). Totally 30 compounds were detected. The identification of phytochemical compounds was based on retention time, peak area, molecular weight, and molecular formula were presented in table.

Among the identified compounds from fraction of A (Chloroform: Methanol, 85:15), Cyclopentadecane and Octadec-9-enoic acid was found to be the major compound attained the largest peak area (30.47%) with the retention time 12.913 minutes. Another major compound 1- Methyl-4-[4, 5- dihydrophenyl]-hexahydropyridine having peak area (26.52%) with retention time 12.053 minutes. The compound Tricosanoic acid having peak area (9.65%) with retention time 10.937 minutes. The compounds Trimethyl [(4-methyl cyclohexyl) oxy] transsilane (Peak area 7.49%) (Retention time 8.526 miuntes), 2- methyl-hexadecanoic acid (Peak area 5.30%) (Retention time 11.391 miuntes), 2, 5- Diisopropyl-6-methyl-oxathiaborinane (Peak area 3.59%) (Retention time 9.944 miuntes), 1, 3- Di (4-bromophenyl) propane (Peak area 3.04%) (Retention time 9.594 miuntes), 4-(2-phenylcyclohex-4-enyl) 4-hydroxybutan-2-one (Peak area 2.42%) (Retention time 6.181 miuntes), Bicyclopentylidene (Peak area 2.39%) (Retention time 7.108 miuntes), 4-Methyl- Cyclopentadecanone and 1-Aminomethyl cyclododecanol (Peak area 1.54%) (Retention time 3.968 miuntes).

Among the identified compounds from fraction of B (Chloroform: Methanol, 95:5), 6, 10, 14- trimethyl-2-pentadecanone was found to be the major compound attained the largest peak area (11.44%) with the retention time 10.597 minutes. Another major compound Diazoprogesterone having peak area (5.87%) with retention time 9.954 minutes. Another one compound Dibutyl Phthalate having peak area (5.29%) with retention time 11.087 minutes. The compound 2-Amino-6, 7- dimethyl 4(1H) - pteridinone (peak area (3.64 %) retention time 7.042minutes), 4- methyl- 1H- benzotriazole (peak area (2.96 %) retention time 8.280 minutes), 4-(4-methyl phenyl)-5-phenoxy-6-phenyl-2-(1H)- pyrimidinone (peak area (2.43 %) retention time 8.706 minutes), 4,6- Dimethyl-3-nitro-2[1H]- Pyridinone (peak area (1.81 %) retention time 7.836 minutes), Cyclopentaneundecanoic acid (peak area (1.75%) retention time 6.692 minutes), 1-Cyclohexene-1-methanol (peak area (1.66%) retention time 9.642 minutes), Diepicedrene-1-oxide (peak area (1.46%) retention time 9.384 minutes).

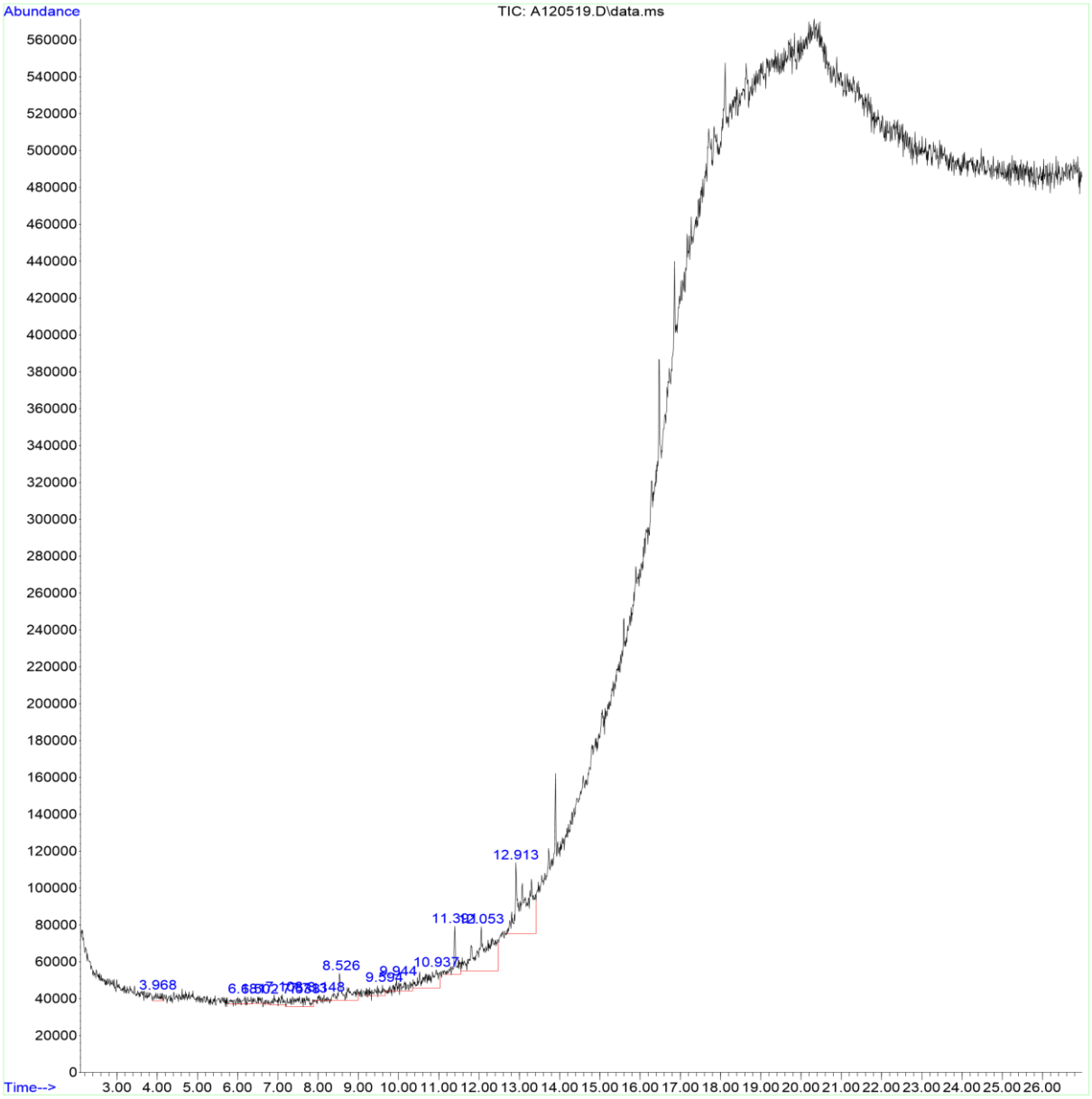


Fig. 8: GC-MS Chromatogram of fraction A from Hydro-alcoholic extract of *Pongamia pinnata*

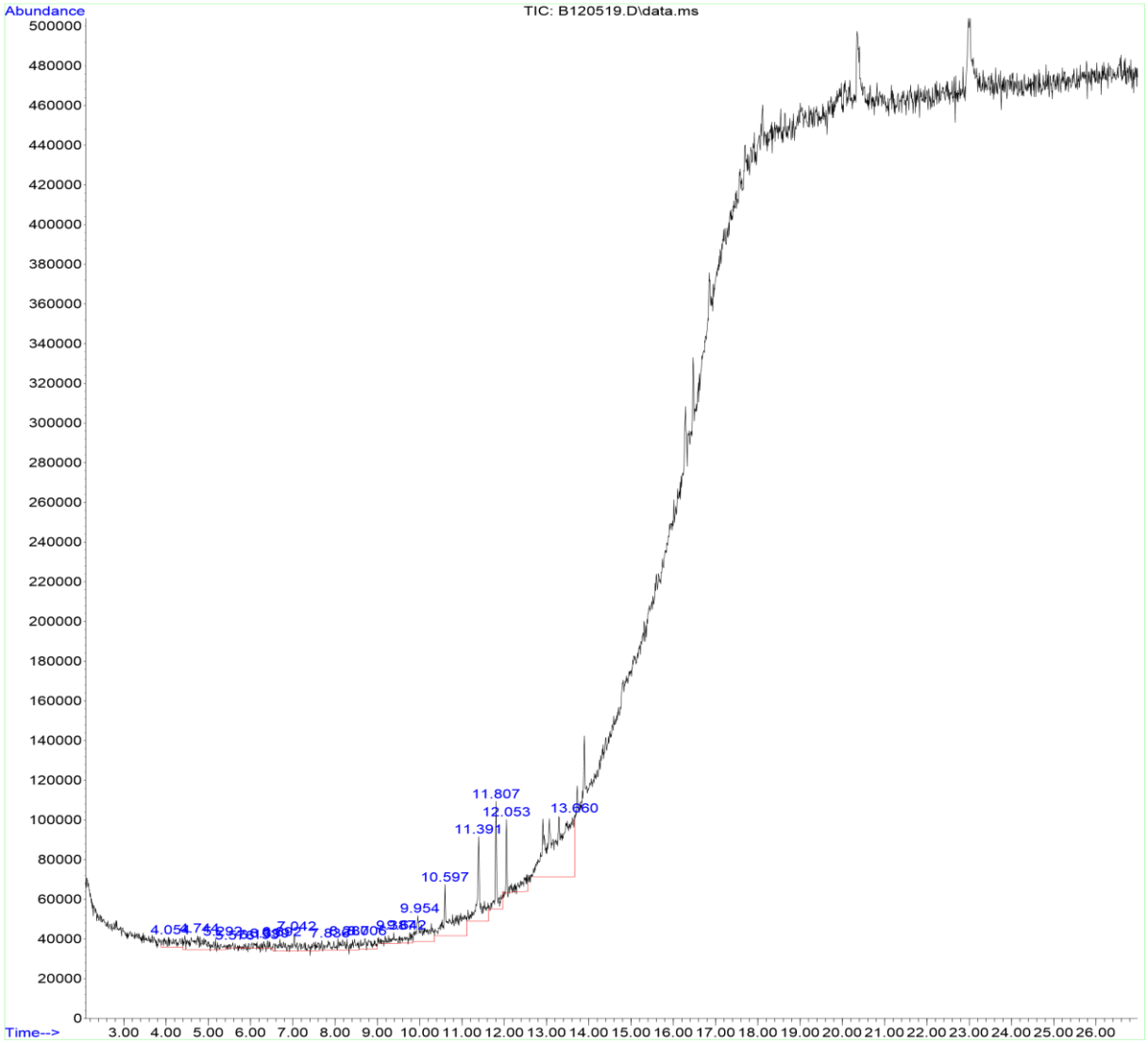


Fig. 9: GC-MS Chromatogram of fraction B from Hydro-alcoholic extract of *Pongamia pinnata*

Table 9: GC-MS report of fraction A from Hydro-alcoholic extract of *Pongamia pinnata*

S. No	Compound code	Retention time (mins)	Peak area (%)	IUPAC Name	Molecular weight
1.	HEEPP A1	3.968	1.54	Oleic acid	282.47g/mol
2.	HEEPP A1	6.181	2.42	4-(2-phenylcyclohex-4-enyl) 4-hydroxybutan-2-one	244.33 g/mol
3.	HEEPP A1	6.502	1.42	7- Nitro imino 2,4,6,8-tetraaza bicycle[3.3.0] octan-3-one	186.13 g/mol
4.	HEEPP A1	7.108	2.39	Bicyclopentylidene	136.24 g/mol
5.	HEEPP A1	7.713	2.09	Hexahydro-5-[3-(-2-phenoxy ethoxy) benzylidene]pyrimidine-2,4,6- trione	352.25 g/mol
6.	HEEPP A1	8.526	7.49	Trimethyl [(4-methyl cyclohexyl)oxy] transsilane	186.37 g/mol
7.	HEEPP A1	9.594	3.04	1,3- Di (4-bromophenyl) propane	354.08 g/mol
8.	HEEPP A1	3.968	1.54	4-Methyl-Cyclopentadecanone	238.41 g/mol
9.	HEEPP A1	9.944	3.59	2,5- Diisopropyl-6-methyl- oxathiaborinane	202.16 g/mol
10.	HEEPP A1	12.913	30.47	Cyclopentadecane	210.40g/mol
11.	HEEPP A1	11.391	5.30	2- methyl-hexadecanoic acid	270.46 g/ mol
12.	HEEPP A1	12.053	26.52	1- Methyl-4-[4,5-dihydrophenyl]-hexahydropyridine	207.27g/mol
13.	HEEPP A1	3.968	1.54	1-Aminomethyl cyclododecanol	213.36 g/mol
14.	HEEPP A1	10.937	9.65	Tricosanoic acid	354.62g/mol
15.	HEEPP A1	12.913	30.47	Octadec-9-enoic acid	282.47 g/mol

Table 10: GC-MS report of fraction B from Hydro-alcoholic extract of *Pongamia pinnata*

S. No	Compound Code	Retention Time (mins)	Peak Area (%)	IUPAC Name	Molecular weight
1.	HEEPP B1	5.292	0.84	3- Hydroxy benzene methanol	124.14 g/mol
2.	HEEPP B1	5.576	0.37	Spiro [4,5] decan-1-one	152.24 g/mol
3.	HEEPP B1	6.153	0.76	N-(Bicyclo [2,2,1]hept-2-yl methyl)-3,4-dihydro-4-oxo-1-phthalazinecarboxamide	297.36 g/mol
4.	HEEPP B1	6.399	0.51	1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene	202.34 g/mol
5.	HEEPP B1	6.692	1.75	Cyclopentaneundecanoic acid	254.41 g/mol
6.	HEEPP B1	7.042	3.64	2-Amino-6,7- dimethyl 4(1H)- pteridinone	191.19 g/mol
7.	HEEPP B1	7.836	1.81	4,6- Dimethyl-3-nitro-2[1H]- Pyridinone	168.15 g/mol
8.	HEEPP B1	8.280	2.96	4- methyl- 1H-benzotriazole	133.15 g/mol
9.	HEEPP B1	8.706	2.43	4-(4-methyl phenyl)-5-phenoxy-6-phenyl-2-(1H)-pyrimidinone	354.41 g/mol
10.	HEEPP B1	5.292	0.84	3-cyclohexyl-1-[4-(3-cyclohexyl propionyl)- 2,5-dimethyl-piperazin-1-yl] propan-1-one	390.61 g/mol
11.	HEEPP B1	9.387	1.46	Diepicedrene-1-oxide	220.3505 g/mol
12.	HEEPP B1	9.642	1.66	1-Cyclohexene-1-methanol	112.17 g/mol
13.	HEEPP B1	9.954	5.87	Diazoprogesterone	338.499 g/mol
14.	HEEPP B1	10.597	11.44	6,10,14- trimethyl-2-pentadecanone	268.48 g/mol
15.	HEEPP B1	11.807	5.29	Dibutyl phthalate	278.35 g//mol

4. Characterization of isolated compound

In isolation process, the Hydro-alcoholic extract was subjected to column chromatographic separation. Only two active isolated compounds were separated. Then the isolated compounds undergoes chemical tests reveals that the presence of flavonoids.

Further it undergoes spectral analysis such as FT-IR, ^{1}HNMR, ^{13}CNMR, and Mass Spectroscopy showed the presence of Compound-I 2-(2-amino-3-methoxyphenyl) chromen-4-one, and Compound-II 3, 4', 5, 7-Tetrahydroxy-3'-methoxyflavone.

Isolated compound- I

The fraction of Benzene: Chloroform in the ratio 80: 20. Evaporation of Benzene: Chloroform shows constituents with enough quantity. Compound I crystalized from chloroform as colourless solid. It is soluble in Chloroform, Dimethyl sulfoxide, and Dimethyl formamide. Melting Point 179˚C. Hence it is subjected to TLC.

Table 11: TLC Solvent system

Solvent system	Ratio	R_f Value
Benzene : Ethyl acetate	6 : 4	0.5
Chloroform : Ethanol	7 : 3	0.6

Spectral evidence for isolated compound -I

FT-IR spetral analysis

The structure of isolated compound- I was elucidated by Shimadzu 8400 series Fourier Transform- Infra Red spectrophotometer in KBr pellet method. IR values are measured in cm^{-1} and results are given below table no.9

Table 12: FTIR spectral data of compound I

S. No	Frequency (cm^{-1})	Groups assigned
1.	3324	-NH Group
2.	2920	–C-H (aromatic) group
3.	1665	–C=O group
4.	1610	–C=C (aromatic)

^{1}HNMR and ^{13}CNMR analysis

The structure of isolated compound –I was proposed by ^{1}HNMR and ^{13}CNMR spectrophotometer. The samples were dissolved in $CDCl_3$ and the values are measured in δ ppm and the results are given below table no. 10 and 11

Table 13: ^{1}HNMR spectral data of compound- I

S. No	Chemical shift value (ppm)	Functional group
1	δ 12.59	s, 2H, 2'-NH_2
2	δ 9.50	Broad hump, 1H, 6-H
3	δ 7.77-7.67	d, 1H, 7-H
4	δ 7.55-7.52	dd, 1H, 5-H
5	δ 6.92- 6.89	d, 1H, 8-H
6	δ 6.41	d, 1H, 2'-H
7	δ 6.18	d, 1H, 3'H
8	δ 2.50	s, 3H, 3'-OCH_3

Table 14: ^{13}CNMR spectral data of compound-I

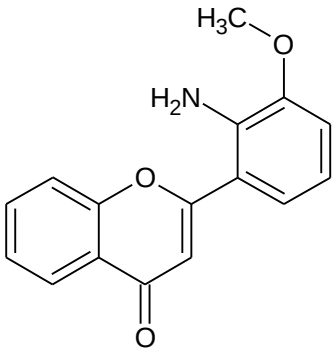
S. No	Chemical shift value (ppm)	Functional group
1	δ 176	C-2
2	δ 165	C-9
3	δ 162	C-6
4	δ 158	C-3
5	δ 148	C-7
6	δ 146	C-4
7	δ 144	C-10
8	δ 133	C-8
9	δ 121.55	C-5
10	δ 121	C-1'
11	δ 117	C-4'
12	δ 116	C-2'
13	δ 115	C-3'
14	δ 94	3'- OCH_3

Mass analysis

The mass spectrum of the isolated compound-I is presented in the spectrum the m/z value of isolated compound of the molecular ion is found as 278 (M+9) which includes the isotopes of corresponding atoms.

Based on the spectral data the tentative structure of compound-I to proposed as 2-(2-amino-3-methoxyphenyl) chromen-4-one are given. The Molecular Formula of the compound-I was deduced as $C_{16}H_{13}NO_3$.

From the above isolated compound-I was confirmed as 2-(2-amino-3-methoxyphenyl) chromen-4-one



2-(2-amino-3-methoxyphenyl) chromen-4-one

Isolated compound- II

The fraction of Ethyl acetate: Ethanol in the ratio 90: 10. Evaporation of Ethyl acetate: Ethanol shows constituents with enough quantity. Compound II crystalized from ethanol as colourless solid. It is soluble in Chloroform, Dimethyl sulfoxide, and Dimethyl formamide. Melting Point 233˚C. Hence it is subjected to TLC.

Table 15: TLC Solvent system

Solvent system	Ratio	R_f Value
Chloroform : Ethanol	9.3 : 0.7	0.7
Acetic acid : Water	7.5 : 2.5	0.3

Spectral evidence for isolated compound -II

FTIR spectral analysis

The structure of isolated compound- II was elucidated by Shimadzu 8400 series Fourier Transform- Infra Red spectrophotometer in KBr pellet method. IR values are measured in cm^{-1} and results are given below table no.13

Table 16: FTIR spectral data of compound II

S. No	Frequency (cm^{-1})	Groups assigned
1.	3335-3465	-OH group
2.	1656-1616	Strong band –C=O group
3.	1061	-C-O-C- group

^{1}HNMR and ^{13}CNMR analysis

The structure of isolated compound –II was proposed by ^{1}HNMR and ^{13}CNMR spectrophotometer. The samples were dissolved in $CDCl_3$ and the values are measured in δ ppm and the results are given below table no. 14 and 15

Table 17: ^{1}HNMR spectral data of compound II

S. No	Chemical shift value (ppm)	Functional group
1	δ 13.06	s, 1H, 3-OH
2	δ 10.76	hump, 1H, 5-OH
3	δ 9.62	s, 1H, 7-OH
4	δ 8.38	s, 1H, 4'-OH
5	δ 6.88- 6.82	d, 1H, 4-OH
6	δ 6.56	1H, 8-H
7	δ 3.75	s, 3H, 3'-OCH_3

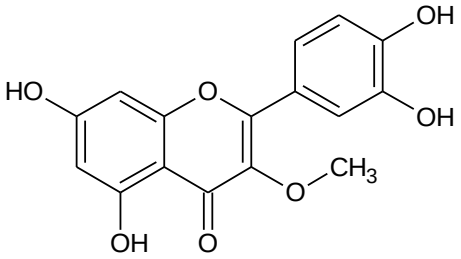
Table 18: ^{13}CNMR spectral data of compound II

S. No	Chemical shift value (ppm)	Functional group
1	δ 181	C-4
2	δ 157.69	C-2
3	δ 157.49	C-9
4	δ 154	C-3
5	δ 153	C-10
6	δ 152	C-6
7	δ 132	C-5
8	δ 130	C-7
9	δ 122.78	C-4'
10	δ 121.23	C-1'
11	δ 121	C-8
12	δ 116	C-5'
13	δ 106	C-2'
14	δ 59.95	3'- OCH_3

Mass analysis

The mass spectrum of the isolated compound II is presented in the spectrum the m/z value of the isolated compound of the molecular ion is found as 316 which includes the isotopes of corresponding atoms. On the basis of spectral data (IR, ^{1}H & ^{13}CNMR and Mass) and tentative structure of isolated compound II was found as 3, 4', 5, 7-Tetrahydroxy-3'-methoxyflavone and the Molecular Formula deduced as $C_{16}H_{1}$.

From the above isolated compound-I was confirmed as, 3, 4', 5, 7-Tetrahydroxy-3'-methoxyflavone



3, 4', 5, 7-Tetrahydroxy-3'-methoxyflavone

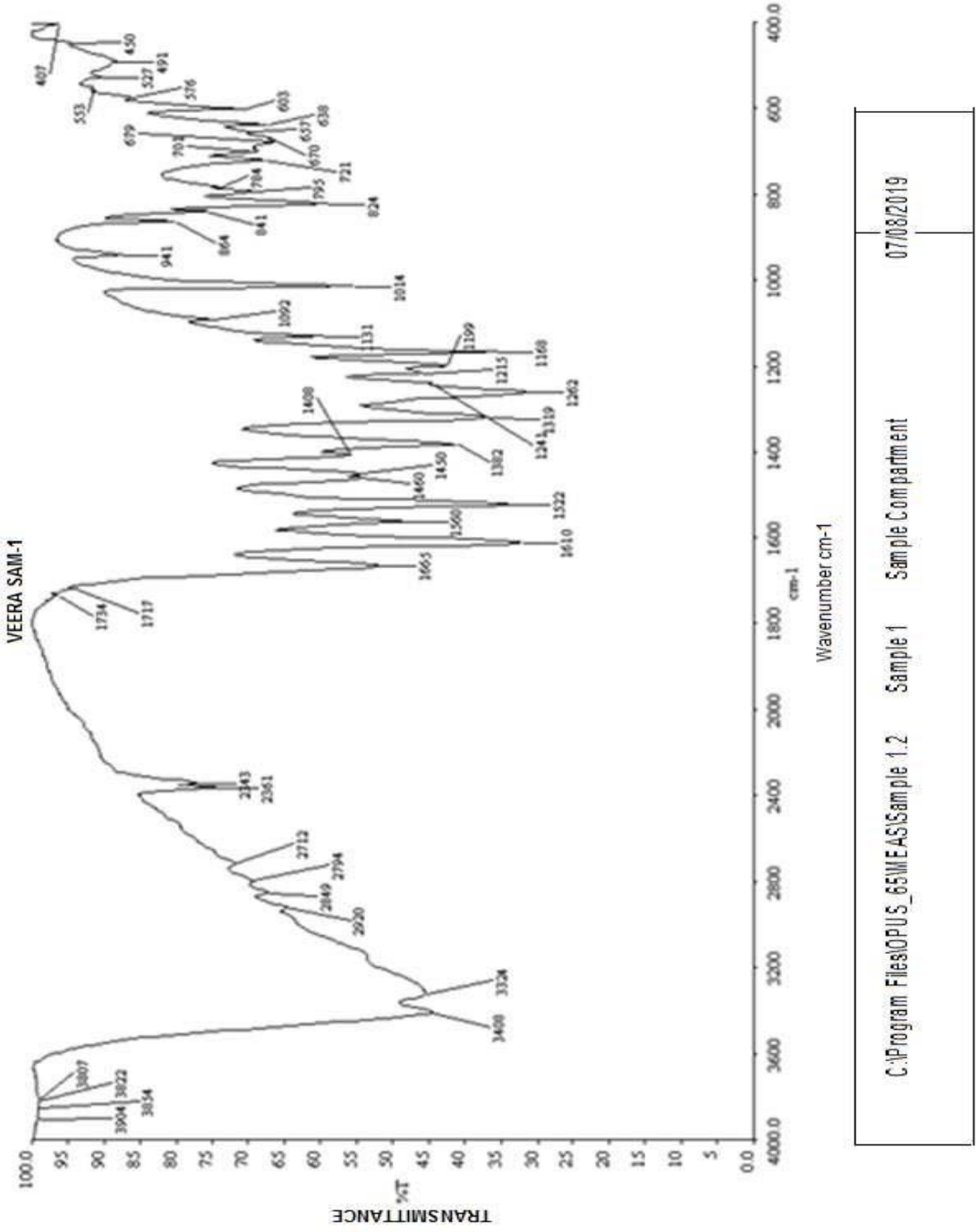


Fig. 10: FT-IR Spectrum of Compound-I

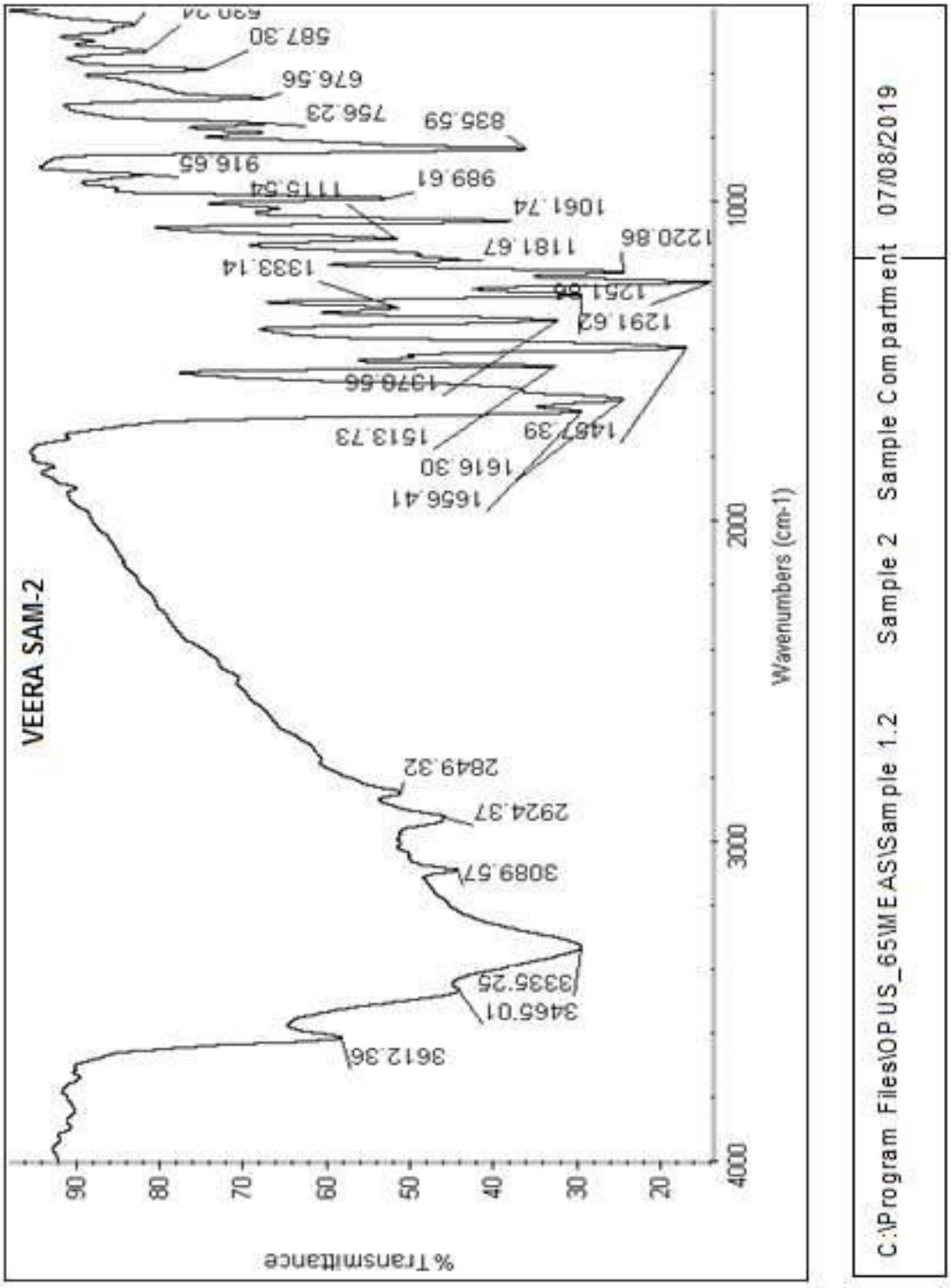


Fig. 11: FT-IR Spectrum of Compound II

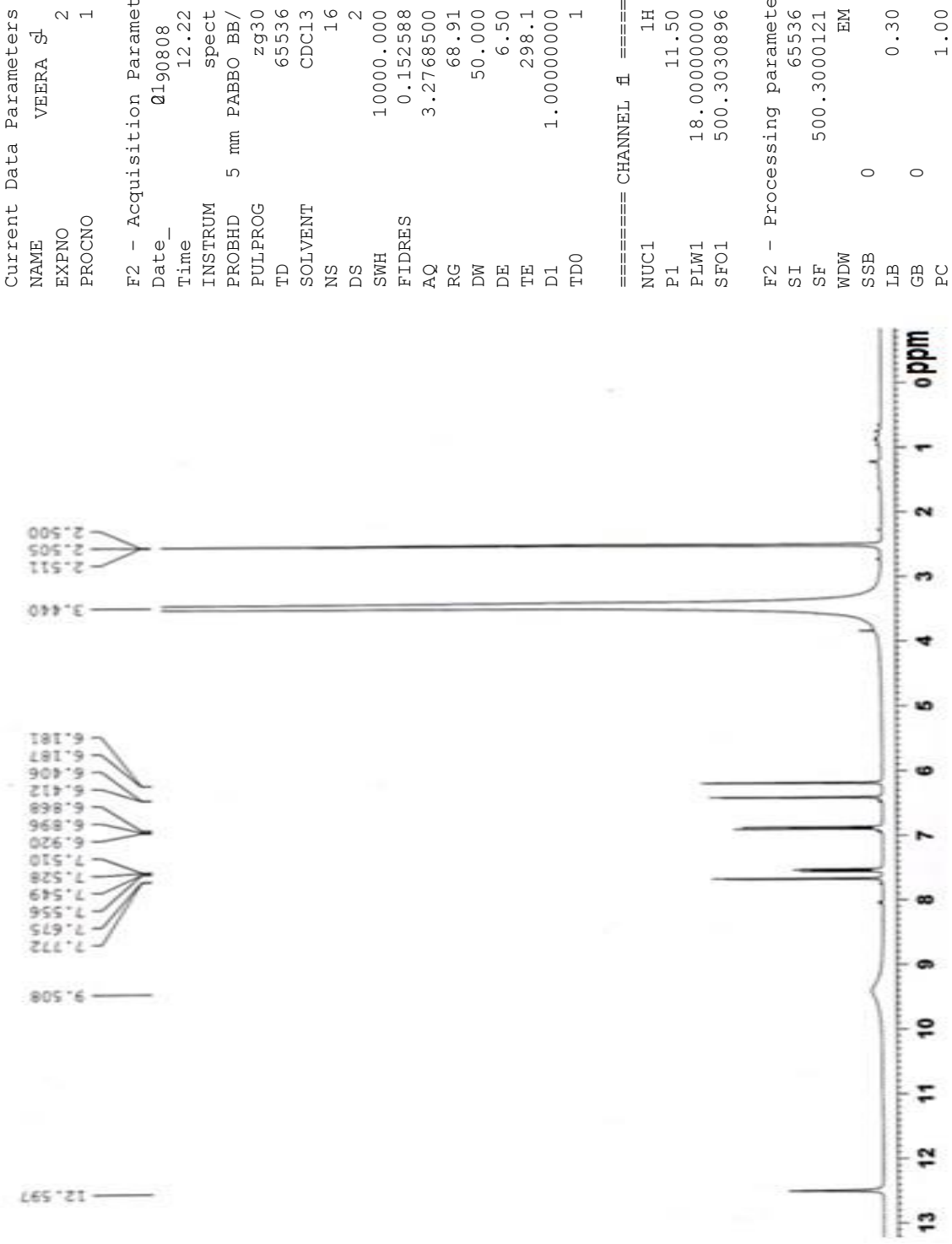
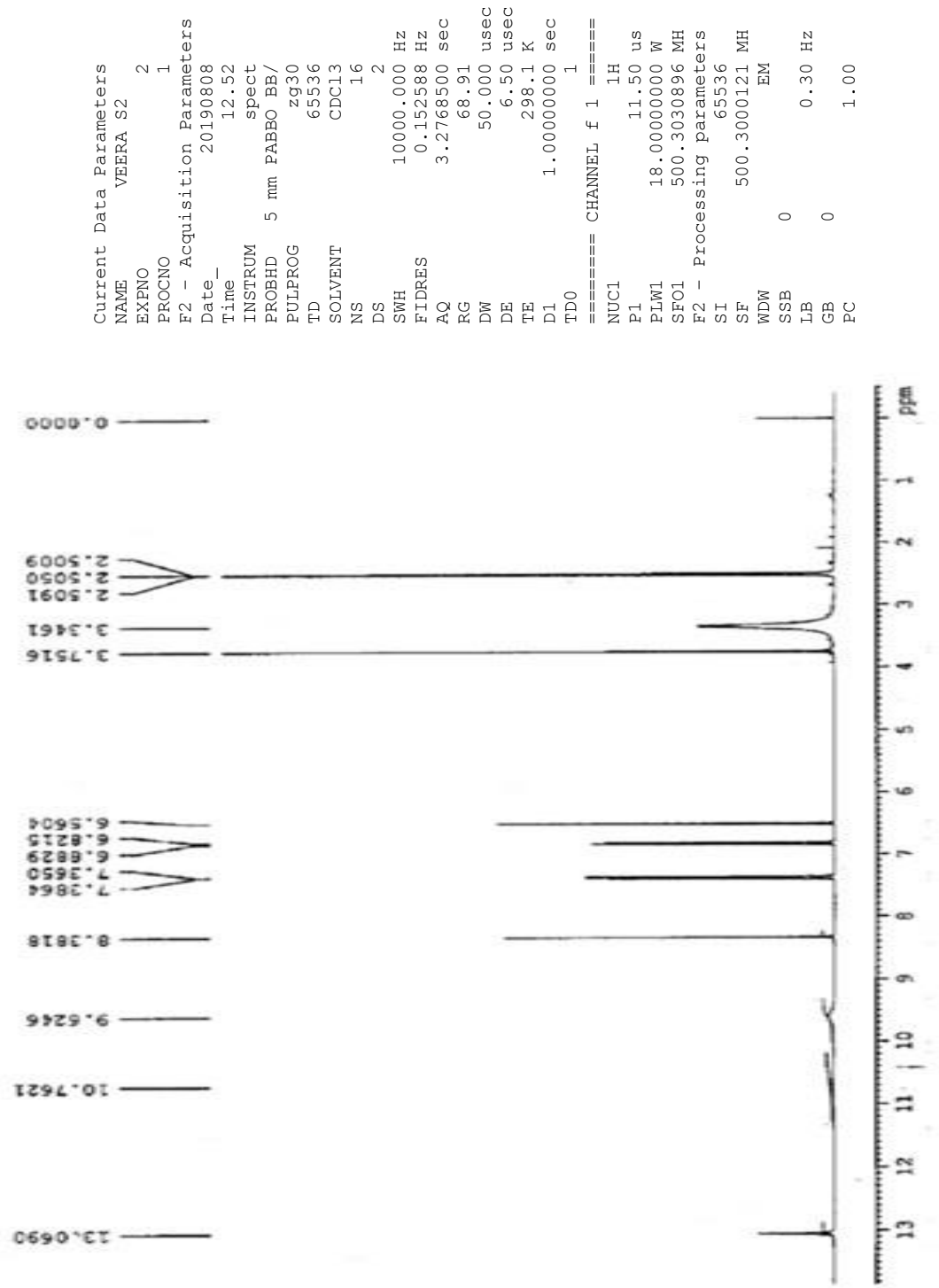


Fig. 12: [1]HNMR Spectrum of Compound-I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PROCNO 1
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PULPROG zg30
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SOLVENT CDC13
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2768500 sec
RG 68.91
DW 50.000 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f 1 ======
NUC1 1H
P1 11.50 us
PLW1 18.00000000 W
SFO1 500.3030896 MH
F2 - Processing parameters
SI 65536
SF 500.3000121 MH
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.0000
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2.5050
2.5009
3.3461
3.7516
6.5604
6.8215
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7.3650
7.3864
8.3818
9.6246
10.7621
13.0690
ppm

Fig. 13: ^{1}HNMR Spectrum of Compound-II

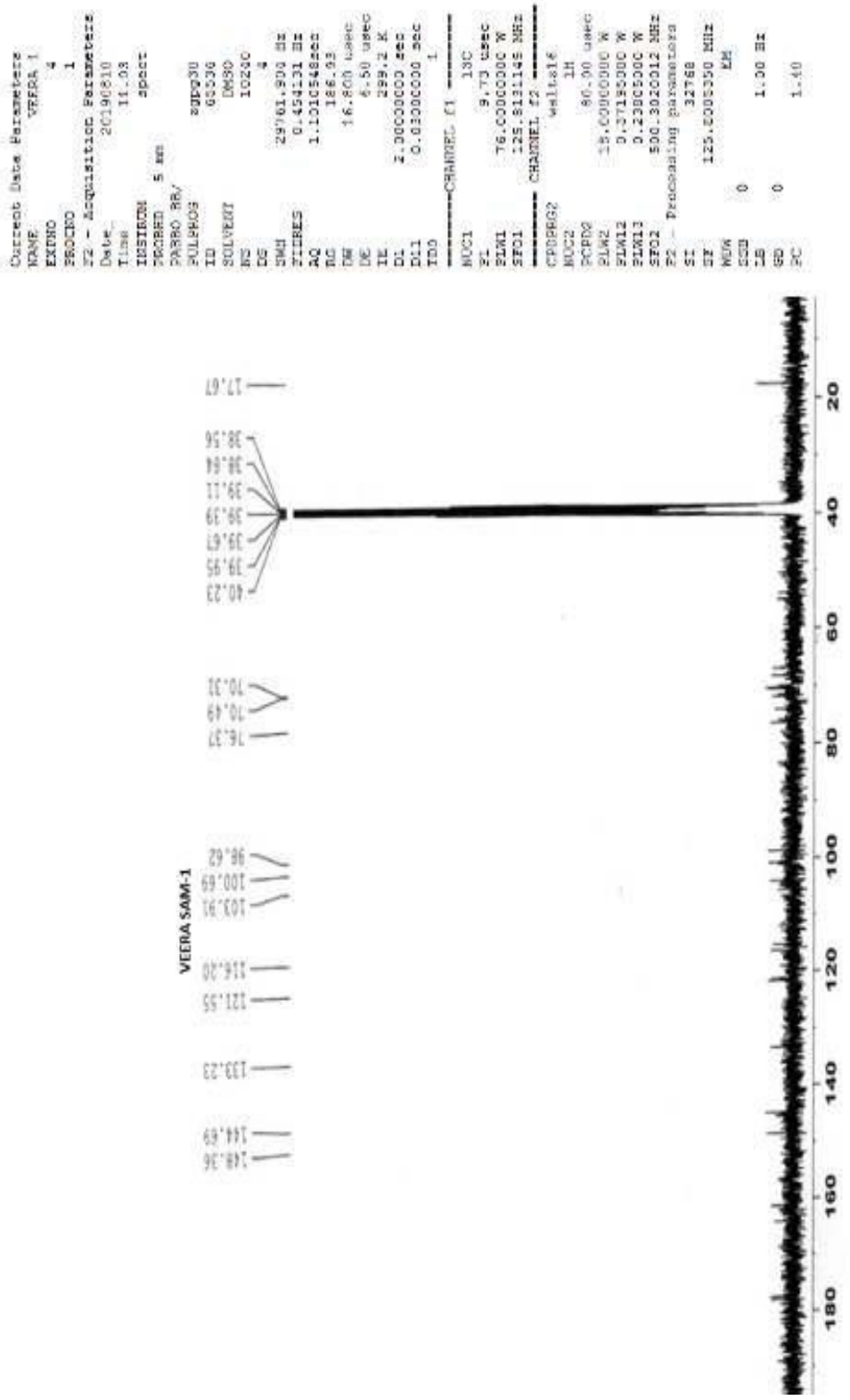

Fig. 14: ^{13}CNMR Spectrum of Compound-I

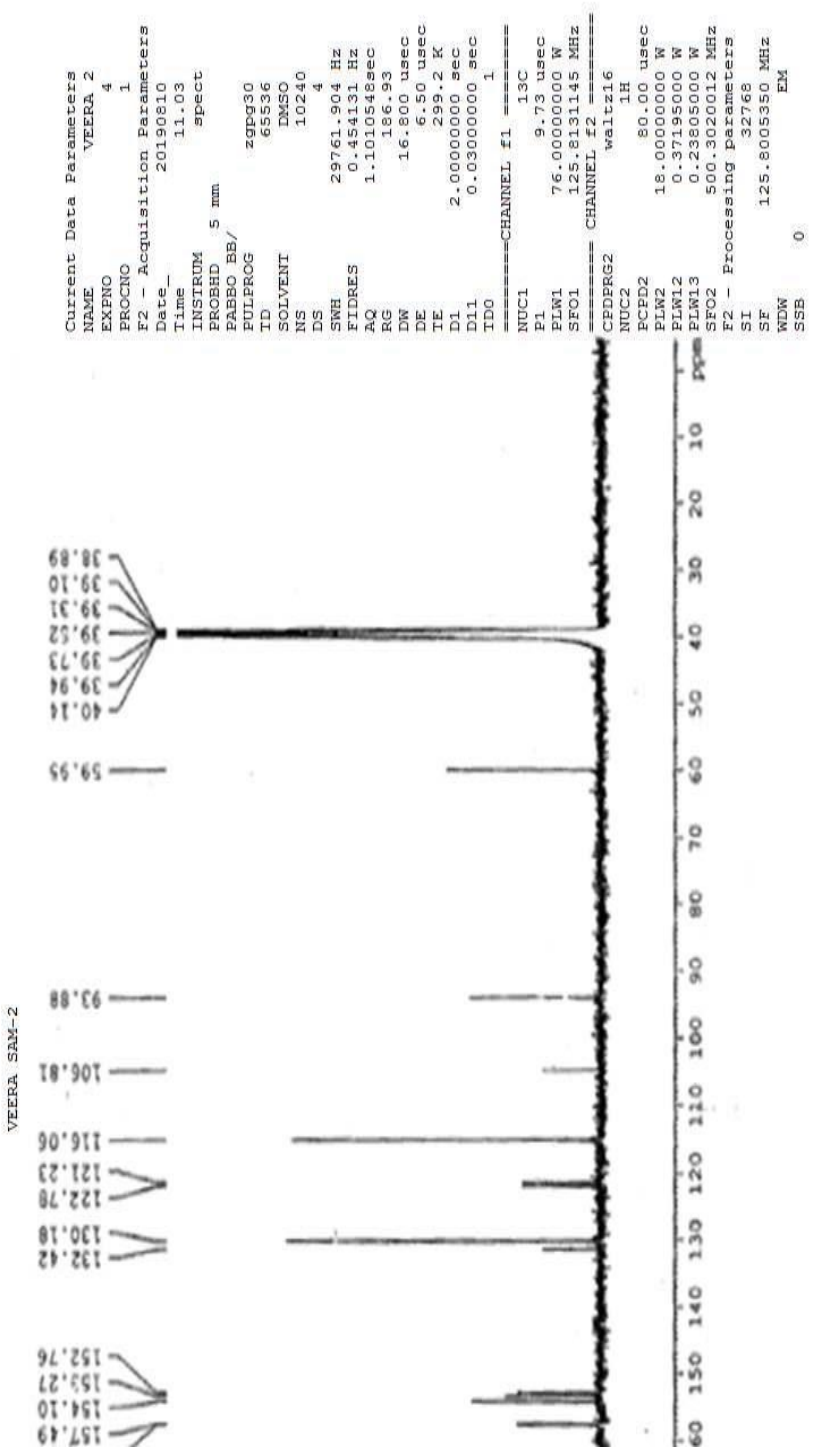

Fig. 15: ^{13}CNMR Spectrum of Compound-II

06-08-2019 13:48:24 1 / 2

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Sample ID : SAMPLE - 1
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Vial# : 1
Injection Volume : 20
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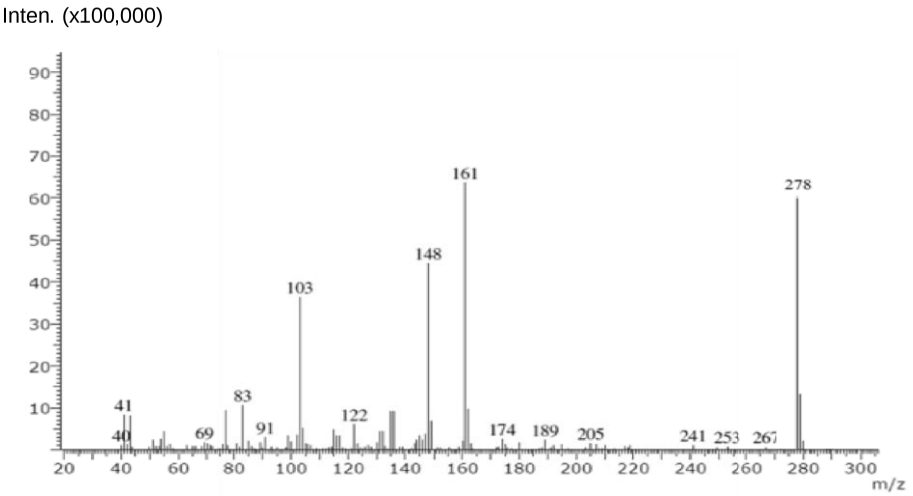
Report Format File : C:\LabSolutions\System\DEFAULT.lsr
Month-Day Acquired : 06-08-2019
Month-Day Processed : 06-08-2019



D:\HPLC+PDA+MS\06-Aug-2019\SAMPLE - 1 2.lcd

Fig. 16: Mass Spectrum of Compound-I

Fig. 17: Mass Spectrum of Compound- II

5. *In-vitro* antioxidant activity of *Pongamia pinnata*

Hydro-alcoholic extract of leaves E and its two fractions (Fraction A and Fraction B) showed less potent when compared to standard drug ascorbic acid in DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

Hydro-alcoholic extract of leaves E and its two fractions (Fraction A and Fraction B) showed moderate potent when compared to standard drug ascorbic acid in ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay**.**

Hydro-alcoholic extract of leaves E and its two fractions (Fraction A and Fraction B) showed moderate potent when compared to standard drug α-tocopherol in hydrogen peroxide scavenging capacity assay.

Table 19: DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay of extract E, fraction A and fraction B

S. No	Sample	Conc.	Mean± SD	IC_{50}
1.	**Standard**	5	26.054±0.345	**17.263**
		10	29.480±0.396	
		20	40.888±0.284	
		40	89.382±0.338	
		80	92.507±0.345	
		160	93.825±0.284	
		320	97.063±0.338	
2.	**E**	5	13.704±0.225	**266.042**
		10	16.942±0.284	
		20	19.201±0.396	
		40	23.757±0.407	
		80	29.216±0.284	
		160	40.474±0.225	
		320	64.194±0.225	
3.	**A**	5	14.646±0.345	**>320**
		10	19.201±0.345	
		20	21.649±0.345	
		40	22.590±0.396	
		80	26.054±0.345	
		160	30.421±0.407	
		320	35.015±0.396	
4.	**B**	5	15.813±0.396	**>320**
		10	18.185±0.345	
		20	20.293±0.284	
		40	21.121±0.456	
		80	23.795±0.345	
		160	26.054±0.235	
		320	32.981±0.284	

Table 20: ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay of extract E, fraction A and fraction B

S. No	Sample	Conc.	Mean± SD	IC_{50}
1.	**Standard**	5	14.158±0.255	**14.49**
		10	36.862±0.337	
		20	68.579±0.320	
		40	87.840±0.389	
		80	92.857±0.255	
		160	95.663±0.382	
		320	96.853±0.447	
2.	**E**	5	4.804±0.389	**36.91**
		10	7.525±0.459	
		20	31.887±0.459	
		40	37.712±0.389	
		80	91.836±0.382	
		160	94.090±0.389	
		320	96.853±0.389	
3.	**A**	5	12.755±0.255	**142.64**
		10	20.280±0.459	
		20	22.789±0.194	
		40	25.297±0.389	
		80	35.714±0.382	
		160	44.345±0.389	
		320	75.085±0.447	
4.	**B**	5	3.954±0.337	**134.926**
		10	13.860±0.320	
		20	15.433±0.255	
		40	24.234±0.255	
		80	32.908±0.255	
		160	44.387±0.337	
		320	80.314±0.320	

Table 21: Hydrogen Peroxide Scavenging Capacity of extract E, fraction A and fraction B

S. No	Sample	Conc.	Mean± SD	IC_{50}
1.	**Standard**	5	25.829±0.476	**17.71**
		10	32.640±0.458	
		20	50.793±0.556	
		40	90.216±0.346	
		80	95.007±0.444	
		160	99.365±0.049	
		320	99.711±0.049	
2.	**E**	5	23.290±0.964	**68.98**
		10	26.031±0.217	
		20	33.968±0.435	
		40	58.066±0.278	
		80	73.650±0.278	
		160	77.979±0217	
		320	85.916±0.435	
3.	**A**	5	24.155±0.312	**90.92**
		10	25.165±0.264	
		20	28.427±0.349	
		40	42.424±0.222	
		80	65.858±0.278	
		160	76.421±0.132	
		320	87.272±1.589	
4.	**B**	5	17.344±0.327	**83.05**
		10	21.731±0.312	
		20	27.763±0.217	
		40	61.471±0.259	
		80	68.455±0.278	
		160	78.787±0.259	
		320	85.772±0.278	

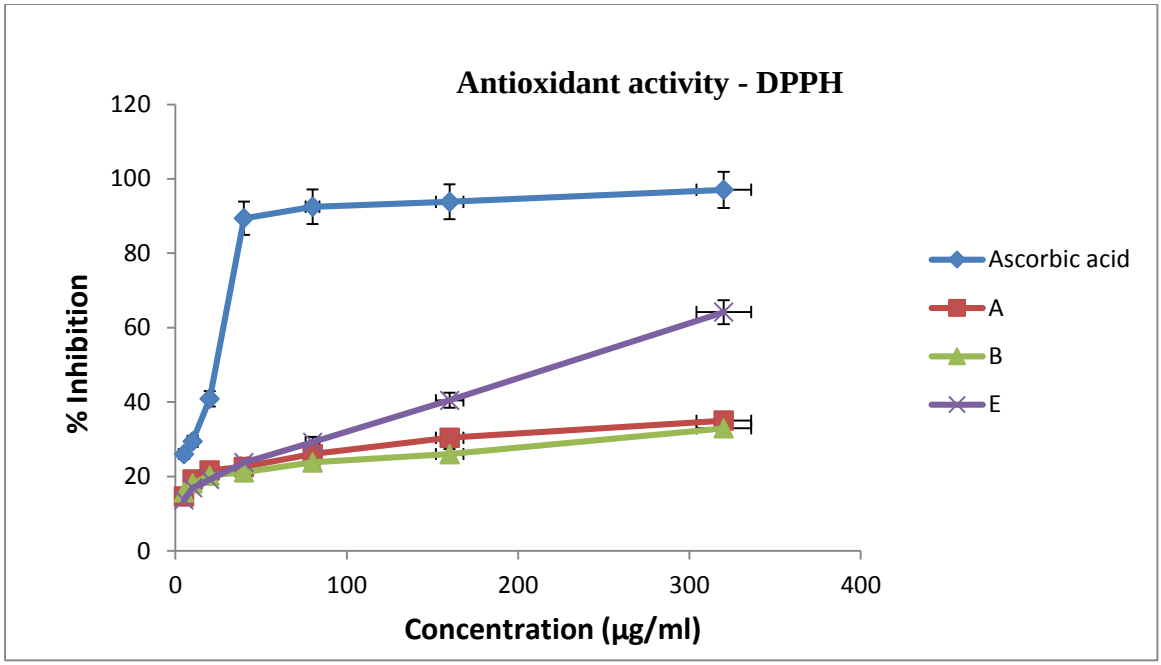


Fig. 18: DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay of extract E, fraction A and fraction B

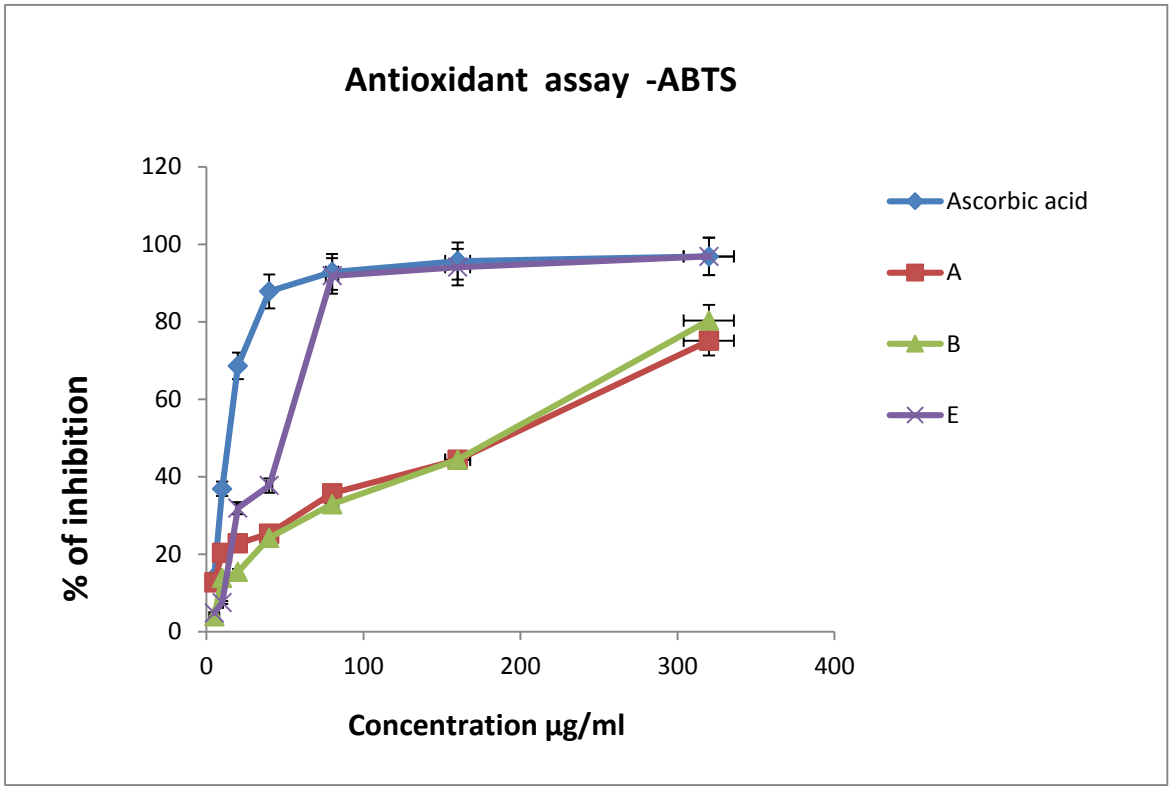


Fig. 19: ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay of extract E, fraction A and fraction B

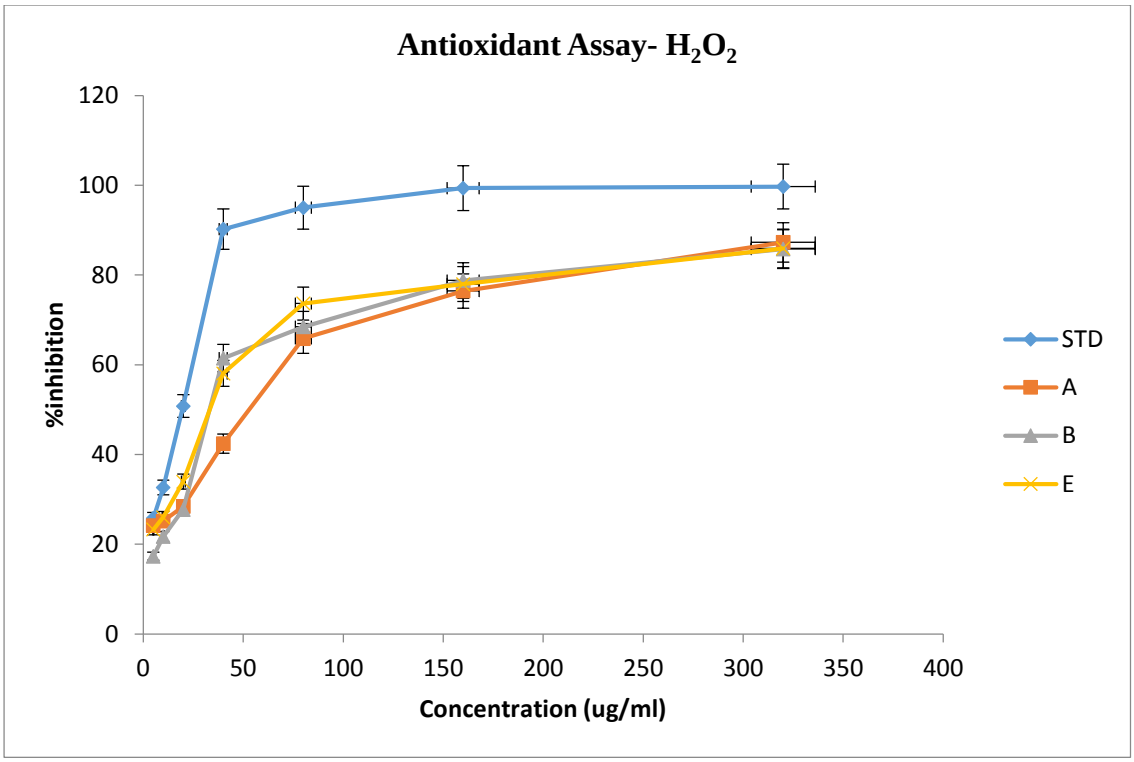


Fig. 20: Hydrogen Peroxide Scavenging Capacity of extract E, fraction A and fraction B

6. In-vitro anticancer activity of *Pongamia pinnata*

Evaluation of anti-lung cancer activity of Hydro-alcoholic leaf extract and fraction A and B from Hydro-alcoholic leaf extract of *Pongamia pinnata* shows mild anti-cancer activity when compared with the standard drug cisplatin.

Table 22: ANTI-CANCER ACTIVITY (LUNG CANCER) OF HYDRO-ALCOHOLIC LEAF EXTRACT OF *PONGAMIA PINNATA* AND ITS FRACTION

S. No	Sample	Conc.	Viability	Mean ± SD	IC_{50} Value
1.	**Standard**	3.12	50.675117	0.615± 0.006	**1.35**
		6.25	25.516671	0.311±0.004	
		12.5	13.089005	0.160±0.005	
		25	8.5422982	0.105±0.004	
		50	6.0898319	0.076±0.008	
		100	2.121797	0.028±0.008	
2.	**E**	3.12	93.937724	1.138± 0.007	**96.20**
		6.25	90.300358	1.094±0.010	
		12.5	80.931386	0.981±0.006	
		25	71.341967	0.865±0.001	
		50	64.673464	0.784±0.007	
		100	42.60127	0.517±0.005	
3.	**A**	3.12	90.0248	1.091±0.007	**51.08**
		6.25	83.686966	1.014±0.007	
		12.5	70.597961	0.856±0.007	
		25	60.788096	0.737±0.007	
		50	53.87159	0.654±0.005	
		100	36.81455	0.447±0.011	
4.	**B**	3.12	87.186553	1.057±0.006	**40.67**
		6.25	78.809589	0.955±0.005	
		12.5	67.594379	0.819±0.006	
		25	58.500964	0.709±0.005	
		50	51.171122	0.621±0.006	
		100	32.24029	0.392±0.007	

Control

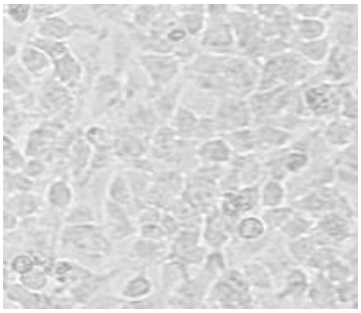


Fig. 21

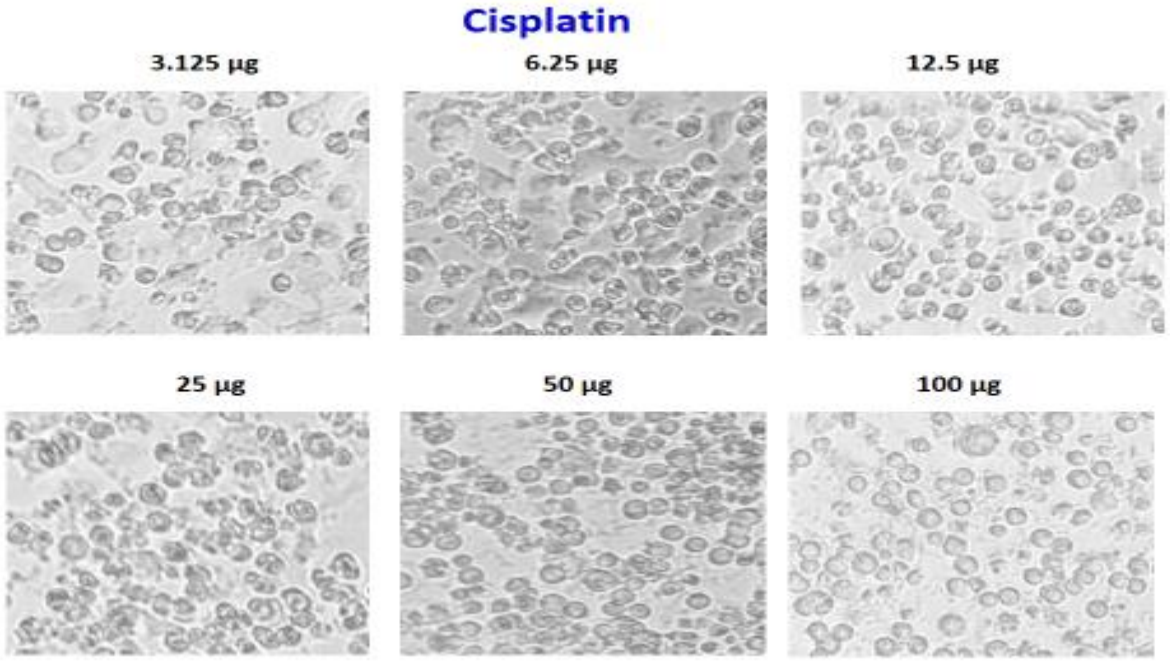


Fig. 22

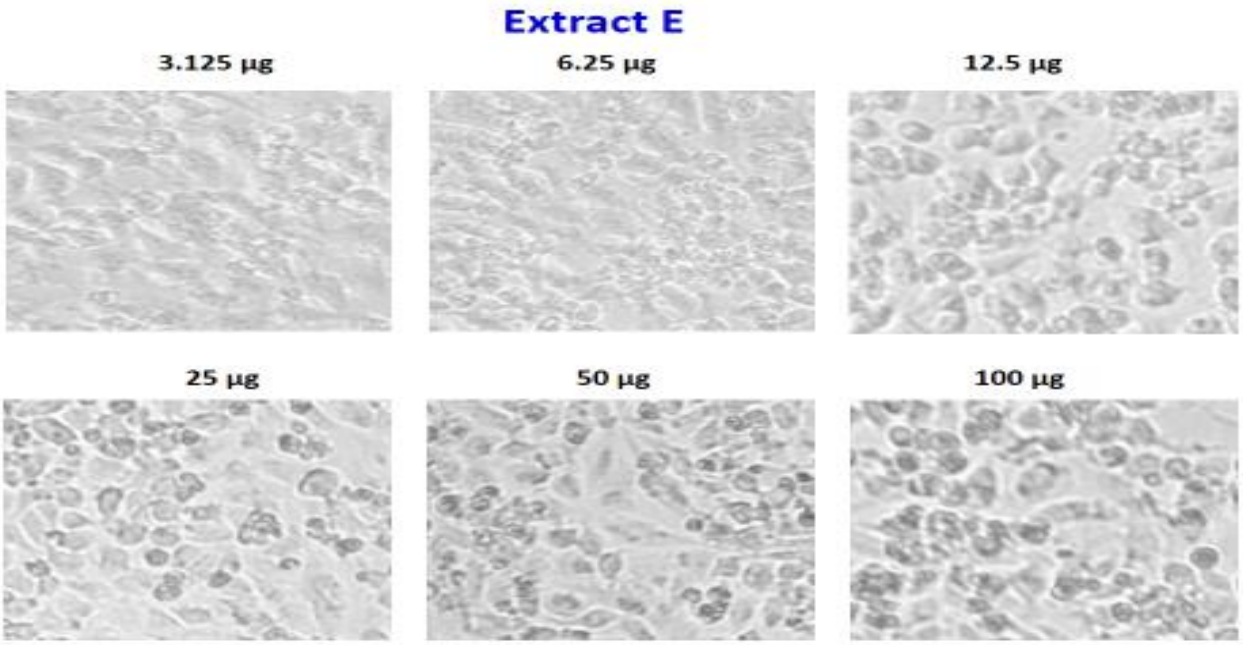

Extract E
3.125 µg
6.25 µg
12.5 µg
25 µg
50 µg
100 µg

Fig. 23

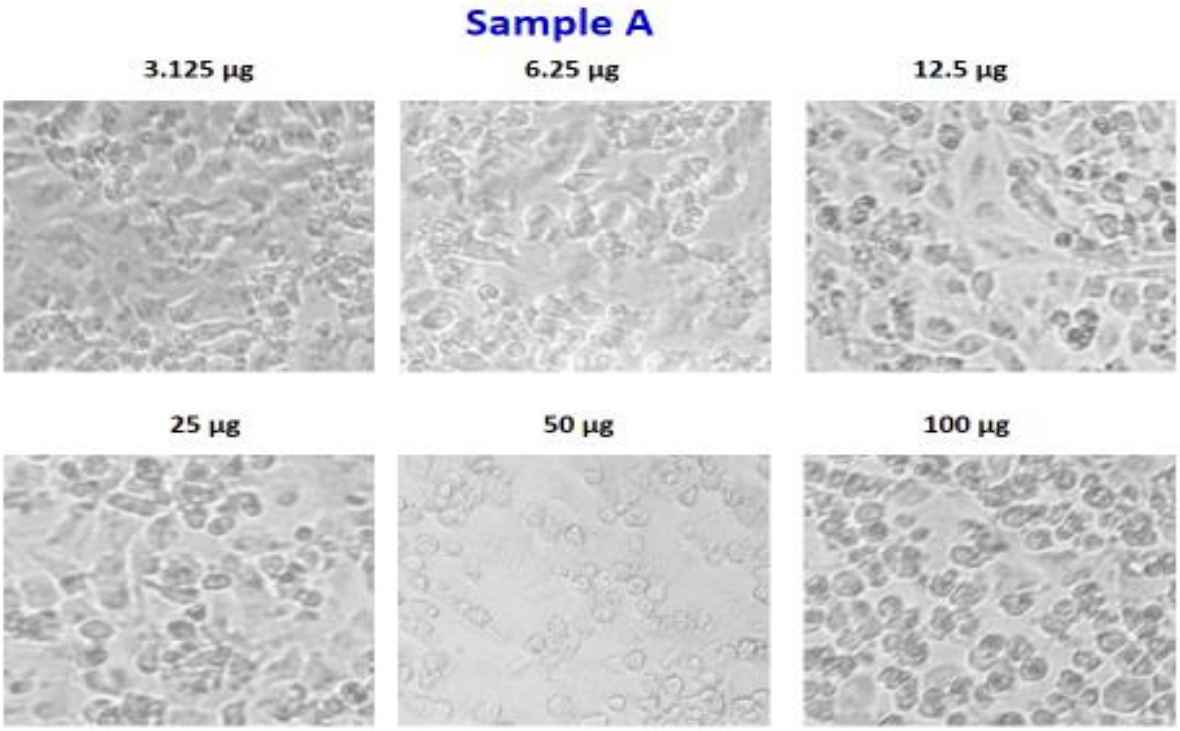

Sample A
3.125 µg
6.25 µg
12.5 µg
25 µg
50 µg
100 µg

Fig. 24

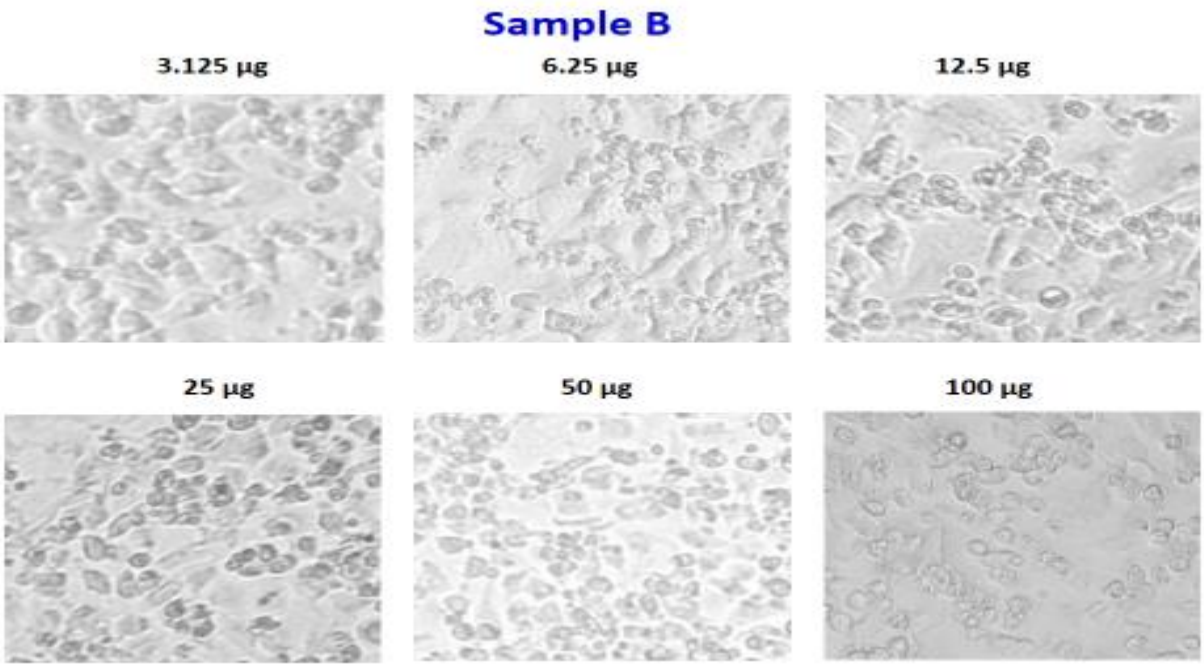


Fig. 25

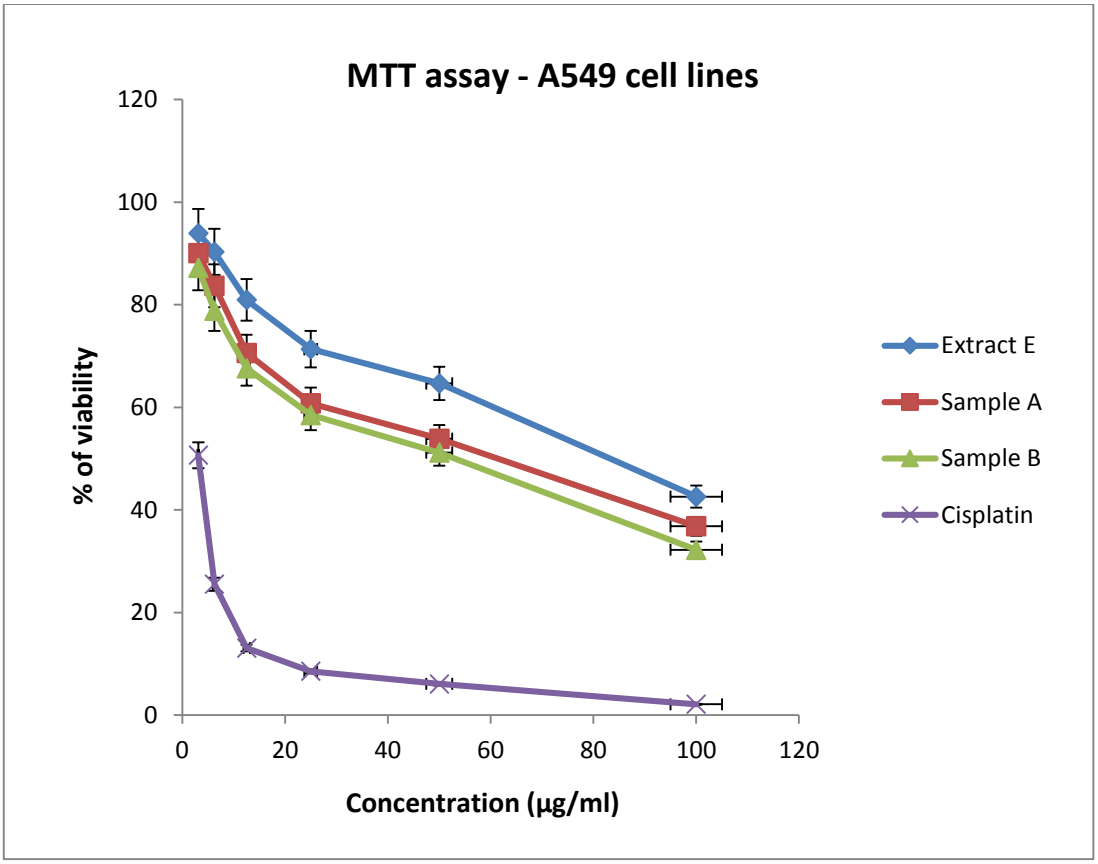


Fig. 26: Anti-cancer activity (lung cancer) Hydro-alcoholic leaf extract of *Pongamia pinnata* and its fraction

CONCLUSION

In summary, our results reported that preliminary phytochemical analysis shows alkaloids, carbohydrates, glycosides, phytosterols, sterols, flavonoids, tannins, phenolic compounds and triterpenoids in Hydro-alcoholic leaf extract of *Pongamia pinnata* (Linn.) Pierre. In GC-MS analysis of fractions of A and B from Hydro-alcoholic leaf extract of *Pongamia pinnata* (Linn.) Pierre reported various compounds. FT-IR, ^{1}HNMR, ^{13}CNMR, and Mass Spectral studies reveals that the compound-I 2-(2-amino-3-methoxyphenyl) chromen-4-one, and Compound-II 3, 4', 5, 7-Tetrahydroxy-3'-methoxyflavone. These extract and its fractions possessed less anti-lung cancer activity due the moderate antioxidant activity. Natural bioactive compounds has been found to interfere and prevent all kinds of cancer. Flavonoids have been used as anti-tumor agents involving a free radicals quenching mechanism. Hence from this current study, it is proved that the plant *Pongamia pinnata* (Linn.) Pierre has moderate antioxidant and anticancer activity. Further study is required to improve its pharmacological potency and identify which compound is behind the reason for its potency. Additionally, will perform extraction using various solvent, various biological activity with docking study.

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