EVALUATION OF ANTICANCER POTENTIAL OF LEAVES AND STEM OF *Azima tetracantha* Lam.

Dissertation submitted in partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 600 032

Submitted by

Reg. No: 261420662

Under the guidance of

Dr. R. RADHA, M.Pharm., Ph.D.,

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI- 600 003

APRIL - 2016

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTICANCER POTENTIAL OF LEAVES AND STEM OF *Azima tetracantha* Lam."**submitted to **The Tamil Nadu Dr. M. G. R. Medical University, Chennai – 600 032,** in partial fulfillment of the requirements for the award of Degree in Master of Pharmacy in Pharmacognosy is a record of bonafide research work done by the candidate bearing **Reg. No.: 261420662**final year M.Pharm (Pharmacognosy) in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, during the academic year 2015 – 2016 under the guidance of **Dr. R. Radha, M.Pharm., Ph.D.,**Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai- 600 003.

Dr. A. JERAD SURESH, M.Pharm., M.B.A., Ph.D.,

Principal, College of Pharmacy,

Madras Medical College,

Chennai - 600 003.

Place: Chennai - 03.

Date:

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTICANCER POTENTIAL OF LEAVES AND STEM OF *Azima tetracantha* Lam."** submitted to **The Tamil Nadu Dr.M.G.R.Medical University, Chennai - 600032,** in partial fulfillment of the requirements for the award of Degree in Master of Pharmacy in Pharmacognosy is a record of bonafide research work done by the candidate bearing **Reg. No.: 261420662** final year M.Pharm (Pharmacognosy) in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, during the academic year 2015-2016 under my guidance and direct supervision.

Dr. R. RADHA, M.Pharm., Ph.D., M.B.A.,
Professsor and Head,
Department of Pharmacognosy,
College of Pharmacy,
Madras Medical College,
Chennai - 600 003.

Place: Chennai - 03.

Date:

ACKNOWLEDGEMENT- THE ETIQUETTE OF THANKING

*I owe a special tribute to **God Almighty,** for the opportunity given to me to take up this research work and also for the inner strength to complete the research work successfully.*

*Grateful gratitude is accorded to our Dean **Dr. R. Vimala, M.D.,** Madras Medical College, Chennai, for granting permission to pursue M. pharmacy in this institution and providing all the facilities to execute my study.*

*I wish to express my sincere thanks to **Dr. A. Jerad suresh, M.Pharm., M.B.A., Ph.D.,** Principal, College of Pharmacy, Madras Medical College, for providing me with all the necessary facilities for the research and continuous encouragement.*

*I would like to express my gratitude to my guide **Dr. R. Radha, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for introducing me to this project, providing excellent scientific advice and giving energetic encouragement throughout my research. She is always supported me, always had time for questions and discussions.*

*A very special thanks goes to **Dr. N. Jayshree, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacology, College of Pharmacy, Madras Medical College, without her motivation and encouragement I would not have considered a graduate career in this research.*

*I express my sincere thanks to **Dr. P. Muthusamy, M.Pharm., Ph.D., B.L.,** Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for sharing expertise, and sincere and valuable guidance and encouragement extended to me.*

*I owe my gratitude to **Dr. R. Vijaya Bharathi, M.Pharm., Ph.D,** Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for the useful comments, remarks and engagement through the learning process of this master research.*

*I would like to express my sincere gratitude to **Dr. R. Vadivu, M.Pharm., Ph.D.,** Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, has been always there to listen and give advice. I am deeply grateful to her for the discussions and guidance that helped me in all the time of research.*

*I extended my thanks to **Mrs. T. S. Lakshmi** and **Mrs. M. Kumuthavalli,** Lab technicians, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for their help during the research.*

*My sincere thanks to **Mr. R. Sathyamoorthi, M.Sc., M.Phill., B.Ed., Ph.D.,** IIT Madras, Chennai and **Mr. M. Krishnaraj, M.Sc., Ph.D.,** Director, Apex Biotechnology Training and Research Institute, Chennai, for their valuable guidance and endless help in spectral studies and invtro assays.*

*I express my special thanks to my classmates **Mrs. P. Karunya, Ms. T. Nandhini, Ms. N. Shantha sheela, Ms. R. Ramya, and Ms. P. Vijayalakshmi** for their cooperation for the completion of my research.*

I take this opportunity with pride and immense pleasure to thank all my Teachers who lit the light of knowledge and wisdom in my life.

I also place on record, my sense of gratitude to one and all, who directly or indirectly, have lent their hand in this venture.

I express my deepest gratitude to my brother ***K. M. Noorulla, M.Pharm., (Ph.D).,*** *for his excellent guidance, caring, patience, and providing me with an excellent atmosphere for doing research. Without his precious support it would not possible to conduct this research. I am grateful to him for enlightening me the first glance of research.*

Last but not the least; I would like to thank my family: my parents ***Mr. M. Sundaresan*** *and* ***Mrs. S. Sentamil Selvi,*** *for giving birth and supporting me spiritually throughout my life and my sisters and brothers for their unconditional sacrifices, love, moral support and encouragement that helped me to reach my dreams. They deserve the credit of success in whatever work I do.*

Reg. No.: 261420662

TABLE OF CONTENTS

LIST OF THE TABLE

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACp	Acyl-Carrier Protein
AS	Airsac
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AJC	American Joint Committee
AST	Aspartate aminotransferase
ANS	Autonomic Nervous System
ABTS	[2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]
A549	Adenocarcinomic human alveolar basal epithelial cells
BC	Before Christ
^{13}C	Carbon
CCL_4	Carbon tetrachloride
CNS	Central Nervous System
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CT Scan	Computerized Tomography
Co	Cortex
Cu	Cuticle
DNA	Deoxyribo Nucleic Acid
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAC	Ehrlich Ascites Carcinoma
EAT	Extract of *Azima tetracantha*
FAA	Formalin, Acetic acid, Absolute ethanol
GC-MS	Gas Chromatography- Mass Spectroscopy
GSH	Glutathione
HeLa	Human cervical carcinoma cells
^{1}H	Hydrogen
HF	Hypodermal fibres
IR	Infrared
IC_{50}	Inhibitory Concentration of 50%
IAEC	Institutional Animal Ethical Committee

IBC	Isolated Bioactive Compound
La	Lamina
L-ADP	L-adenosine diphosphate
LDH	Lactate Dehydrogenase
LV	Lateral vein
LE	Lower epidermis
MS	Mass Spectroscopy
MCF-7	Human Brest cancer cells
MTT	Microculture Tetrazolium Assay
MR	Midrib
NCCS	National Coalition for Cancer Survivorship
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
ND	Not Detected
OECD	Organisation for Economic Co-operation and Development
PAC	Paclitaxel
Pa	Palisade cells
PGK	Papua New Guinean Kina
PF	Pericyclic fibres
Ph	Phloem
Pig	Pigments
PDB	Protein Data Bank
RPMI	Roswell Park Memorial Institute
SOD	Small-molecule superoxide dismutase
Sch	Sclerenchyma
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
Sp.	Species
SI	Stomatal Index
TZN	Tetrazosin
TBARS	Thiobarbituric acid reactive substances

TLC	Thin Layer Chromatography
TSA	Tumor Surface Antigen
UV	Ultra-violet
UICC	Union for International Cancer Control
USA	United States of America
UE	Upper Epidermis
VB	Vascular bundle
Xy	Xylem
WHO	World Health Organization
2D	Two- Dimensional
3D	Three-Dimensional

LIST OF SYMBOLS

(+)	Present	**δ**	delta
(-)	Absent	**R_f**	Retention factor
%	Percentage	**Al**	Aluminium
ºC	Degree Centigrade	**Ar**	Arsenic
g	gram	**Ca**	Calcium
ng	nanogram	**Cl**	Chloride
µg	microgram	**Co**	Cobalt
mg	Milligram	**Cu**	Copper
nm	nanometer	**Fe**	Iron
ml	milli litre	**Pb**	Lead
kcal/mol	kilocalories/ mole	**Mg**	Magnesium
sq mm	square milli meter	**K**	Potassium
%w/v	percentage weight/ volume	**Ag**	Silver
%v/v	percentage volume/ volume	**SO_4**	Sulphate
ν_{max}	Maximum absorbance	**KBr**	Potassium bromide
cm^{-1}	centimeter inverse	**HCl**	Hydrochloric acid

ABSTRACT

Cancer is considered to be a very atrocious disease since the time man started to imagine about therapeutics. It is one of the leading causes of morbidity and mortality worldwide. The majority of world's population in developing countries still relies on herbal medicine to meet their health needs. The impact of toxicity/ ADR of the synthetic anticancer drug also paved the way for the focus on herbal medicines. *Azima tetracantha* Lam., is belonging to the family Salvadoraceae used in the Folk medicine to treat various ailments. In the present work, the various extracts of leaves and stem of *Azima tetracantha* were subjected to pharmacognostical, phytochemical and pharmacological studies. The plant extracts were evaluated for *in vitro* anticancer activity against MCF-7 cell lines using MTT assay. Based on MTT assay, the active extract was subjected to bioactivity guided isolation by column chromatography using hexane, chloroform and ethanol. All the fractions and isolated compounds were evaluated for their *invitro* antioxidant activity and the compound that showed high antioxidant effect on DPPH further evaluated for *invivo* acute toxicity studies and spectral studies. From all the fractions tested, the more effecient fraction (IBC32) subjected to *invitro* anticancer activity on various cell lines (MCF-7, HeLa and A549) using MTT assay. Docking studies were carried out for the isolated bioactive compound (IBC32) with various targeting enzymes such as PDB ID- 4O33, 2Y3I, 3UEN and 3ZOZ.

CHAPTER 1

INTRODUCTION

Cancer is an uncontrolled growth of cells, with loss of differentiation and commonly with metastasis, spread of the cancer to other tissues and organs.[1,2] Cancer is a genetic disease as it can be traced for alteration within specific genes, but in most cases, it is not an inherited disease. The genetic alterations that lead to most cancers arise in the deoxyribonucleic acid (DNA) of somatic cell. Because of these genetic changes, cancer cells proliferate uncontrollably, producing malignant tumour that invade the surrounding healthy tissues.[3,4]

Cancer is also called as *Neoplasia* which literally means "new growth". The new growth produced is called 'neoplasm' or 'tumour'. Neoplastic cells are said to be *transformed* because they continue to replicate, apparently oblivious to the regulatory influences that control normal growth. Therefore, satisfactory definition of a neoplasm or tumour is *'a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells even after cessation of stimulus for growth which caused it'.* The study of tumours is called *oncology* (from *oncos, "tumour",* and *logos, "study of"*).[5-7]

Neoplasm may be benign when they are slow growing and localized without causing much difficulty to the host or malignant when they proliferate rapidly, spread throughout the body and may eventually cause death of the host.[4,5]

1.1. HISTORY

Hippocrates (460-377 BC) coined the term *karkinos* for cancer of the breast. The word *'cancer'* means crab, thus reflecting the true character of cancer since *'it sticks to the part stubbornly like a crab'*.[8] This name comes from the appearance of the cut surface of a solid malignant tumor, with "the veins stretched on all sides as the animal the crab has its feet, hence it derives its name".[9] Galen stated that "cancer of the breast is so called because of the fancied resemblance to a crab given by the lateral prolongations of the tumor and the adjacent distended veins".[10]

Old theories about cancer[11]

Humoral theory

Hippocrates believed that the body contained 4 humors (body fluids) such as blood, phlegm, yellow bile and black bile. Any imbalance of these fluids will result in disease and excess of black bile in a particular organ site was thought to cause cancer. This theory of cancer was standard through the Middle Ages for over 1300 years.

Lymph theory

This theory proposed that cancer formation by fluid called lymph. Life was believed to consist of continuous movement of the fluids like as blood and lymph in the body. The lymph theory was supported in 17th century that tumors grow from lymph constantly thrown out by the blood.

Blastema theory

Muller demonstrated that cancer is made up of cells but not with lymph in 1838. Virchow (1821-1902) determined that all cells including cancer cells were derived from other cells.

Chronic irritation theory

Virchow proposed that chronic irritation was the cause of cancer. Later Thiersch was showed that cancers metastasize through the spread of malignant cells and not through some unidentified fluid.

Trauma theory

From the late 1800s until the 1920s, cancer was thought to be caused by trauma.

Parasite theory

Till 18th century, scientists believed that cancer was contagious and spreads through parasite.

1.2. EPIDEMIOLOGY

Worldwide, it is estimated that about 20% of all deaths are cancer-related; in US, cancer is the second most common cause of deaths, next to heart disease.[5,6] In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers),[12] and in 2010 nearly 7.98 million people died.[13] Cancers as a group account for approximately 13% of all deaths each year with the most common being: lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths).[14] This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world. **Table 1** showed worldwide incidence of different forms of cancer in men, women and children.

Table 1: Five most common primary cancers in the world

S. No	Men	Women	Children
1.	Lung (Oral cavity in India)	Breast (Cervix in India)	Acute leukemia
2.	Prostate	Lung	CNS tumour
3.	Colorectal	Colorectal	Bone sarcoma
4.	Urinary bladder	Endometrial	Endocrine
5.	Lymphoma	Lymphoma	Soft tissue sarcoma

1.3. TYPES OF CANCER[15,16]

Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. These types include:

- ***Carcinoma:*** Cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in the aged, and include nearly all those developing in the breast, prostate, lung, pancreas and colon.
- ***Sarcoma:*** Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchymal cells outside the bone marrow.
- ***Lymphoma and Leukemia:*** These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30%.[17]

- ***Germ cell tumor:*** Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- ***Blastoma:*** Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

1.4. PRODUCTION OF CANCER CELL

Cancer is when abnormal cells divide in an uncontrolled way. Some cancers may eventually spread into other tissues. Cancer grows as cells multiply over and over. Cancer starts when gene changes make one cell or a few cells begin to grow and multiply too much. This may cause a growth called a tumour. A primary tumour is the name for where a cancer starts. Cancer can sometimes spread to other parts of the body is called as secondary tumour or metastasis.[18] Cancer is a broad term for a class of diseases characterized by abnormal cells that grow and invade healthy cells in the body.[19]

Cancer develops when normal cells in a particular part of the body begin to grow out of control. There are different types of cancers; all types of cancer cells continue to grow, divide and re-divide.[20]

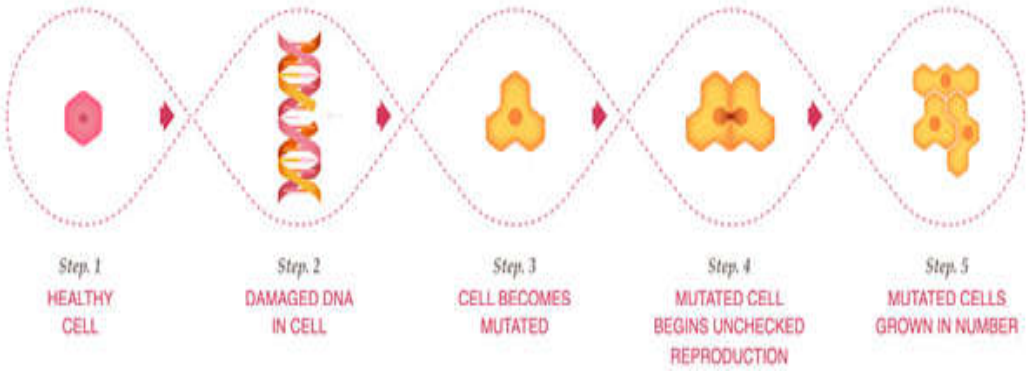


Fig 1: Production of cancer cell

Global comparison shows that India has high incidence rates of cancers of oral cavity, pharynx & cervix. The age standardized cancer incidence in Indian registries as compared to incidence in certain developed countries is about half to one third in men and about half in women.[21]

Pathophysiology of cancer

Genetics

Cancer is fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes that regulate cell growth and differentiation must be altered.[22]

The affected genes are divided into two broad categories. Oncogenes are genes that promote cell growth and reproduction. Tumor suppressor genes are genes that inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in many genes are required to transform a normal cell into a cancer cell.[23]

Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA. **Figure 2** showed the pathophysiology of cancer.

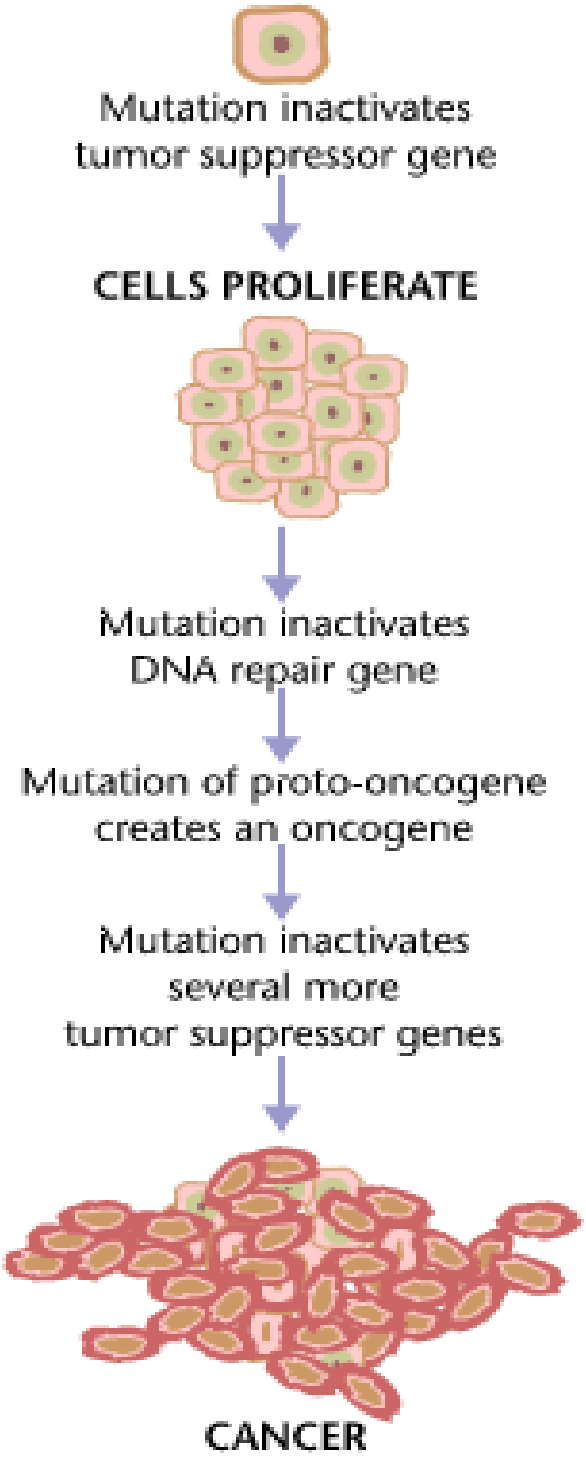


Fig 2: Pathophysiology of cancer: Cancers are caused by a series of mutations.

Carcinogenesis: Molecular basis of Cancer[5,6]

Carcinogenesis or oncogenesis or tumorigenesis means mechanism of induction of tumours (pathogenesis of cancer); agents which can induce tumours are called carcinogen (etiology of cancer).

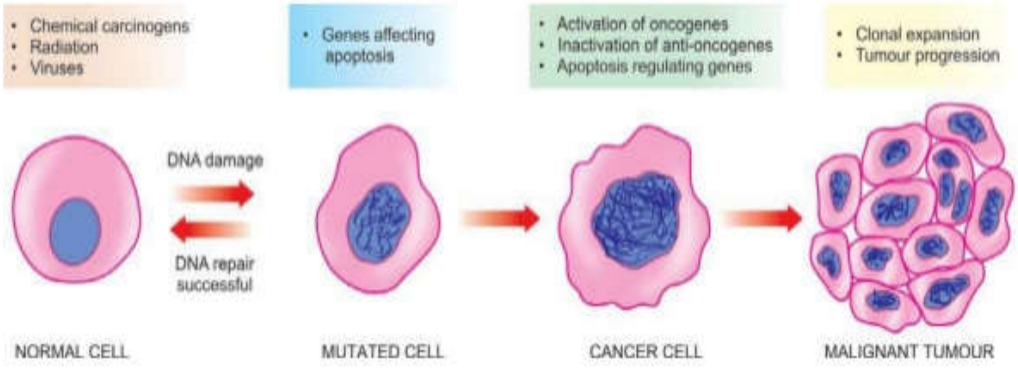


Fig 3: Molecular basis of cancer

The fundamental principles involved in the molecular basis of cancers are:

- Non-lethal genetic damage lies at the heart of carcinogenesis. Such genetic damage may be acquired by the action of environmental agents, such as chemicals, radiation or viruses.
- Four classes of normal regulatory genes- growth promoting proto oncogenes, growth inhibiting tumour suppressor genes, genes that regulate programmed cell death (i.e.) apoptosis and genes involved in DNA repair are the principal targets of genetic damage.
- Oncogenes are genes that induce a transformed phenotype when expressed in cells.
- Tumour suppressor genes are genes that normally prevent uncontrolled growth and when mutated or lost from a cell, allow the transformed phenotype to develop.

- Genes that regulate apoptosis and DNA repair may act like proto-oncogenes (loss of one copy is sufficient) or tumour suppressor genes (loss of both copies).

Etiology of Cancer (Carcinogenic agents)[5,6]

Genetic damage lies at the heart of carcinogenesis. Three classes of carcinogenic agents are chemicals, radiant energy and microbial agents. Chemical and radiant energy causes cancer in humans and oncogenic viruses are involved in the pathogenesis of tumour in several animals and human.

1.5. SIGNS AND SYMPTOMS[24,25]

Cancer is a group of diseases that can cause almost any sign or symptom. The signs and symptoms will depend on where the cancer is, how big it is, and how much it affects the organs or tissues. If a cancer has spread (metastasized), signs or symptoms may appear in different parts of the body.

As a cancer grows, it can begin to push on nearby organs, blood vessels and nerves. This pressure causes some of the signs and symptoms of cancer. If the cancer is in a critical area, such as certain parts of the brain, even the smallest tumor can cause symptoms.

But sometimes cancer starts in places where it won't cause any signs or symptoms until it has grown quite large. Cancers of the pancreas, for example, usually don't cause symptoms until they grow large enough to press on nearby nerves or organs (this causes back or belly pain). Others may grow around the bile duct and block the flow of bile. This causes the eyes and skin to look yellow (jaundice). By the time a pancreatic cancer causes signs or symptoms like these, it's usually in an advanced stage. This means it has grown and spread beyond the place it started- the pancreas.

Cancer may also cause symptoms like fever, extreme tiredness (fatigue), or weight loss. This may be because cancer cells use up much of the body's energy supply, or they may release substances that change the way the body makes energy from food. Cancer can also cause the immune system to react in ways that produce these signs and symptoms.

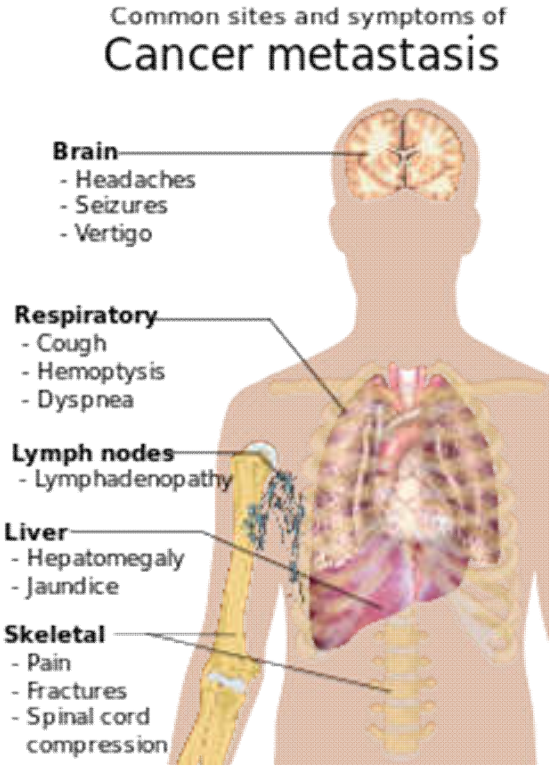


Fig 4: Signs and symptoms of cancer

Sometimes, cancer cells release substances into the bloodstream that cause symptoms that are not usually linked to cancer. For example, some cancers of the pancreas can release substances that cause blood clots in veins of the legs. Some lung cancers make hormone-like substances that raise blood calcium levels. This affects nerves and muscles, making the person feel weak and dizzy.

1.6. DIAGNOSIS

Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. Cancer are investigated with medical tests include,

- Blood tests
- X-rays
- CT scan
- Endoscopy

Grading of cancer[5,6]

Grading and staging are the two systems to predict tumour behavior and guide therapy after a malignant tumour is detected. Grading is defined as the gross and microscopic degree of differentiation of the tumour, while staging means extent of spread of the tumour within the patient.

Cancer may be graded grossly and microscopically. Grading is largely based on two important histological features:

- Degree of anaplasia
- Rate of growth

Borders proposed the system of grading for dividing squamous cell carcinoma into 4 grades depending upon the degree of differentiation

Grade I: Well-differentiated (less than 25% anaplastic cells)

Grade II: Moderately-differentiated (25-50% anaplastic cells)

Grade III: Moderately-differentiated (50-75% anaplastic cells)

Grade IV: Poorly-differentiated or anaplastic (less than 255 anaplastic cells)

Staging of Cancer[5,6]

The extent of spread of cancer can be assessed by 3 ways- clinical examination, by investigations and by pathologic examination of tissue removed. Two important staging systems currently followed are: TNM staging and AJC staging.

TNM staging

TNM (T for primary tumour, N for regional nodal involvement and M for distant metastases) was developed by the UICC (Union for International Cancer Control, Geneva). For each of the 3 components namely T, N and M, numbers are added to indicate the extent of involvement.

T_0 to T_4: *In situ* lesion to largest and most extensive primary tumour.

N_0 to N_3: No nodal involvement to widespread lymph node involvement.

M_0 to M_2: No metastasis to disseminated haemotogenous metastases.

AJC staging

American Joint Committee staging divides all cancers into stage 0 to IV and takes into account all the 3 components of the preceding system (primary tumour, nodal involvement and distant metastases) in each stage. TNM and AJC staging systems can be applied for staging most malignant tumours.

1.7. TREATMENTS

Malignant neoplastic diseases may be treated by various approaches:[26]

- ✓ Surgery
- ✓ Radiation therapy
- ✓ Immunotherapy
- ✓ Hormonal therapy
- ✓ Chemotherapy
- ✓ Targeted therapy

Surgery

Surgery is the primary method of treatment of most isolated solid cancers and may play a role in palliation and prolongation of survival. It is typically an important part of making the definitive diagnosis and staging the tumor as biopsies are usually required. In localized cancer surgery typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area. For some types of cancer this is all that is needed to eliminate the cancer.[25]

Radiation therapy

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer. It works by damaging the DNA of cancerous tissue leading to cellular death. To spare normal tissues (such as skin or organs, which radiation must pass through to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose there than in the surrounding, healthy tissue. As with chemotherapy, different cancers respond differently to radiation therapy.[27]

Immunotherapy

A variety of therapies using immunotherapy, stimulating or helping the immune system to fight cancer, have come into use since 1997, and this continues to be an area of very active research.[28]

Hormonal therapy

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an

important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.[29]

Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. The term encompasses any of a large variety of different anticancer drugs, which are divided into broad categories such as alkylating agents and antimetabolites.[30] Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells.

Targeted therapy is a form of chemotherapy that targets specific molecular differences between cancer and normal cells. The first targeted therapies to be developed blocked the estrogen receptor molecule, inhibiting the growth of breast cancer. Another common example is the class of Bcr-Abl inhibitors, which are used to treat chronic myelogenous leukemia (CML).[31] Currently, there are targeted therapies for breast cancer, multiple myeloma, lymphoma, prostate cancer, melanoma and other cancers.

The efficacy of chemotherapy depends on the type of cancer and the stage. In combination with surgery, chemotherapy has proven useful in a number of different cancer types including: breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer, and certain lung cancers.[25] The overall effectiveness ranges from being curative for some cancers, such as some leukemias[32,33] to being ineffective, such as in some brain tumors,[34] to being needless in others, like most non-melanoma skin cancers.[35] The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Even when it is impossible for chemotherapy to

provide a permanent cure, chemotherapy may be useful to reduce symptoms like pain or to reduce the size of an inoperable tumor in the hope that surgery will be possible in the future.

Disadvantages of Cancer treatments[36]

- Surgery is traumatic larger, and some parts of surgery is difficult, not valid for sub-clinical metastases, cannot completely eliminate the cancer cells, cancer cells in a certain period of time will be long.
- Radiation therapy equipment is expensive, the treatment expense is higher.
- Radiation therapy long period, which normally takes 1 to 2 months.
- Radiation is more complicated, and even loss of function caused by sector.
- Radiation treatment is not good, and cannot completely eradicate cancer cells; cancer cells in a certain period of time will be long.
- No chemical drugs to kill cancer cells specifically, to kill cancer cells also kill normal cells, chemotherapy will shorten the survival of patients over time.
- The tumor is not sensitive to the drug, the use of chemotherapy, no clinical value.
- Chemotherapy cannot completely kill all the cancer cells *in vivo*, in a certain time, cancer will relapse or metastasis.

1.8. HERBS AND CANCER

For the treatment of all possible measures are being tried and still worldwide search is going on to explore the antineoplastic agents from various sources. Since the allopathic system of medicine (chemotherapy, radiation, surgery etc.,) may cause several unwanted side effects on the normal host tissue. The indigenous system of medicine has become popular throughout the world in the field of oncology. The

medicinal plants play a major role in the life of human and animals. These serve not only as a medicinal source, but also maintain the health and vitality of individuals, and may cure various diseases including cancer without causing any toxicity.

Phytotherapeutic Approach

A phytotherapeutic approach is more useful in our condition in the search for anticancer drugs. In this approach, the emphasis is on the development of a new drug or biologically active substance whose extraction and isolation has emanated on the basis of anticancer activity.

Natural products and defense against Carcinogenesis

The role of natural products as a source for remedies has been recognized since ancient times.[37] There are some examples of agents derived from natural sources that are currently used in clinical practice.[38] Paclitaxel was originally isolated from the bark of the yew tree *Taxus brevifolia,*[39] a finite source of a compound. Approximately 60% of approved drugs of chemotherapeutic agents are derived from natural compounds.[37] Herbs that promote a healthy liver function include dandelion (Taraxacum), milk thistle (Silybum) and artichoke (Cynara). Camptothecin (from *Camptotheca acuminata*) inhibits the action of topoisomerase I, resulting in cell death. Another prominent molecule is podophyllotoxin, which has been synthetically modified into etoposide and is used to treat lung and testes cancer.

Other important molecules include vincristine, vinblastine, colchicine, ellipticine, flavopiridol (a chromone alkaloid from Rohitukine) and a pyridoindole alkaloid (from Ochrosia spp). [40] The incidence of cancer can thus be substantially reduced by diet modification. Diets rich in vegetables, fruits and legumes contain large quantities of antioxidants that protect against the deleterious action of free radicals

which may lead to cancer development. It has been shown that synthetic cancer drugs cause nonspecific killing of cells, natural products offer protective and therapeutic actions to all cells with low toxicity.

Advantages[41]

There are number of advantages associated with herbal medicines as opposed to synthetic drugs. Examples include the following:

- ***Reduced risk of side effects:*** Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than synthetic drugs.
- ***Regulation of acid-base balance:*** Acid-base balance by regulating the body fluids, improving physical fitness, so that cancer cells do not survive, can completely control the soaring cancer cells, so that no recurrence and metastasis of cancer cells.
- ***Effectives with chronic conditions:*** Herbal medicines tend to be more effective for long standing health complaints that don't respond well to synthetic drugs.
- ***Cost effective:*** Herbs cost much less than synthetic drugs. Research, testing, and marketing add considerably to the cost of synthetic drugs. Herbs tend to be inexpensive compared to drugs.
- **Widespread availability:** Herbs are available without a prescription. In some remote parts of the world, herbs may be the only treatment available to the majority of people.

Chemical groups of natural products with anticancer properties[26]

Plant derived natural products with documented anticancer and antitumor properties can be classified into the following chemical groups (Kintzios and Barberaki, 2004):

Table 2: Natural products containing chemical groups with anticancer properties

Aldehydes	*Cinnamomum cassia, Mondia whitei, Rhus vulgaris, Sclerocarya caffra*
Alkaloids	*Aconitum napellus, Acronychia baueri, A. halophylla, Brucea antidysenterica, Calycodendron milnei, Cassia leptophylla, Chamaecyparis sp., Chelidonium autumnale, Ervatamia microphylla, Eurycoma longifolia,*
Flavonoids	*Acrougehia porteri, Angelica keiskei, Annona densicoma, A. reticulate, Claopodium crispifolium, Eupatorium altissimum, Glycyrrhiza inflate, Gossypium indicum, Polytrichum obioense, Psorospermum febrifigum, Rhus succedanea, Zieridium pseudobtusifolium*
Glycosides	*Phlomis armeniaca, Phyllanthus sp., Plumeria rubra (iridoids), Scutellaria salviifolia, Wikstroemia indica*
Lignans	*Brucea sp., Juniperus virginiana, Magnolia officinalis, Plumeria sp., Wikstroemia foetida*
Lipids	*Nigella sativa, Sho-saiko-to, luzen-taiho-to*
Quinones	*Kigelia pinnata, Koelreuteria henryi, Landsburgia quercifolia, Mallotus japonicas, Nigella sativa, Rubia cordifolia, Sargassum tortile, Wikstroemia indica*
Phenols and derivatives	*Acronychia laurifolia, Angelica gigas, A. decursiva, A. keiskei, Gossypium indicum*

Although many plants are used to treat tumours in Indian system of medicine, most of these plants are not scientifically validated. A systemic and intensive ethno pharmacological study is carried on one or more plants used in traditional system are sure to provide effective anticancer drugs. For the present study, the plant *Azima tetracantha* Lam., claimed to possess anticancer activity was selected for the research.

CHAPTER 2

LITERATURE REVIEW

The aim of the literature survey is to establish a complete knowledge, based upon various pharmacological approaches attempted in the plant. The survey also aimed to understand the pharmacognosy and phytochemistry for the selection of plant. Thus, a detailed literature survey was carried out based on pharmacognostical, phytochemical and pharmacological aspect.

2.1. BASED ON PHARMACOGNOSY

1. **Balakrishnan M *et al.,*** [42] **(2010)** reported the studies on pharmacognostical specifications of *Azima tetracacntha* Lam.

2.2. BASED ON PHYTOCHEMISTRY

2. **Vinoth B and Manivasagaperumal R,** [43] **(2015)** reported the phytochemical analysis of *Azima tetracantha* of various leaf extracts revealed that the presence of alkaloids, phenol, protein and flavonoids, glycosides, tannins, saponins, steroids and terpenoids.
3. **Gowthami M *et al*.,** [44] **(2012)** reported the separation of phytocompounds such as alkaloids, flavonoids and sterols from leaf extracts of *Azima tetracacntha.*
4. **Muthuswamy P *et al.,*** [45] **(2012)** performed the preliminary phytochemical screening and physicochemical constants of leaf extracts of *Azima tetracantha.*
5. **Gayathri G *et al.,*** [46] **(2012)** evaluated the bioactive compounds present in the methanolic leaf extracts of *Azima tetracantha* using Gas chromatography-Mass spectroscopy method.

6. **Bennett RN *et al.,*** [47] **(2004)** reported profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of *Azima tetracantha* Lam.(Salvadoraceae). They also found some dimeric piperidine alkaloids like azimine, azcarpine, and carpaine in all tissues of *A. tetracantha.*
7. **Abirami H *et al.,*** [48] **(2015)** identified 47 chemical constituents from the hexane extracts of *Azima tetracantha* by GC-MS analysis and concluded that, it is mainly composed of terpenoids and sterols.

2.3. BASED ON PHARMACOLOGY

8. **Vinoth B and Manivasagaperumal R,** [43] **(2015)** studied different extracts of *Azima tetracantha* root against human pathogenic bacterial and fungal strains using disc diffusion method.
9. **Vinoth B *et al*.,** [49] **(2014)** carried out *in-vitro* antimicrobial activity of *Azima tetracantha* leaves against various human bacterial and fungal pathogens using disc diffusion method.
10. **Gowthami M *et al*.,** [44] **(2012)** evaluated the antibacterial activity of phytocompound separation from alkaloids, flavonoids and sterol were tested against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*. Maximum zone of inhibition was observed in sterol compound when compared with alkaloids and flavonoids.
11. **Hema TA *et al*.,** [50] **(2012)** reported the antimicrobial effect of leaves of *Azima tetracantha* against the clinical pathogens by using agar well diffusion method.

12. **Gayathri G *et al.,*** [51] ***(*2011)** proved that the methanolic extract of *A.tetracantha* leaves showed greater antimicrobial activity than chloroform extract.
13. **Duraipandiyan V *et al.*,** [52] **(2010)** evaluated the antifungal activity of *Azima tetracantha* extracts and its isolated compound (friedelin) against fungi using micro dilution method.
14. **Maruthi TE *et al.,*** [53] **(2010)** evaluated the anthelmintic and antimicrobial activities of *Azima tetracantha*. The results revealed that, the alcoholic extract was found to possess antibacterial and antifungal activities while chloroform extract showed only antibacterial activity.
15. **Vinoth B *et al.,*** [54] **(2015)** reported the different extracts of *A. tetracantha* root for antioxidant potential using different *invitro* assays such as inhibition of DPPH, ABTS, hydroxyl radical and superxide anions. The total phenolic contents and ferric reducing antioxidant power of the extracts were also determined by using standard phytochemical reaction methods.
16. **Muthuswamy P *et al.,*** [45] **(2012)** performed the antioxidant study of methanol and ethyl acetate leaf extract of *Azima tetracantha* Lam. They also determined the inhibition of the LPO, DPPH, superoxide anion, hydroxyl radical.
17. **Gayathri G *et al.,*** [51] ***(*2011)** studied the antioxidant effect of leaf extracts of *Azima tetracantha* Lam. The report concluded that the leaves of *A. tetracantha* were proved to be good source of natural phenolic compounds.
18. **Hepsibha BT *et al.,*** [55] **(2010)** evaluated antioxidant and free radical scavenging activities of *Azima tetracantha* Lam. leaf extracts. The result suggested that the methanolic extract of *A. tetracantha* leaves showed better free radical capacity against different reactive oxygen /nitrogen species.

19. **Antonisamy P *et al.*,** [56] **(2011)** evaluated the anti-inflammatory, analgesic and anti-pyretic effects of friedelin. The effects of friedelin on inflammation were studied by using carrageenan-induced hind paw oedema, croton oil-induced ear oedema, acetic acid-induced vascular permeability, cotton pellet-induced granuloma and adjuvant-induced arthritis.
20. **Ismail TS *et al.,*** [57] **(1997)** investigated the anti-inflammatory activity of *Salacia oblonga* root bark powder and *Azima tetracantha* leaf powder in male albino rats using carrageenan-induced rat paw oedema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methods. The study concluded that both the crude drugs were optimally active at a dose of 1000 mg/kg.
21. **Konda VR *et al.,*** [58] **(2015)** evaluated the nephroprotective effect of root extract of *Azima tetracantha* in glycerol-induced acute renal failure in wistar albino rats. The study report suggested that there was a considerable improvement in biochemical parameters and histopathological changes when compared with glycerol treated group.
22. **Manikandaselvi S *et al.,*** [59] **(2012)** studied the significant elevation in the levels of urea, GGT and creatinine in ferrous sulphate induced group compared to control. After treatment with *Azima tetracantha* Lam., there was a significant decrease in the levels near to normal compared to ferrous sulphate induced group.
23. **Soumya A and Nagarajan V** [60] **(2014)** performed that the aqueous extracts of *Azima tetracantha* showed a significant reduction in all the biochemical parameters of liver damage glucose, protein, bilirubin, cholesterol, ALP, SOD, CAT, Vitamin E, TBARS, Albumin and globulin elevated by ferrous sulphate.

24. **Sunil C *et al.,*** [61] **(2013)** reported the antioxidant, free radical scavenging and liver protective effects of friedelin isolated from the leaves of *Azima tetracantha* Lam. They also concluded that, friedelin restored the levels of SGOT, SGPT, LDH, SOD, catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) and showed liver protection, compared to standard drug silymarin.

25. **Sambasivam M *et al.,*** [62] **(2013)** reported that the hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate-induced toxicity in rat revealed that the hydroalcoholic extract of leaf powder of *Azima tetracantha* and the fruit powder of *Tribulus terrestris* retrieved the liver parameters to usual level and possesses significant hepatoprotective activity.

26. **Balakrishnan M *et al*.,** [63] **(2012)** reported that the ethanolic extract (50%) of *Azima tetracantha* Lam. (EEAT) root bark afforded significant protection against carbon tetrachloride (CCl4) induced hepatocellular injury.

27. **Nargis BT *et al*.,** [64] **(2011)** revealed that, the rats treated with ethanolic extract of *A. tetracantha* showed a significant reduction in all the five-biochemical parameters of liver damage (AST, ALT, ALP, ACP and total bilirubin) elevated by carbon tetrachloride.

28. **Arthika S *et al*.,** [65] **(2011)** reported that the EEAT reduced the hepatotoxin intoxication induced elevated biochemical parameters and decrease the protein synthesis and accumulation of triglycerides leading to fatty liver.

29. **Maruthi TE *et al*.,** [66] **(2010)** provided the scientific evidence on the correlative effects of hepatoprotective activities of *Azima tetracantha* Lam. It indicates that the chloroform and ethanol extracts of *A. tetracantha* reduced the hepatotoxin

intoxication induced elevated biochemical parameters and decreased the protein synthesis.

30. **Antonisamy P *et al.,*** [67] **(2015)** reported the protective effects of friedelin isolated from *Azima tetracantha* Lam., against ethanol-induced gastric ulcer in rats. The result from the study showed that the friedelin isolated from the hexane extract of leaves of *Azima tetracantha* protected from ethanol caused severe gastric damage.

31. **Muthusamy P *et al.,*** [68] **(2009)** performed that, EEAT showed notable dose-dependent ulcer protective effect against cold restraint stress and aspirin plus pylorus ligation induced gastric ulcers.

32. **Nargis BT *et al.,*** [69] **(2009)** studied the anticancer activity of *Azima tetracantha* leaf extract on Ehrlich Ascites Carcinoma (EAC) in mice. Ethanol extract of *Azima tetracantha* (EEAT) was evaluated the parameter such as mean survival time, tumor volume, viable tumor cells count and non-viable tumor cells count. They concluded that, the oral administration of EEAT increased the survival time, and also reduce the solid tumor volume, viable tumor cells count and increased the non-viable tumor cells count.

33. **Begum HV *et al.,*** [70] **(2013)** reported that, the aqueous crude extract of the leaves of *Azima tetracantha* for its antidiarrhoeal activity using castor oil-induced diarrhoea and castor oil-induced enteropooling in rats.

34. **Nargis BT *et al.,*** [71] **(2009**) investigated that, the ethanolic leaf extract of *Azima tetracantha* Lam., for its hypoglycemic and hypolipidemic activity in alloxan-induced diabetic albino rats. They also concluded that, the reduction in the plasma glucose and in the lipid profile was slightly less than that achieved with the standard drug glibenclamide.

CHAPTER 3

RATIONALE FOR SELECTION

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors, which play a direct and/ or indirect role in the induction and deterioration of cancers.

Diet with high consumption of anti-oxidant rich fruits and vegetables significantly reduces the risk of many cancer diseases suggesting that confident anti-oxidants could be effective agents for the inhibition of cancer spread.

These agents present in the diet are a group of compounds with low toxicity, safe and generally accepted.

Recent researches revolve round the urgency to evolve suitable chemotherapy consistent with new discovery in cell biology for the treatment of cancer with no toxic effects.

Presently various therapies are available for the treatment of cancer such as chemotherapy, radiotherapy etc. besides these expensive therapies, phytotherapy play a significant role for the treatment of cancer.

Since medieval times, plants have been the source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain even in the 21st century an integral part of the health care in different countries, especially in the developing ones.

In the late 90's, the WHO stated that a big percentage of the world's population depends on plant based therapies to cover the needs of the primary health care (WHO 1999).

Moreover, towards the end of the 20^{th} century, plant based products, nutraceuticals and food supplements comprising the complementary and alternative therapies have gained a big share in the drug market of developing countries.

Considering the vast potentiality of plants as a source of traditional medication a systematic investigation was carried out to evaluate their anticancer properties.

Therefore, efforts are still being made for the search of effective naturally occurring anti-carcinogen that would prevent, slow or reverse cancer development.

Plants have a special place in the treatment of cancer.

So, this was the inspiration to find an herb having anticancer activity which has been claimed scientifically on extract and the present research work is to isolate the bioactive compound which is responsible for anticancer activity.

CHAPTER 4

AIM AND OBJECTIVE

4.1. AIM

The project directly aims to isolate bioactive compounds from *Azima tetracantha* Lam., which will have a curative effect on cancer disease.

4.2. OBJECTIVE

- To standardize the leaves and stem of *Azima tetracantha* Lam., by carrying out the pharmacognostical and phytochemical studies.
- To prepare various extracts (Ethanol and Hexane extracts of *Azima tetracantha* Lam. leaf and stem).
- To evaluate the *invitro* anticancer activity of various extracts to identify the active extract using MTT assay.
- To isolate the bioactive compounds from the active extract by Bioactivity guided isolation using Column chromatography.
- To evaluate the *invitro* antioxidant activity (DPPH assay) of all the fractions and isolated bioactive compounds eluted from the column to identify the active compounds.
- Structural characterization of isolated bioactive compounds by UV, IR, NMR and Mass spectroscopy.
- To evaluate the *invivo* acute toxicity of all isolated bioactive compounds.
- To evaluate *in-vitro* anticancer activity of isolated bioactive compound by MTT assay.
- Docking studies of isolated bioactive compound with various targeting enzymes (PDB-ID 4O33, 2Y3I, 3UEN, 3ZOZ).

CHAPTER 5

PLAN OF WORK

5.1. PHARMACOGNOSTICAL STUDIES

- Collection and identification of the plant material
- Organoleptic characters
- Macroscopic Study
- Microscopic study
  - Powder microscopy
  - Quantitative Microscopy
    - ✓ Determination of Stomatal Number and Stomatal Index
    - ✓ Determination of Vein islet Number and Veinlet termination Number
- Physicochemical Studies
  - Determination of Foreign organic matter
  - Determination of Loss on Drying
  - Ash value
    - ✓ Determination of Total ash
    - ✓ Determination of Acid insoluble ash
    - ✓ Determination of water soluble ash
    - ✓ Determination of Sulphated ash

- Extractive value
  - ✓ Determination of water soluble extractive value
  - ✓ Determination of Ethanol soluble extractive value
  - ✓ Determination of Ether soluble extractive value
- Determination of volatile oil content
- Determination of Swelling Index
- Determination of Foaming Index

➢ Inorganic metal analysis

- Qualitative analysis of Inorganic elements

5.2. PHYTOCHEMICAL STUDIES

➢ Extraction of Plant material

➢ Qualitative phytochemical screening

➢ Fluorescence analysis

➢ Thin layer chromatography

➢ **Selection of Active Extract**

- *Invitro* anticancer activity (MTT Assay)

➢ **Bioactivity Guided Isolation**

- Column Chromatography
- Thin layer chromatography

- **Selection of Active Fraction**
  - *Invitro* antioxidant activity (DPPH assay)
- Spectral studies
  - Ultraviolet Spectroscopy
  - Infrared Spectroscopy
  - Nuclear Magnetic Resonance Spectroscopy
  - Mass Spectroscopy

5.3. PHARMACOLOGICAL STUDIES

- Acute toxicity studies

  By Acute toxic class method (OECD Guidelines 402)
- *Invitro* anticancer activity of bioactive compound

5.4. DOCKING STUDIES

Docking with various anticancer drug targeting enzymes

- PDB-ID 4O33
- PDB-ID 2Y3I
- PDB-ID 3UEN
- PDB-ID 3ZOZ

CHAPTER 6

PLANT PROFILE

Azima tetracantha Lam.

6.1. TAXONOMICAL STUDIES

Family[72] **:** Salvadoraceae

Synonyms[72,73]**:** *Monetia barlerioides* L'Herit.

Azima nova J. F. Gmel.

Kandena spinosa Rafin.

Monetia angustifolia Boj. Ex A. DC.

Monetia tetracantha (Lam.) Salisb.

Scientific classification[73,74]

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Capparales

Family : Salvadoraceae

Genus : Azima

Species : *Azima tetracantha* lam.

Vernacular names[73, 75]

Sanskrit : Kundali

Hindi : Kanta- gur-kamai

Malayalam : Essanku, Sankukuppi, yeshenku

English : Bee sting bush, Fire thorn, Needle bush, Mistletoe

Tamil : Sung-ilai

6.2. DESCRIPTION[76]

Azima tetracantha Lam**.,** belongs to Salvodoraceae and known as Kundali in Ayurvedic medicine. *Azima tetracantha* is a perennial shrub growing upto 3m in hot, dry riverine scrub, particularly on alluvial or saline soil. The plant is dioecious, erect shrub with (1–)2 spines 0.5–5 cm long in each leaf axil, sometimes scandent with stems up to 8 m long; branchlets are terete or quadrangular, glabrous to densely hairy. The leaves of the plant are elliptical in shape and are rigid, pale green colored. The flowers are small, greenish white (or) yellow colored, unisexual in axillary fasciles. Fruits are globular, white shiny. Seeds are compressed, circular. It occurs naturally in central, eastern and southern Africa as well as in the Indian Ocean Islands, and extends through Arabia to tropical Asia.

Fig 5: Plant- *Azima tetracantha* Lam.

Chemical Constituents[47]

Table 3: The chemical constituents present in *Azima tetracantha.*

Class	Chemical Constituents
Glucosinolates and glucosinolate derived compounds	3- indolylmethylglucosinolate N- hydroxyl- 3- indolylmethyl-glucosinolate N- methoxy- 3- indolylmethyl-glucosinolate Neoascorbigen
Dimeric piperidine alkaloids	Azimine Azcarpaine Carpaine

Medicinal importance of *A. tetracantha*[72]

Azima tetracantha is a potent diuretic[76] to treat rheumatism, dropsy, dyspepsia, chronic diarrhorea; it is used as stimulant tonic after child birth. *A. tetracantha* is used to treat cough, phthisis, asthma, small pox and diarrhorea. Rheumatism has been cured by its leaves, root and root bark.[77]

Traditional Uses

Traditionally, root used as diuretic. In Siddha, root is used in the treatment for dropsy and rheumatism. It is also used as analgesic[78] and anti-inflammatory.[57]

Leaves are used as stimulant, expectorant and antispasmodic. It is also used in cough and asthma. Bark is used as antiperiodic, astringent and expectorant.

In western India, juice of the leaves is applied as ear drops against earache and crushed leaves are placed on painful teeth.

In India and Sri Lanka the root, root bark and leaves were administered with food as a remedy for rheumatism, dropsy, dyspepsia, chronic diarrhea and is considered as stimulant tonic and given to pubertal women immediately after confinement.[79,80]

Locally, the traditional healers from Tirunelveli district of Tamilnadu use the root bark (paste with buttermilk) as potent remedy for jaundice.[80]

CHAPTER 7

PHARMACOGNOSTICAL STUDIES

The study of plant drugs from the pharmacognostical stand point would include the study of the habitat, general characters of the plant from which the drug is derived, its place in the botanical system, the organ or the organs of the plant used, their gross, minute structures in the whole and in the powdered conditions and the chemistry of the constituents especially of those which may be used in therapeutics.

The macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials. This should be carried out before any tests are undertaken. Lack of proper standards of medicinal plants may result in the usage of improper drugs which in turn will cause damage not only to the individual using it, but also to respect gained by the well-known ancient system of medicine and the entire work on the plant becomes invalid. Thus, in recent years there has been an emphasis in pharmacognostical standardization of medicinal plants of therapeutic potential. So, the present study is undertaken to standardize *Azima tetracantha* Lam., pharmacognostically which will help in the correct identification of the drug.

7.1. MATERIALS AND METHODS

Collection and identification of Plant materials

The plant materials (leaves and stem) of *Azima tetracantha* Lam., were collected from Vellore district, Tamilnadu, India. The collected plant materials were botanically identified and authenticated by the Dr. G.V.S Murthy, Scientist 'F', Botanical Survey of India, under reference number **BSI/SRC/5/23/2016/Tech./178.**

The herbarium specimen was prepared and deposited at Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for future reference. The shade dried leaves and stem were coarsely powdered and used for further studies.

Organoleptic characters[81]

Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of particular drug. Organoleptic characters such as shape, size, colour, odour, taste and fracture of stem bark, leaf structure like margin, apex, base surface, venation, and inflorescence etc were evaluated.

MACROSCOPIC STUDY[82-84]

The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens.

MICROSCOPIC STUDY[85-92]

The microscopic study is the anatomical study which is done by taking appropriate section of the plant parts.

Staining method

Fixation of Plant organ- Different organ samples were cut and fixed in FAA solution (Formalin- 5ml, Acetic acid- 5ml, 70% Ethanol- 90ml)

Dehydration of specimen- After 24 hours of fixing, the plant parts like leaves, stem and thorns were graded with series of tert- butyl alcohol, as per the standard method.

Infiltration of specimen- It was carried out by gradual addition of 58-60°C melting pointed paraffin wax until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections was done by customary procedures. Since toluidine blue is a polychromatic stain, the sections were stained as per the method published by O'Brein *et al*., (1964) the staining results were remarkably good.

The dye rendered pink colour to the cellulose walls, blue to the lignifies cells, dark green to suberin, violet to mucilage, blue to protein bodies etc.

Photomicrographs

Microscopic description of tissues was supplemented with micrographs wherever necessary.

- Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used.
- For the study of crystals and lignified cells, polarized light was used. Since, these structures have bifringent property under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

Powder microscopy[83-86]

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features using various staining reagents.

Powder was stained with 1% phloroglucinol in 90% ethanol, conc. hydrochloric acid and glycerin further observed under microscope. All the lignified

cells stained pink colour. Calcium oxalate crystals were observed under polarized light microscope.

Quantitative Microscopy[83]

Quantitative microscopic parameters such as stomatal number, stomatal index, vein islet number and veinlet termination number were studied in the present study.

Determination of Stomatal Number and Stomatal Index

Stoma (plural- stomata) is a minute epidermal opening covered by two kidney shaped guard cells in dicot leaves. These guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomata perform the functions of gaseous exchange and transpiration in plants. The nature of the stomata, as well as, the stomatal index and stomatal number are important diagnostic characteristics of dicot leaves.

Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf. The actual number of stomata per sq mm may vary for the leaves of the same plant grown in different environment or under different climatic conditions. It is, however, shown that the ratio of the number of stomata to the total number of epidermal cells in a given area of epidermis is fairly constant for any age of the plant and under different climatic conditions.

Stomatal index is the percentage which the number of stomata form by the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following equation.

$$S.I = \frac{S}{E+S} \times 100$$

Where S.I = Stomatal Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Procedure

- Clear the fragments of the leaf from the middle lamina by boiling with 70% alcohol and 5% sodium hydroxide, subsequently washed with water. Peel out upper and lower epidermis separately.
- Prepare the mounts of low and upper epidermis separately in glycerin water.
- Draw a square of known dimensions by means of a stage micrometer and camera lucida on a drawing paper.
- Replace the stage micrometer by the cleared leaf preparation, focus under the same magnification and trace the epidermal cells and stomata by looking through the microscope when a superimposed image of the leaf is seen at the same time.
- The number of epidermal cells and stomata within the square was counted.

Determination of Vein islet Number and Veinlet termination Number

The mesophyll of the leaf in dicot leaves is divided into small portions by branching of the veins throughout the tissues. The small areas of the green tissue outlined by the veinlets are termed as vein-islets. *Vein-islet number* is defined as the number of vein-islets per sq mm of the leaf surface midway between midrib and the margin. It is a constant for a given species of the plant and used as a characteristic for the identification of the allied species. *Veinlet termination number* is defined as the number of veinlet termination per sq mm of the leaf surface midway between midrib and the margin. A vein termination is the ultimate free termination of veinlet.

Procedure

- Boil a few leaves in chloral hydrate solution and placed in a boiling water-bath.
- Mount the preparation in glycerin water.

- Set up the camera lucida and divide the paper into squares of 1 sq mm by means of stage micrometer.
- Replace the stage micrometer by the cleared leaf preparation and trace the veins in four continuous squares.
- Trace the vein-islets and veinlet termination by looking through the microscope when a superimposed image of the leaf and paper is seen at the same time.
- The number of epidermal cells and stomata within the square was counted.

PHYSICOCHEMICAL STUDIES[93-99]

The powdered plant materials were morphologically and organoleptically screened and subjected to physicochemical analysis in accordance with standard guidelines.

Determination of Foreign organic matter

Medicinal plant materials should be entirely free from visible sign of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioriation should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign matter or residue.

Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

Procedure

100g of the plant sample to be examined was weighed and spread out in a thin layer. The foreign matter was detected by inspection with the unaided eye, separated, weighed and calculated the percentages of foreign matters present.

Determination of Loss on Drying

Loss on drying is the loss in weight in %w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. If the sample is in the form of large crystals, reduce the size by rapidly crushing to a powder.

Procedure

About 5g of the powdered drug was weighed accurately in a tarred petridish which was previously dried at 105°C in hot air oven and weighed. The drug was distributed as evenly as practicable in the petridish and dried at 105°C to constant weight. Calculate the percentage loss in weight with reference to the air-dried powdered drug.

Ash value

Ash values are used to determine the quality and purity of a crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The residue remaining after incineration is called ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it, in the form of adulteration. Hence, an ash value determination furnished the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration or contamination with inorganic matter.

Determination of Total ash

Place about 2g of the ground air-dried material, accurately weighed in a previously ignited and tared crucible. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. Calculate the content of total ash in mg per g of air-dried material.

Determination of Acid insoluble ash

The acid insoluble ash consist mainly silica and indicate contamination with earthy material. To the crucible containing the total ash, add 25 ml of 2M hydrochloric acid for 15 minutes. The insoluble matter was collected in an ashless filter paper, washed with hot water, ignited and cooled in a dessicator and weighed. Calculate the content of acid insoluble ash in mg per g of air-dried material.

Determination of water soluble ash

The water soluble ash is used to estimate the amount of inorganic compound present in drugs. To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in an ashless filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg per g of air-dried material.

Determination of Sulphated ash

About 3g of accurately weighed air dried powdered drug was taken in a silica crucible, which was previously ignited and weighed. Then ignite gently until the drug was thoroughly charred. The crucible was cooled and the residue was moistened with 1 ml of concentrated sulphuric acid, heat gently until the white fumes were no longer evolved and ignited at 800±25°C until all the black particles has disappeared. The crucible was allowed to cool, add few drops of sulphuric acid and heated. The ignition was carried out as before, allowed to cool and weighed to a constant weight (difference is not exceeding 0.5g between two consecutive reading). Calculate the percentage of sulphated ash with reference to the air-dried drug.

Extractive value

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

Determination of water soluble extractive value

5g of macerated and air dried coarse powder of sample was mixed with 100 ml of chloroform water and kept in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105C and weighed. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Determination of Ethanol soluble extractive value

5g of macerated and air dried coarse powder of drug was mixed with 100 ml of 95% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105C and weighed. Calculate the percentage of ethanol soluble extractive with reference to the air-dried drug.

Determination of Ether soluble extractive value

5g of macerated and air dried coarse powder of sample was mixed with 100 ml of ether and kept in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a

tared flat-bottomed shallow dish, dried at 105C and weighed. Calculate the percentage of ether soluble extractive with reference to the air-dried drug.

Determination of volatile oil content

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. Chemically, they are usually composed of mixtures of, for example, monoterpenes, sesquiterpenes and their oxygenated derivatives. Aromatic compounds predominate in certain volatile oils.

Determination of Swelling Index

Many herbal materials are of specific therapeutic or pharmaceutical utility because of their swelling properties-especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose.

The *swelling index* is the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual herbal material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

The mixing of whole herbal material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

Procedure

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the herbal material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16 mm, the

length of the graduated portion about 125 mm, marked in 0.2- ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the herbal material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of herbal material.

Determination of Foaming Index

Many herbal materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index.

Procedure

Reduce about 1 g of the herbal material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume. Pour the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows:

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.

If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (a) is used to determine the index. If this tube is the

first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

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

$$\frac{1000}{a}$$

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

INORGANIC METAL ANALYSIS

Plant minerals play a vital role in metabolism and osmolality. Presence of elements vary with the soil, climatic conditions etc. There are essential and non essential elements which may be beneficial or harmful to living things. Non-essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium, silver bring about toxic effects resulting in intoxication. Hence, qualitative and quantitative estimation of inorganic elements in the leaves and stem of *Azima tetracantha* Lam., were carried out

Qualitative analysis of Inorganic elements[81]

To the ash of powder material, 50% v/v HCl was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium

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

Arsenic

Arsenous salts in neutral solution react with solution of copper sulphate to form green precipitate (Scheele's green) which on boiling ggives a red precipitate of cupric oxide.

Borate

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

Calcium

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

Carbonates

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

Chlorides

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

Copper

An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron

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

Lead

Strong solution of lead salts, when treated with HCl, yield a white precipitate which is soluble in boiling water and is deposited as crystals when the solution is cooled.

Magnesium

Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

Mercury

Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

Nitrate

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

Phosphates

Solution of phosphate when treated with silver nitrate, dilute ammonia solution and in dilute nitric acid yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.

Potassium

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

Silver

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

Sulphates

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

Quantitative estimation of Inorganic elements[100,101]

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

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

Quantitative analysis of Heavy metals

Instrumentation parameters:

Instrument name: Inductive coupled plasma-Optical emission spectroscopy

Instrument Model: PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial **Detector system**: Charge coupled detector, (UV-Visible detector which is maintaining at -40°C) to detect the intensity of the emission line.

Light source (Torch): Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror

located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow gem tip**.**

Preparation of sample by acid digestion method

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water. Blank solution was prepared as above without sample.

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

Detection

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

7.2. RESULTS

Organoleptic characters

Colour - Dark green to pale green

Odour - Characteristic odour

Taste - Tasteless

MACROSCOPIC STUDY

Azima tetracantha is a perennial shrub growing upto 3m in hot, dry riverine scrub, particularly on alluvial or saline soil. The plant is dioecious, erect shrub with (1–)2 spines 0.5–5 cm long in each leaf axil, sometimes scandent with stems up to 8 m long; branchlets are terete or quadrangular, glabrous to densely hairy. The leaves of the plant are elliptical in shape and are rigid, pale green colored. The flowers are small, greenish white (or) yellow colored, unisexual in axillary fasciles. Fruits are globular, white shiny. Seeds are compressed, circular.

A) Leaf

B) Stem with thorns

C) Flower

D) **Fruits**

E) Length of leaf D) Breadth of Leaf

Fig 6: Macroscopy of *Azima tetracantha* Lam.

Table 4: Macroscopic evaluation of *Azima tetracantha* Lam.

S. No	Particulars	Observations
1.	Apex	Mucronate, sharp-tipped
2.	Base	Acute base
3.	Margin	Simple and entire
4.	Shape	Aristate with a spine on tip
5.	Vein	Cross venulate
6.	Stipules	Absent or rudimentary
7.	Leaflets	Pinnate

MICROSCOPICAL STUDY

Leaf

The transverse section of leaf shown dorsiventral nature. Some of the important features were follows,

Midrib: The midrib is flat on the adaxial side and hemispherical on the abaxial side.

Vascular bundle: Single, top shaped and consists of a few parallel, short, radial multiples of vessels and an abaxial arc shaped phloem.

Lamina: 230 μm thick. Both adaxial and abaxial sides are smooth and even.

Epidermal tissues: The epidermal tissue as seen in paradermal section.

Stomata: Anisocytic with four unequal subsidiary cells.

Stem

Epidermis: Thin and the epidermal cells are squarish or rectangular, coated with thick cuticle.

Vascular bundles: Radially elongated; a thick, semicircular mass of sclerenchymatous bundle.

Pith: Wide, homogenous and parenchymatous, thick walled, vary in size.

Thorns

Epidermis: Vascular bundle is surrounded by a compact layer of thin walled parenchymatus cells.

Pigments: Abundantly present

Airsac: Air space present at the centre.

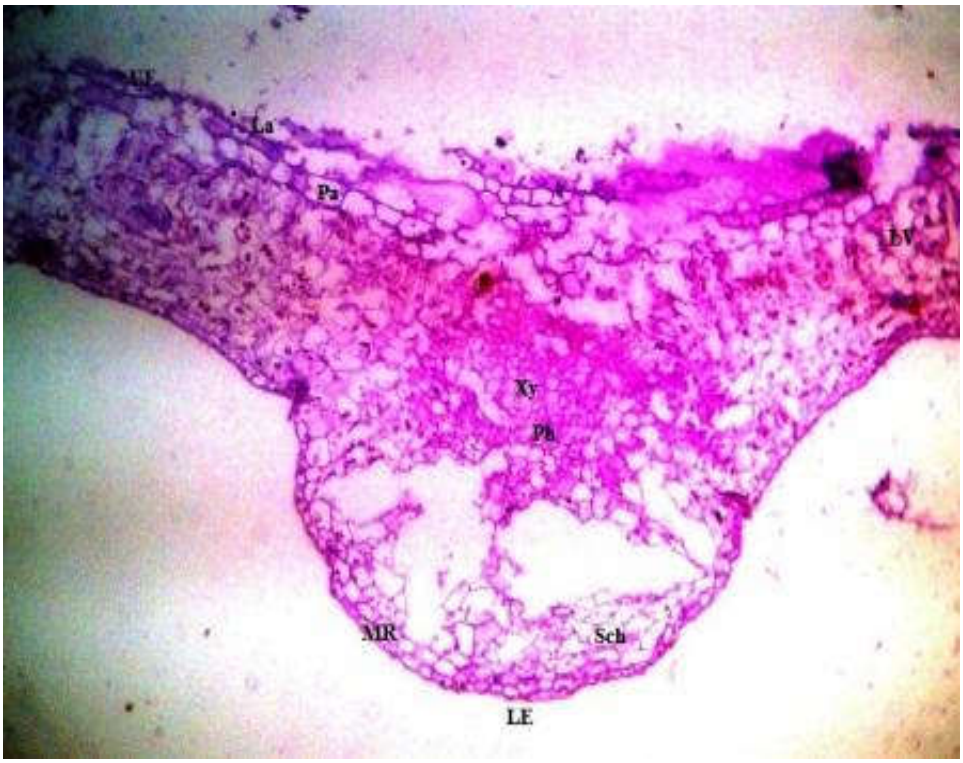


Fig 7: Transverse section of *Azima tetracantha* Leaf

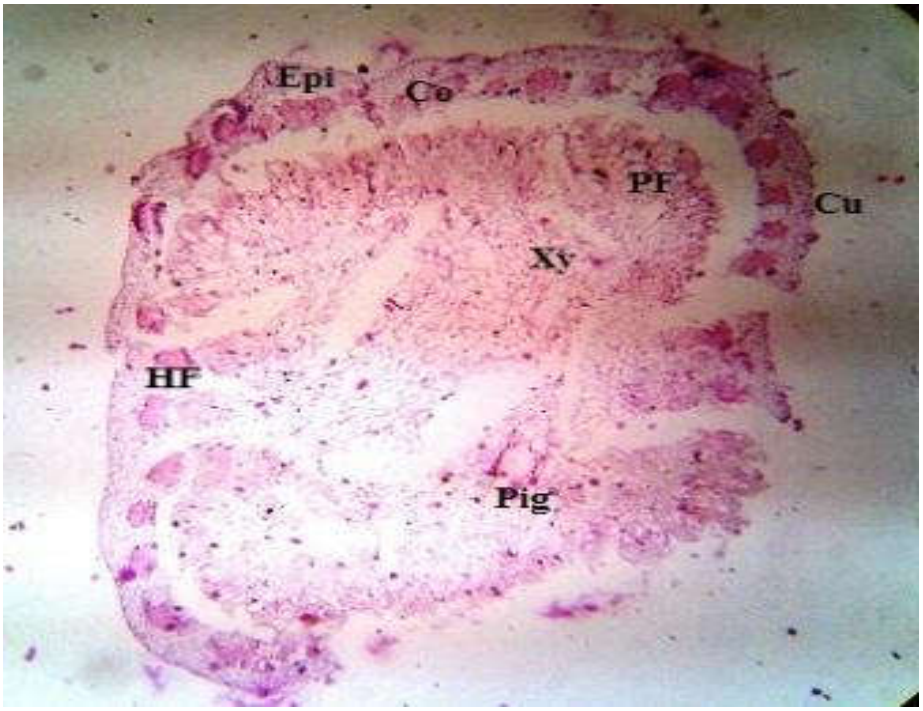


Fig 8: Transverse section of *Azima tetracantha* Stem

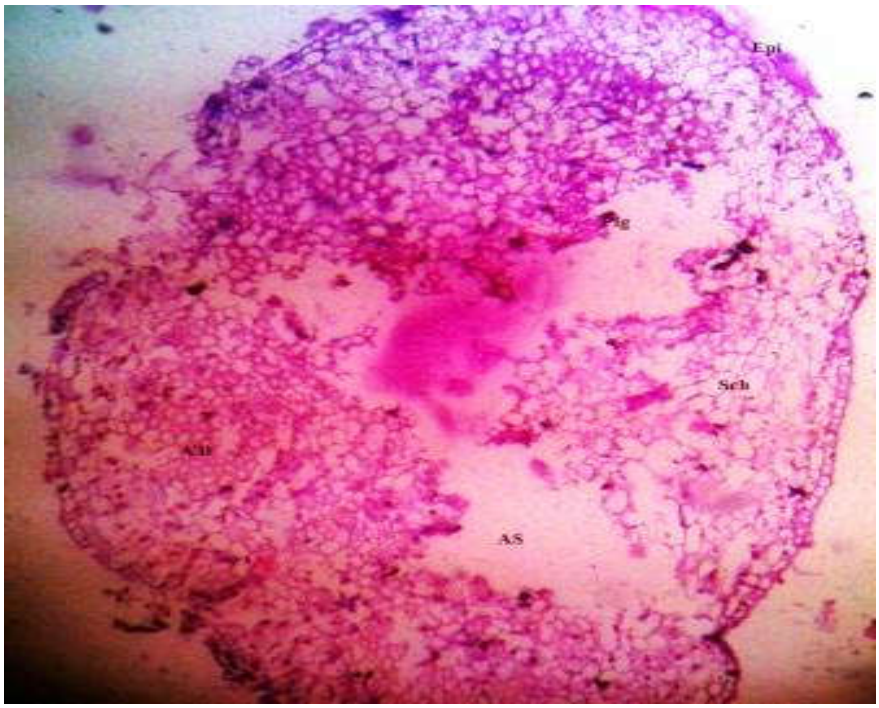
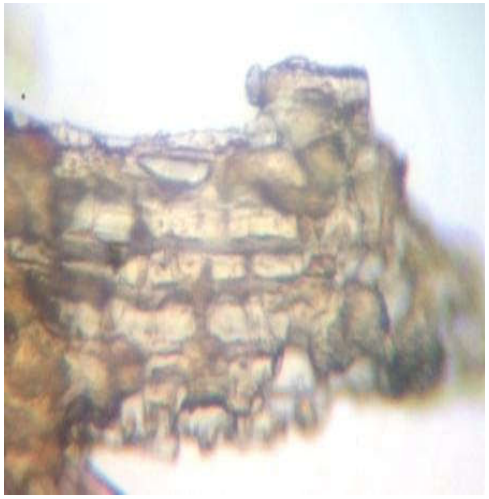


Fig 9: Transverse section of *Azima tetracantha* Thorn

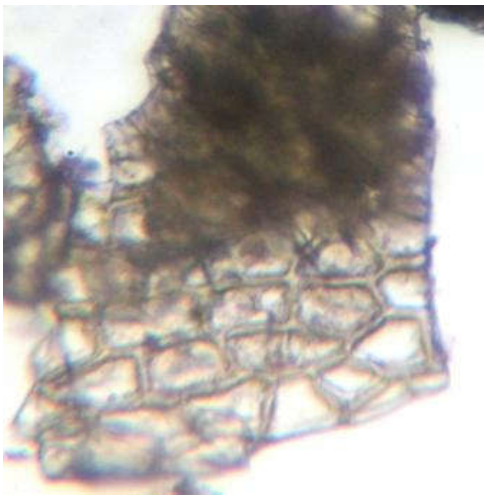
UE- Upper epidermis, La- Lamina, Pa- Palisade cells, Xy- Xylem, Ph- Phloem, MR- Midrib, LE- Lower epidermis, Sch- Sclerenchyma, LV- Lateral vein, AS- Airsac, VB- Vascular bundle, Pig- Pigments, HF- Hypodermal fibres, PF- Pericyclic fibres, Cu- Cuticle, Co- Cortex

Powder microscopy

The leaf powder showed the presence of parenchyma, sclerenchyma, trichomes, fibres, anisocytic stomata, crystal sheath of calcium oxalate and xylem vessels. The stem powder showed the presence of epidermis, fibres, pigments and xylem vessels. The powder characteristics of leaf and stem were shown in the **Figure 10** and **Figure 11** Respectively



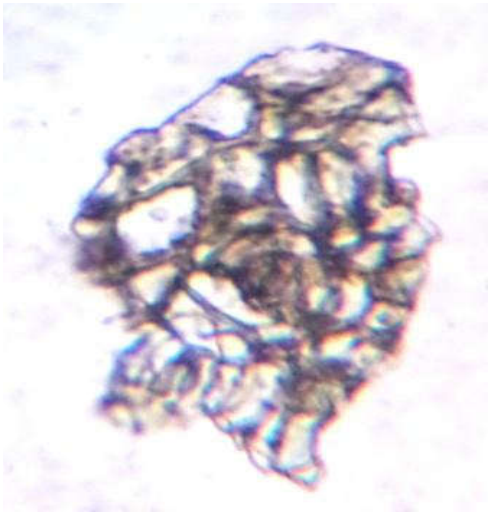
A) Parenchyma



B) Sclerenchyma

C) Trichomes

D) Fibres



E) Stomata

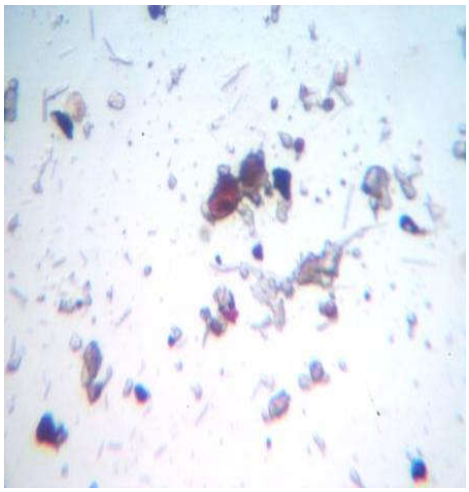
F) Crystal sheath

G) Xylem vessels

Fig 10: Powder microscopy of *Azima tetracantha* Leaf powder

A) Epidermis

B) Fibres



C) Pigments

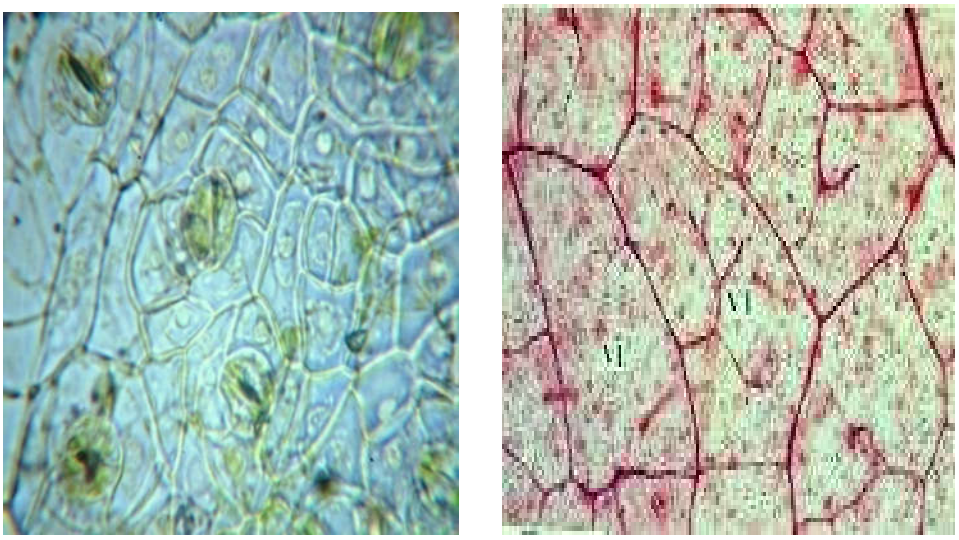
D) Xylem vessels

Fig 11: Powder microscopy of *Azima tetracantha* Stem powder

Quantitative microscopy

Table 5: Quantitative microscopy of *Azima tetracantha* Leaf

S. No	Parameters	Values
1.	Stomatal number- Lower epidermis	23-27
2.	Stomatal index- Lowder epidermis	19-20
3.	Vein islet number- Lowder epidermis	9-15
4.	Veinlet termination number- Lowder epidermis	15-19



A **B**

Fig 12: Quantitative microscopy of *Azima tetracantha* Leaf

A) Stomata and B) vein-islets

PHYSICOCHEMICAL STUDIES

Table 6: Physicochemical analysis of the leaves and stem of *Azima tetracantha*

S. No	Parameters	Leaves	Stem
1.	Total ash	7.45%	21.039%
2.	Acid insoluble ash	1.06%	0.493%
3.	Water soluble ash	6.98%	13.985%
4.	Sulphated ash	15.35%	18.84%
5.	Loss on drying	15.27%	11.95%
6.	Water soluble extractive value	1.593%	1.394%
7.	Ethanol soluble extractive value	1.197%	0.399%
8.	Foreign organic matter	3.6%	No
9.	Volatile oil content	1.5%v/w	No
10.	Swelling Index	No	No
11.	Foaming Index	<100	<100

n = 3 times

INORGANIC METAL ANALYSIS

Table 7: Qualitative estimation of Inorganic elements in *Azima tetracantha* Lam., Leaf and stem

S. No	Inorganic elements	Observation	
		Leaves	Stem
1.	Aluminium	+	+
2.	Arsenic	+	+
3.	Borate	-	-
4.	Calcium	+	+
5.	Carbonate	-	-
6.	Chlorides	-	-
7.	Copper	+	+
8.	Iron	+	+
9.	Lead	+	+
10.	Magnesium	+	+
11.	Mercury	-	-
12.	Phosphate	-	-
13.	Potassium	+	+
14.	Silver	+	-
15.	Sulphate	+	+

(+)- Present, (-)- Absent

Table 8: Quantitative estimation of inorganic elements of *Azima tetracantha*

S.No	Inorganic Elements	Total Amount (%W/W)	
		Leaves	**Stem**
1	Aluminium	0.028	0.012
2	Chloride	0.052	0.037
3	Copper	0.009	0.011
4	Calcium	0.010	0.006
5	Sulphate	0.028	0.023
6	Borate	0.005	0.002
7	Potassium	0.020	0.0017
8	Silver	0.008	0.003

Quantitative estimation of Heavy metals by ICP OES method

The quantification of the individual heavy metals was analyzed for the powdered mixture of *Azima tetracantha* Lam., by ICP-OES technique the following metals like arsenic, lead, cadmium were detected and quantified, results are given in the following **Table 9.**

Table 9: Quantitative estimation of heavy metals in *Azima tetracantha* Lam., Leaf and stem

S. No	Metals	Results		Specification
		Leaves	Stem	
1.	Mercury	ND	ND	Not more than 0.5ppm
2.	Arsenic	0.084	0.013	Not more than 5.0ppm
3.	Lead	0.046	0.009	Not more than 10 ppm
4.	Cadmium	0.031	0.011	Not more than 0.3ppm

7.3. DISCUSSION

Pharmacognostical standardization was carried out on the basis of detailed botanical evaluation of the leaves and stem which includes morphology and microscopy as well as WHO recommended physico-chemical studies. The results of the standardization may throw an immense light on the botanical identity of the leaves of *Azima tetracantha* Lam., which may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its adulterants and other species.

The macroscopic characters were examined to identify the right crude drug.

Microscopical Features

Transverse section of the leaves showed a fairly prominent midrib, lateral veins and dorsiventral nature. The lamina has smooth even surface with two layers of palisade cells and anisocytic stomata with four unequal subsidiary cells.

Transverse section of the stem showed epidermis with thick cuticle, vascular bundle and homogenous and parenchymatous, thick walled pith.

Powder Characters

The powder characters of a drug are mainly used in the identification of the drug in the powder form. The leaf powder is pale green in colour with characteristic odour. On micrsocopical examination the powder showed presence of parenchyma, sclerenchyma, trichomes, fibres, anisocytic stomata, crystal sheath of calcium oxalate and xylem vessels. The stem powder showed the presence of epidermis, fibres, pigments and xylem vessels.

Quantitative Microscopy

Quantitative microscopic data has been highly relied upon pioneer pharmacognosists and are found to be constant for a species. These values are

especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant.

Physico-chemical Constants

The physico-chemical parameters are mainly used in judging the purity and quality of the drug. An ash value of a drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug.

The ash values are important since ash may be derived from the plant itself (physiological or natural ash) as well as from the extraneous matter, especially sand and soil adhering to the surface of the drug (non physiological ash). The determination of physiological and non physiological ash together is called as total ash. The total ash may vary within wide limits for specimen of genuine drug due to variable natural or physiological ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid- insoluble ash which represents most of the ash from the contaminating soil. The ash values of the powdered leaves revealed a high percentage of sulphated ash. Any significant deviation in the percentage of ash reported in this work may indicate adulteration of the drug.

Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs. The result suggested that the powdered leaves have high water soluble extractive value.

The loss on drying reveals the percentage of moisture present in the drug, since moisture facilitates the enzyme hydrolysis or growth of microbes which leads to deterioration. The swelling and foaming index was also studied.

Inorganic Mineral Analysis

The presence of inorganic metals like aluminium, arsenic, calcium, carbonate, chloride, copper, iron, lead, magnesium, mercury, phosphate and sulphate in the leaf and stem powder were analyzed. It showed the presence of elements such as Al, Ar, Ca, Co, Fe, Pb, Mg, K, and SO_4. The heavy metals are also present within the limits.

This detailed pharmacognostical studies on the leaves of *Azima tetracantha* Lam., may substantiate as an essential data for the identification of raw material and also used to differentiate the plant from its allied species and adulterants.

CHAPTER 8

PHYTOCHEMICAL STUDIES

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants.[102] Techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation of natural products as well as various chromatographic techniques. It is used for the discovery of new drugs. Phytochemical studies are used to find out the phytochemical constituents in the plants.

Phytochemistry deals with the study of secondary metabolites synthesized in plants. It deals with the enormous variety of organic substances embedded in it. The phytochemistry also deals with chemical structure and its biological function. The beneficial therapeutic outcome of plant is based on its secondary metabolites.

8.1. MATERIALS AND METHODS

8.1.1. EXTRACTION OF PLANT MATERIAL

Freshly harvested leaves and stem were air-dried at room temperature for a period for 2 weeks. Dried materials were coarsely grounded and stored in an air- tight container. About 200g of dried powdered leaves and stem were packed well in **soxhlet apparatus** and was subjected for hot extraction with hexane and ethanol (99.9%) at 18 hrs. The extract was filtered while hot and concentrated under Rotary vacuum evaporator in order to remove the solvent completely, dried and kept in a desiccator till further use.

Determination of Extract yield

The percentage yield of the leaves and stem of *Azima tetracantha* was determined by weighing the pulverized dried leaves and stem before extraction and the concentrated extracts obtained after extraction and then calculated using the formula.

$$\text{Percentage Yield} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of crude powder taken (g)}} \times 100$$

Qualitative phytochemical analysis[103-108]

The extracts were tested for the presence of phytoconstituents using following standard methods.

Test for Saponins

To 0.5g of plant extracts, distilled water was added and heated for few minutes. Foam formation indicated the presence of saponins.

Test for Tannins

To 0.5g of plant extracts, 10ml of distilled water was added and filtered. To the filtrate 0.1% of ferric chloride solution was added. Formation of brownish green indicated the presence of tannins.

Test for Steroids

To 0.5g of plant extracts, 2ml of acetic anhydride and 2ml of sulphuric acid was added. Formation of violet-blue colour indicated the presence of steroids.

Test for Flavonoids

To 0.5g of plant extracts, a few drops of acetone was added and heated in a water bath until the acetone got evaporated and then filtered. The filtrate was cooled and 5ml sodium hydroxide was added. Presence of yellow colour indicated the presence of flavonoids.

Test for Alkaloids

To 0.5g of plant extracts, 3ml of methanol was added with 10% acetic acid and ammonium hydroxide was added. Formation of precipitate indicated the presence of alkaloids.

Test for Phenols

To 0.5g of plant extracts, distilled water was added and heated, to that 2ml of ferric chloride was added. Blue / green colour formation indicated the presence of phenol.

Test for Glycosides

To 0.5g of plant extracts, 1ml of glacial acetic acid was added, and then ferric chloride and 1ml of sulphuric acid was added. Reddish brown colour appeared at the junction of two layers and the upper layer turned bluish green which indicated the presence of glycosides.

Test for Carbohydrates

300mg of 50% alcoholic extracts was dissolved in water and filtered. The filtrate was treated with concentrated sulphuric acid and then with Molisch's regent. Appearance of pink or violet colour indicated the presence of carbohydrates. The filterate was boiled with Fehling's and Benedict solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non- reducing sugars respectively.

Test for Triterpenoids

5 ml of each extracts was mixed in 2ml of chloroform, and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoid.

Test for Proteins

Extracts were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purple violet colour might be indicated the presence of protein.

Fluorescence Analysis[109-111]

The Fluorescence analysis of leaf and stem powder were carried out by mixing the powder with various chemical reagents and observing it under daylight, near UV and far UV. The extracts were also observed under UV light.

Thin Layer Chromatography[112]

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

TLC plates

Precoated readmade silica gel on an aluminium plates were used as a stationary phase.

Sample application

The extracts to be analyzed were diluted with respective solvent and then spotted with the help of capillary tube just above 2cm above the bottom of the TLC plate.

Selection of mobile phase

Several mobile phases were tried for the separation of maximum components. Solvents were used in the order of increasing polarity. From the vast analysis, best solvents were selected as mobile phase which showed good separation with maximum number of components.

Solvent system

Hexane: ethyl acetate and ethyl acetate: ethanol: water of various ratios of mobile phase was used. The spots were visualized by exposing to iodine vapors and UV light. R_f values were noted down for each selected extracts after elution.

8.1.2. SELECTION OF ACTIVE EXTRACT

There has been an enormous interest in the development of alternative medicines for cancer, especially screening of phytochemicals with the ability to prevent or inhibit cancer growth. The goal of the present study was to provide *invitro* evidence for potential inhibition of cancer growth. Various extracts were examined at different concentrations of plant extracts using an *invitro* method to access possible effect on cancer cell line. The extracts with highest inhibitory effect on cancer cell line were selected for the isolation of bioactive compounds.

In vitro anticancer activity (MTT Assay)[113]

Extracts used: Hexane and Ethanol extracts of leaves and stem of *Azima tetracantha* Lam.

Cell line used: MCF-7 Cell line

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture

MCF-7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37◦C in a humidified atmosphere of 5% CO2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay

Cell viability was measured with the conventional MTT reduction assay. Briefly, MCF 7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 hrs, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations of test compound was added and incubated for 48 hrs. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37 ◦C for 4 h and then with DMSO at room temperature for 1 hr. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments.

Cell viability (%) = Mean OD/Control OD x 100

8.1.3. BIOACTIVITY GUIDED ISOLATION

Bioactivity guided isolation method is commonly employed in drug discovery research due to its effectiveness to directly link the analyzed extract and targeted compounds using fractionation procedure that followed with certain biological activity.

In pharmacognosy, a typical protocol to isolate a pure chemical agent from natural origin is bioactivity guided isolation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity**.**

Column Chromatography

Column chromatography is the oldest form of chromatographic technique. A tube is packed with a solid stationary phase, the sample mixture applied to the top of the column and the mobile phase is allowed to move down through the column under gravity. The constituents of the mixture move at different rates through the column as bands. Eventually they reach the end of the column and elute in solution in the mobile phase.[114]

Isolation of compounds

The crude ethanolic extract of *Azima tetracantha* (8g) was subjected to column chromatography (Silica gel 100- 200 mesh) with gradient elution using solvents of increasing polarity, hexane, hexane: chloroform, chloroform, chloroform: ethanol, ethanol; starting at 100:0; 95:5; 90:10; 85:15; 0:100%. Totally 45 fractions were eluted. The column flow rate was 1ml/min approximately 25 ml of 45 fractions was collected. TLC was carried out simultaneously for each fraction using hexane: ethyl acetate (7:3) and chloroform: ethanol (5:5). The spots were visualized either by exposing to iodine vapors and/or UV light. The fractions with similar R_f values were pooled together, concentrated to get pure compound.

8.1.4. SELECTION OF ACTIVE FRACTION

The reactive oxygen species of endogenous and exogenous source involved in etiologie of human diseases such as arteriosclerosis, neurogenerative disease, cancer and ischaemic geart disease. Highly reactive molecules called free radicals can cause tissue damage by reacting with polyunsaturated fatty acid in cell membrane, nucleotides, DNA and critical sulfyhydral bonds in proteins.[115]

The extent of tissue damage is the result of imbalance between free radicals generated and antioxidants protective defense system. Literature data pointed out the indigenous natural antioxidants helps to prevent the deleterious outcome of oxidative stress. Thus an effort was taken to highlight the antioxidant potency of *Azima tetracantha* by DPPH assay. All the 15 fractions were screened for its *invitro* antioxidant activity.

DPPH Assay

DPPH is a stable free radical which produces deep purple colour by accepting proton from any proton donor substance and widely used to test free radical scavenging effect. DPPH become a stable diamagnetic molecule by accepting either an electron or proton.[116] The reduction of DPPH molecule is determined by its decrease in the absorbance at 515nm due to colour change

In vitro antioxidant activity (DPPH assay)[117]

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). Two ml of 6×10^{-5} M methonolic solution of DPPH were added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of

absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound (Vitamin C) were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration as follows.,

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula.[118]

$$IP = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min

$A_{A(t)}$ is the absorbance of the antioxidants at $t = 16$ min.

8.1.5. SPECTRAL STUDIES

The bioactive compounds isolated from the ethanolic extract of leaves of *Azima tetracantha* using Bioactivity guided isolation in Column chromatography were characterized by spectral studies.

Ultra-Violet Spectroscopy[119]

Ultraviolet-visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals.

Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. IR spectrum is considered as vibrational- rotational spectra. KBr pellet technique was used for solid compound and Nujol mull method was followed if the compound was liquid.

Infrared spectroscopy is a very useful technique in the preliminary stages of investigation of a compound, as it provides data regarding the presence of functional groups, such as hydroxyl, carbonyl of all kind (aldehydes, ketones, carboxylic acids, esters, lactones etc.) in different environments.[120]

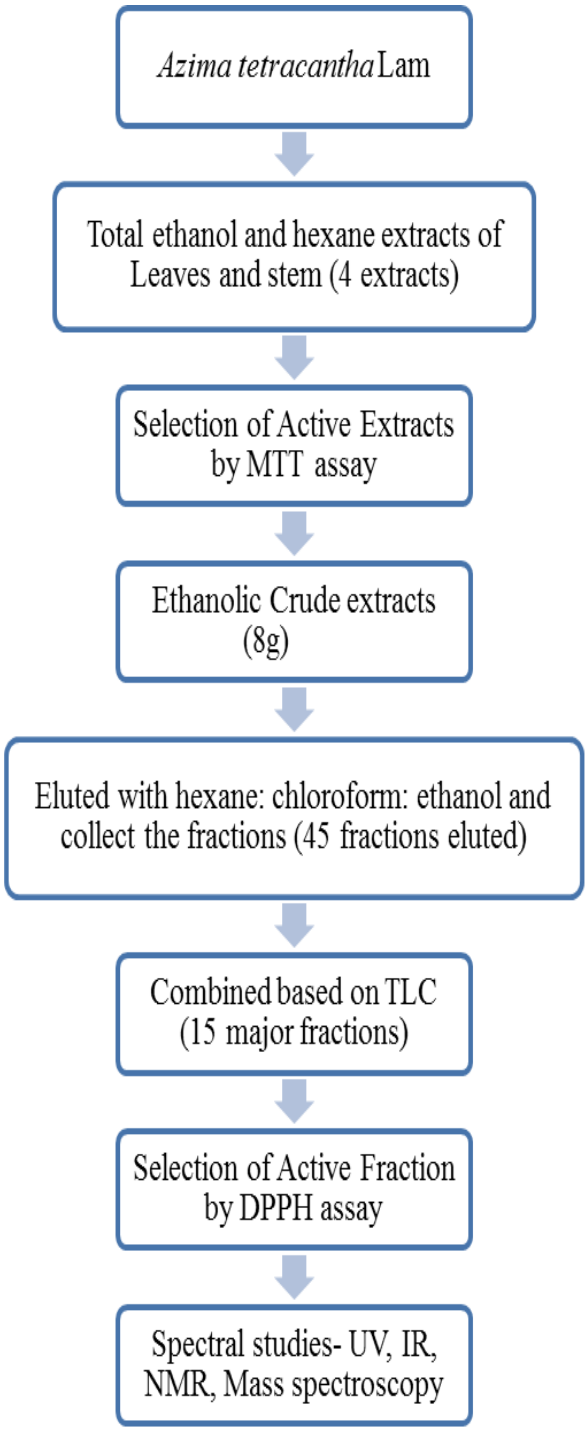
Nuclear Magnetic Resonance Spectroscopy[121]

NMR spectroscopy is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

Mass Spectroscopy[122]

Mass Spectroscopy (MS) is an analytical technique that sorts ions based on their mass (or "weight"). Mass spectroscopy is used for chemical analysis and the spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectroscopy works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

Fig 13: Schematic representation of Bioactivity guided isolation of *Azima tetracantha* Lam.



8.2. RESULTS

Phytochemistry involves mainly secondary metabolites which are biosynthesized from plants. Most of the herbal medicines are sum of their constituents present in it. It is a distinct discipline which finds a remarkable position between natural product chemistry and plant biochemistry being closely related to both.

EXTRACTION

The percentage yield of various extracts of Leaves and stem of *Azima tetracantha* Lam were given in **Table10.**

Table 10: Percentage yield of various extracts of Leaves and stem of *Azima tetracantha* Lam.

S. No	Extracts	Parts	Method of extraction	Yield (%)
1.	Ethanol	Leaf	Soxhlet extraction	16.7
2.	Hexane	Leaf		1.8
3.	Ethanol	Stem		2.3
4.	Hexane	Stem		1.2

Qualitative phytochemical screening

Preliminary phytochemical analyses of the extracts were done for the detection of various phytochemical constituents. All the extracts were showed the presence of a variety of phytochemicals. The preliminary phytochemical analysis of various extracts were reported in **Table 11.**

Table 11: Preliminary phytochemical test of various extracts of *A. tetracantha*.

Phytochemical Test	Ethanolic leaf extract	Hexane leaf extract	Ethanolic stem extract	Hexane stem extract
Alkaloids	+	-	+	-
Carbohydrates	-	-	-	-
Glycosides	-	-	-	-
Protein & Aminoacids	-	-	-	-
Flavonoids	+	+	+	+
Tannins	-	-	-	-
Steriods	+	+	+	+
Oil	+	+	+	+

Fluorescence Analysis

Fluorescence characteristics of leaf, stem powder and various extracts were displayed in **Table 12** and **Table 13.**

Table 12: Fluorescence characteristics of leaves of *Azima tetracantha*

Treatment	Day light	UV light	
		254nm	365nm
Powder	Pale-brownish green	Pale green	Brown
Powder + 1N NaOH (aqueous)	Yellowish green	Pale yellow	White
Powder + 1N NaOH (alcoholic)	Pale green	Brownish green	Brownish green
Powder + 1N Hydrochloric acid	Pale green	Brown	Black
Powder + 50% Sulphuric acid	Pale green	Bluish green	Pale green
Powder + 50% Nitric acid	Green	Yellowish green	Black
Powder + Picric acid	Dark yellow	Brown	Pale brown
Powder + Acetic acid	Pale green	Brownish green	Pale green
Powder + Ferric chloride	Orange	Brown	Pale brown
Powder +Con. Nitric acid	Brown	Brown	Black
Powder + Nitric acid + Ammonia	Green	Green	Black

Table 13: Fluorescence characteristics of the extracts of *Azima tetracantha*

Treatment	Day light	UV light	
		254nm	365nm
Ethanolic Leaf extract	Green	Greenish black	Greenish black
Hexane Leaf extract	Green	Dark green	Green
Ethanolic Stem extract	Green	Black	Black
Hexane Stem extract	Green	Black	Black

Thin Layer Chromatography

Thin layer chromatography of hexane and ethanol extracts of leaves and stem were given in **Table 14.**

Table 14: Thin layer chromatography of various extracts of *Azima tetracantha*

S. No	Extracts	Solvent System	No. of Spots	R_f Value
1.	Ethanol- Leaf	Hexane: Ethyl acetate (3.5:1.5)	8	0.32, 0.47, 0.49 0.53, 0.67, 0.75, 0.78, 0.83
2.	Hexane- Leaf	Ethyl acetate: ethanol: water (5:4:1)	4	0.45, 0.47, 0.52, 0.69
3.	Ethanol- Stem	Hexane: Ethyl acetate	5	0.33, 0.49, 0.55, 0.59, 0.61
4.	Hexane- Stem	Ethyl acetate: ethanol: water (5:4:1)	3	0.52, 0.71, 0.80

SELECTION OF ACTIVE EXTRACT

Invitro anticancer activity (MTT Assay)

The ethanolic leaf extract of *Azima tetracantha* showed inhibition of MCF-7 cell lines in the ng range tested dose and other three extracts (hexane - leaf, stem and ethanolic stem) also displayed anticancer activities against MCF-7 cell line. *Invitro* anticancer activities of various extracts were carried out using MCF-7 Cell lines. The results were given in **Table 15, 16,17 and 18.**

Table 15: *Invitro* anticancer activity of Ethanolic Leaf Extract

Plant Extract	Concentration	Absorbance	% Inhibition	IC_{50} Value
Ethanolic extract of *Azima tetracantha* Leaves	1 ng	0.572	30.95	40.809µg/ml
	10 ng	0.578	31.01	
	100 ng	0.486	41.98	
	1 µg	0.384	54.14	
	10 µg	0.365	56.41	
	100 µg	0.343	59.09	

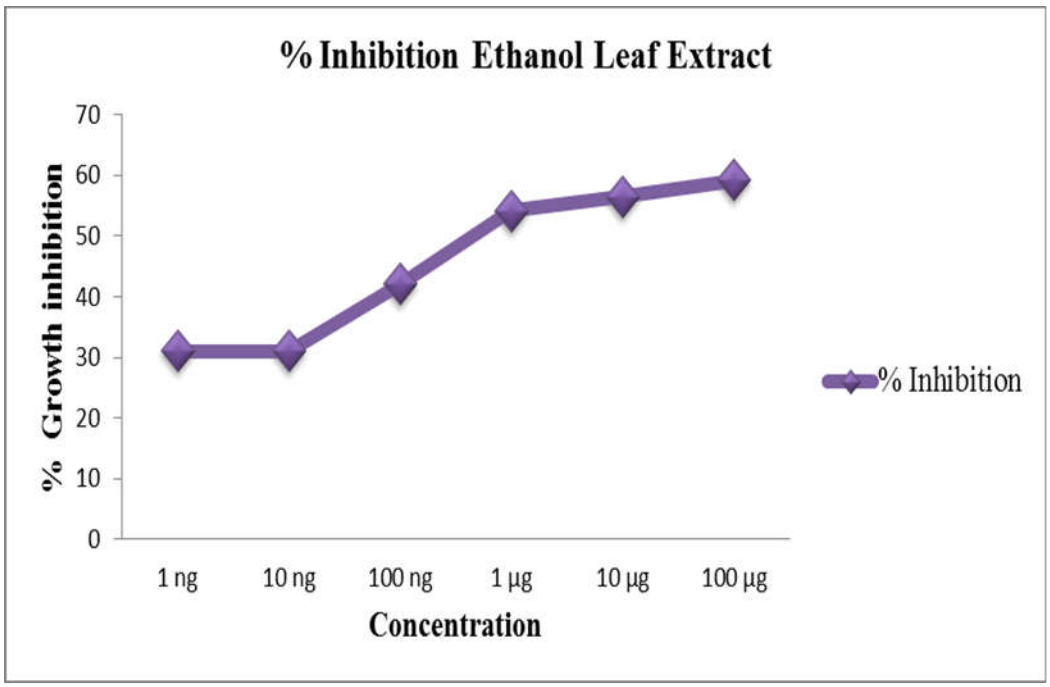


Fig 14: *Invitro* anticancer activity of Ethanolic Leaf Extract

Fig 15: MCF-7 cell line inhibition of ethanolic extract of leaf in different concentrations.

Table 16: *Invitro* anticancer activity of Hexane Leaf Extract

Plant Extract	Concentration	Absorbance	% Inhibition	IC_{50} Value
Hexane extract of *Azima tetracantha* Leaves	1 ng	0.675	19.43	40.948µg/ml
	10 ng	0.578	31.01	
	100 ng	0.481	42.58	
	1 µg	0.409	51.16	
	10 µg	0.313	62.61	
	100 µg	0.282	66.31	

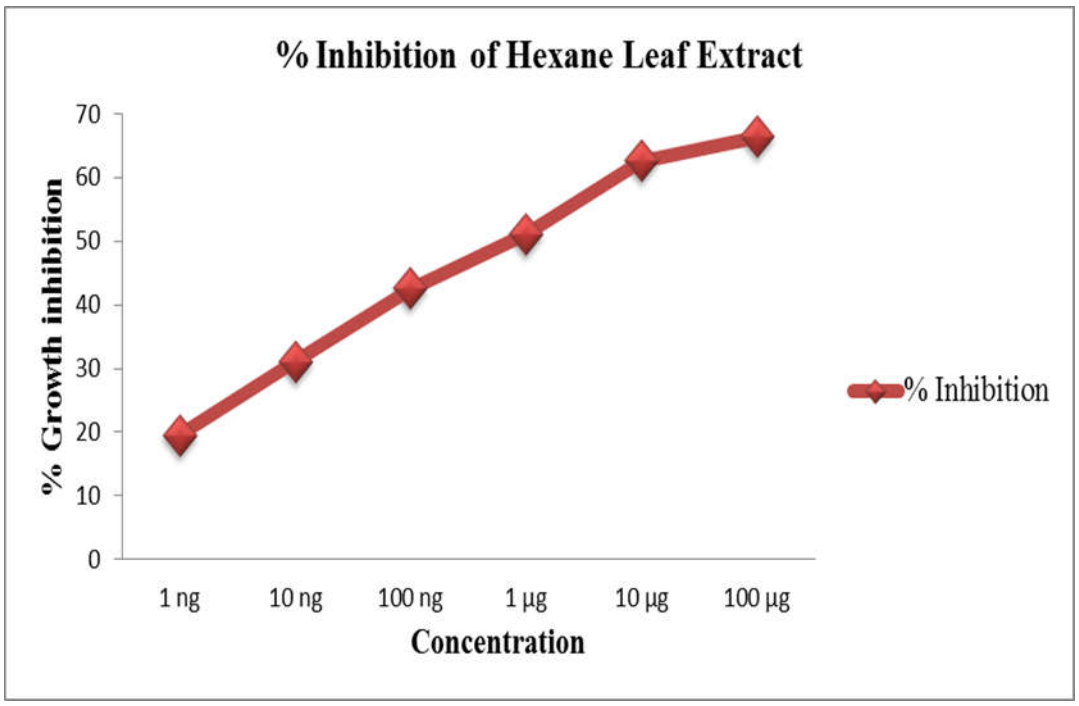


Fig 16: *Invitro* anticancer activity of Hexane Leaf Extract

Fig 17: MCF-7 cell line inhibition of hexane extract of leaf in different concentrations.

Table 17: *Invitro* anticancer activity of Ethanol Stem Extract

Plant Extract	Concentration	Absorbance	% Inhibition	IC_{50} Value
Ethanolic extract of *Azima tetracantha* Stem	1 ng	0.590	29.58	41.226µg/ml
	10 ng	0.535	36.20	
	100 ng	0.456	45.56	
	1 µg	0.424	49.37	
	10 µg	0.350	58.20	
	100 µg	0.318	62.08	

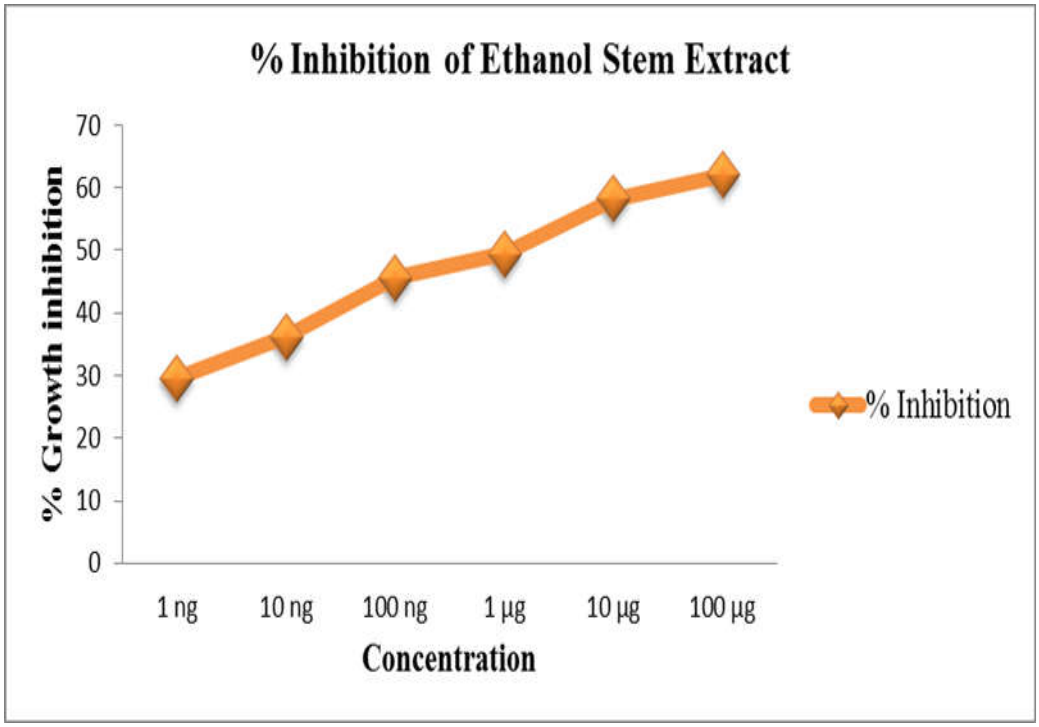


Fig 18: *Invitro* anticancer activity of Ethanol Stem Extract

Fig 19: MCF-7 cell line inhibition of ethanolic extract of stem in different concentrations.

Table 18: *Invitro* anticancer activity of Hexane Stem Extract

Plant Extract	Concentration	Absorbance	% Inhibition	IC_{50} Value
Hexane extract of *Azima tetracantha* Stem	1 ng	0.572	25.40	36.384µg/ml
	10 ng	0.625	32.32	
	100 ng	0.567	43.47	
	1 µg	0.474	59.51	
	10 µg	0.339	71.55	
	100 µg	0.238	73.46	

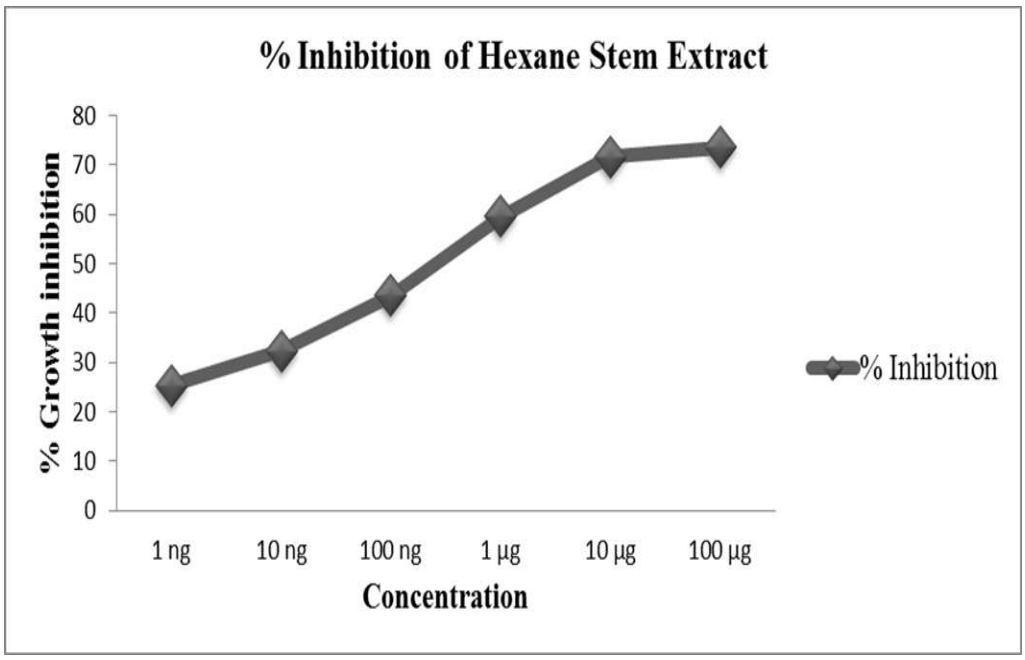


Fig 20: *Invitro* anticancer activity of Hexane Stem Extract

Fig 21: MCF-7 cell line inhibition of hexane extract of stem in different concentrations.

BIOACTIVITY GUIDED ISOLATION

Column chromatography

Based on MTT assay, the active extract was selected and performed column chromatography using various solvents of increasing polarity. 45 fractions were eluted and pooled together based on its R_f value. Fractions obtained from column chromatography were shown in **Table 19.**

Table 19: Bioactivity guided isolation of crude ethanolic extract of *Azima tetracantha* Leaves

Eluent	Solvent ratio	Fraction codes	Fractions
Hexane	100	F1	F1
Hexane: Chloroform	95:5	F2-F6	F2
Hexane: Chloroform	90:10	F7-F15	F3
Hexane: Chloroform	80:20	IBC16	F4
Hexane: Chloroform	50:50	F17-F26	F5
Hexane: Chloroform	20:80	IBC27	F6
Hexane: Chloroform	10:90	F28-F31	F7
Chloroform	100	IBC32	F8
Chloroform: Ethanol	95:5	F33-F35	F9
Chloroform: Ethanol	90:10	F36	F10
Chloroform: Ethanol	80:20	F37	F11
Chloroform: Ethanol	50:50	F38	F12
Chloroform: Ethanol	20:80	F39, F40	F13
Chloroform: Ethanol	10:90	F41-F44	F14
Ethanol	100	IBC45	F15

SELECTION OF ACTIVE FRACTION

Invitro Antioxidant activity (By DPPH assay)

Invitro antioxidant activity of all the fractions were done using DPPH assay and resulted in **Table 20.**

Table 20: DPPH Scavenging Effect of various fractions

Fractions	Control	Concentrations µg/ml									
		200		400		600		800		1000	
	OD	OD	%	OD	%	OD	%	OD	%	OD	%
Vit C	0.595	0.531	10.76	0.425	28.57	0.31	47.90	0.247	58.49	0.133	77.65
F1	0.595	0.578	2.86	0.544	8.57	0.512	13.95	0.489	17.82	0.477	19.83
F2	0.595	0.565	5.04	0.537	9.75	0.521	12.44	0.519	12.77	0.501	15.80
F3	0.595	0.58	2.52	0.576	3.19	0.558	6.22	0.527	11.43	0.508	14.62
F4	0.595	0.511	14.12	0.467	21.51	0.422	29.08	0.386	35.13	0.351	**41.01**
F5	0.595	0.593	0.34	0.581	2.35	0.578	2.86	0.556	6.55	0.532	10.59
F6	0.595	0.568	4.54	0.543	8.74	0.415	30.25	0.403	32.27	0.377	**36.64**
F7	0.595	0.581	2.35	0.572	3.87	0.481	19.16	0.422	29.08	0.407	31.60
F8	0.595	0.533	10.42	0.506	14.96	0.46	22.69	0.369	37.98	0.261	**56.13**
F9	0.595	0.591	0.67	0.574	3.53	0.512	13.95	0.493	17.14	0.469	21.18
F10	0.595	0.571	4.03	0.542	8.91	0.521	12.44	0.497	16.47	0.473	20.50
F11	0.595	0.588	1.18	0.571	4.03	0.535	10.08	0.503	15.46	0.477	19.83
F12	0.595	0.593	0.34	0.582	2.18	0.566	4.87	0.549	7.73	0.521	12.43
F13	0.595	0.559	6.05	0.532	10.59	0.511	14.12	0.496	16.64	0.47	21.01
F14	0.595	0.584	1.85	0.563	5.38	0.551	7.39	0.532	10.59	0.514	13.61
F15	0.595	0.552	7.23	0.516	13.28	0.451	24.20	0.382	35.80	0.317	**46.72**

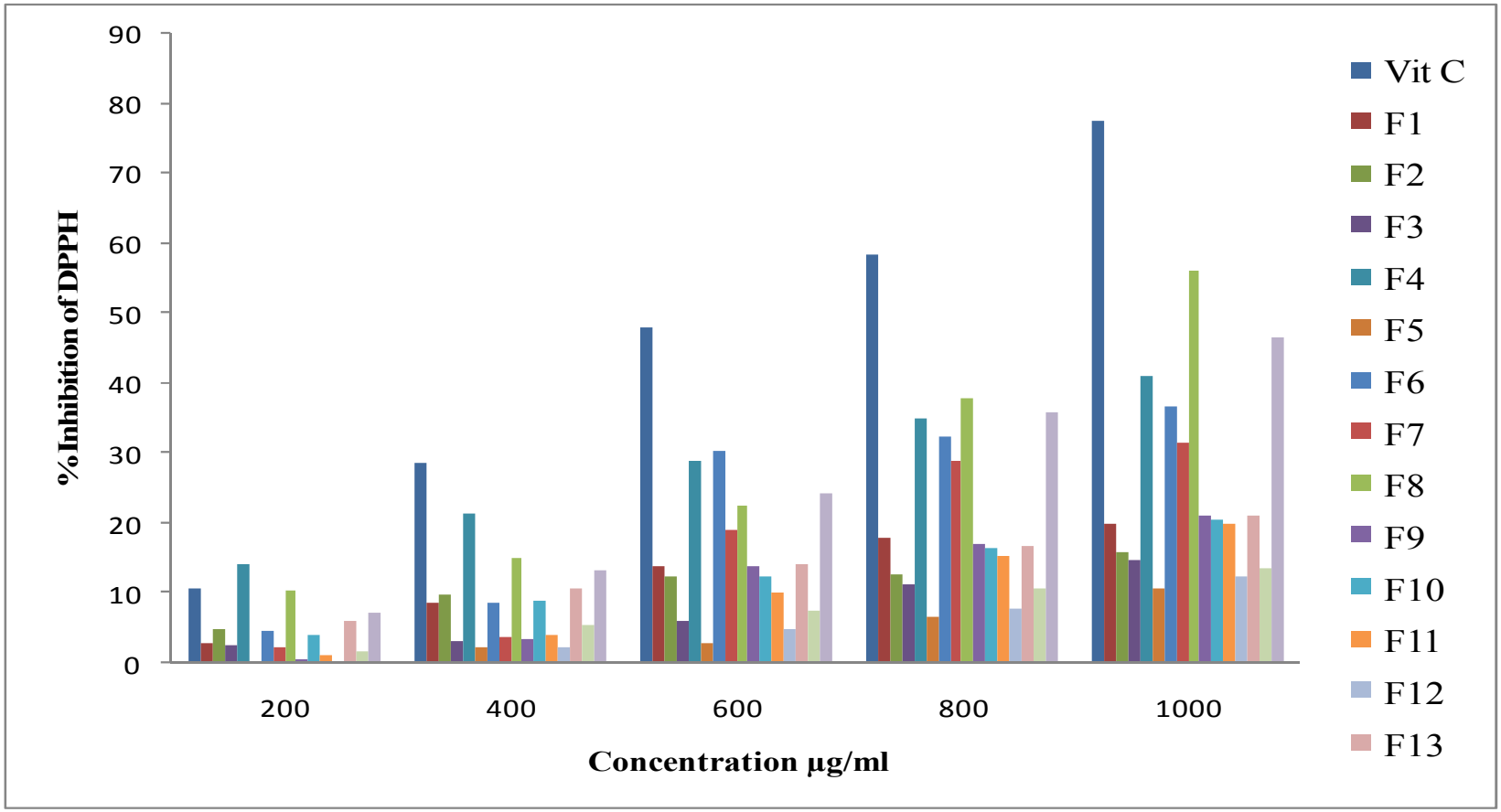
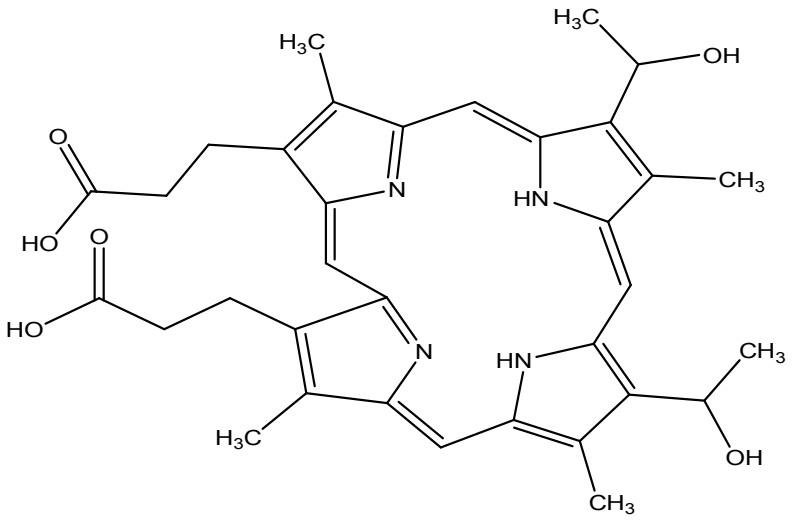


Fig 22: Graphical representation of percentage inhibition of DPPH of various fractions

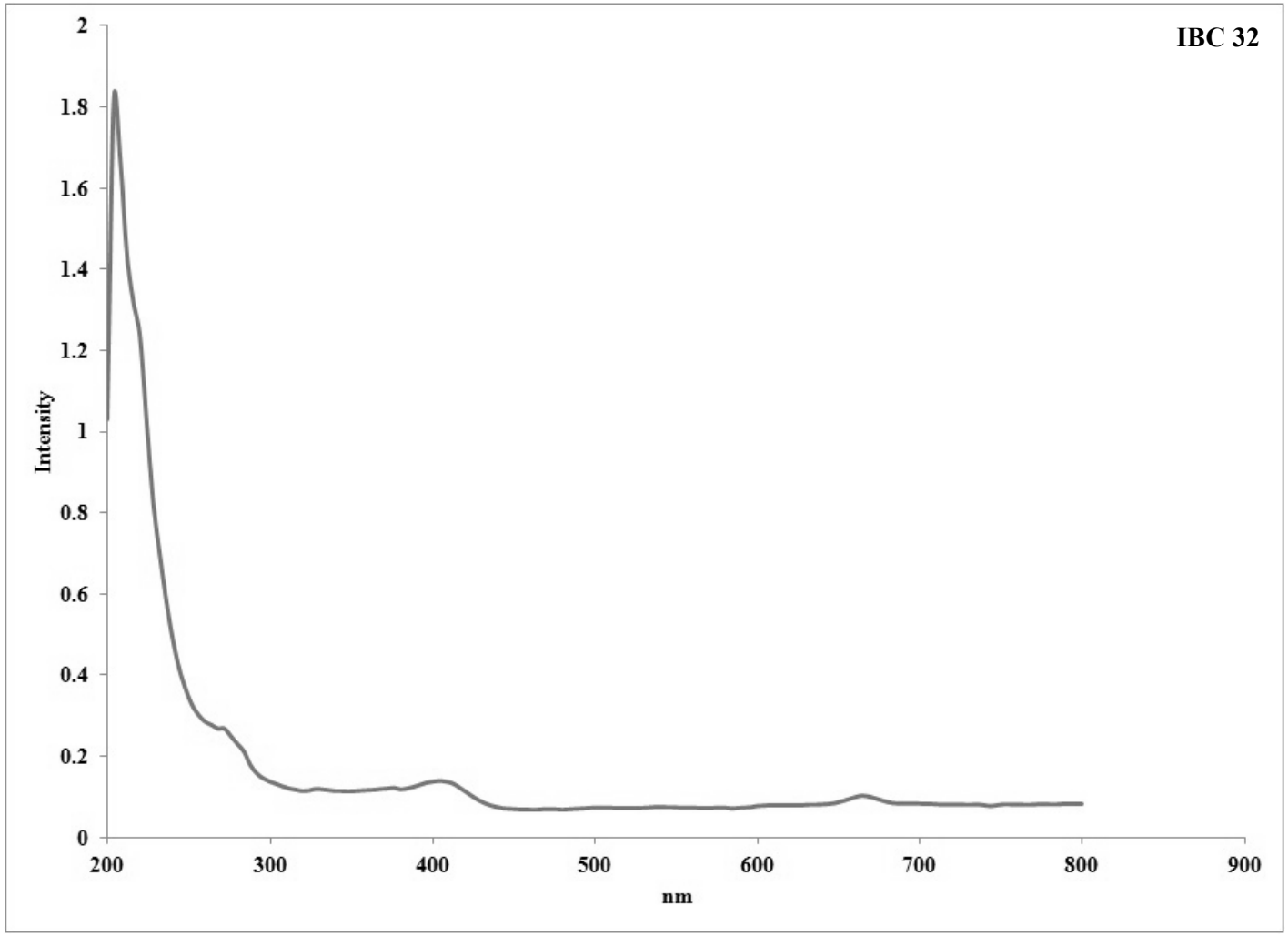
SPECTRAL STUDIES OF ISOLATED BIOACTIVE COMPOUNDS

IBC 32

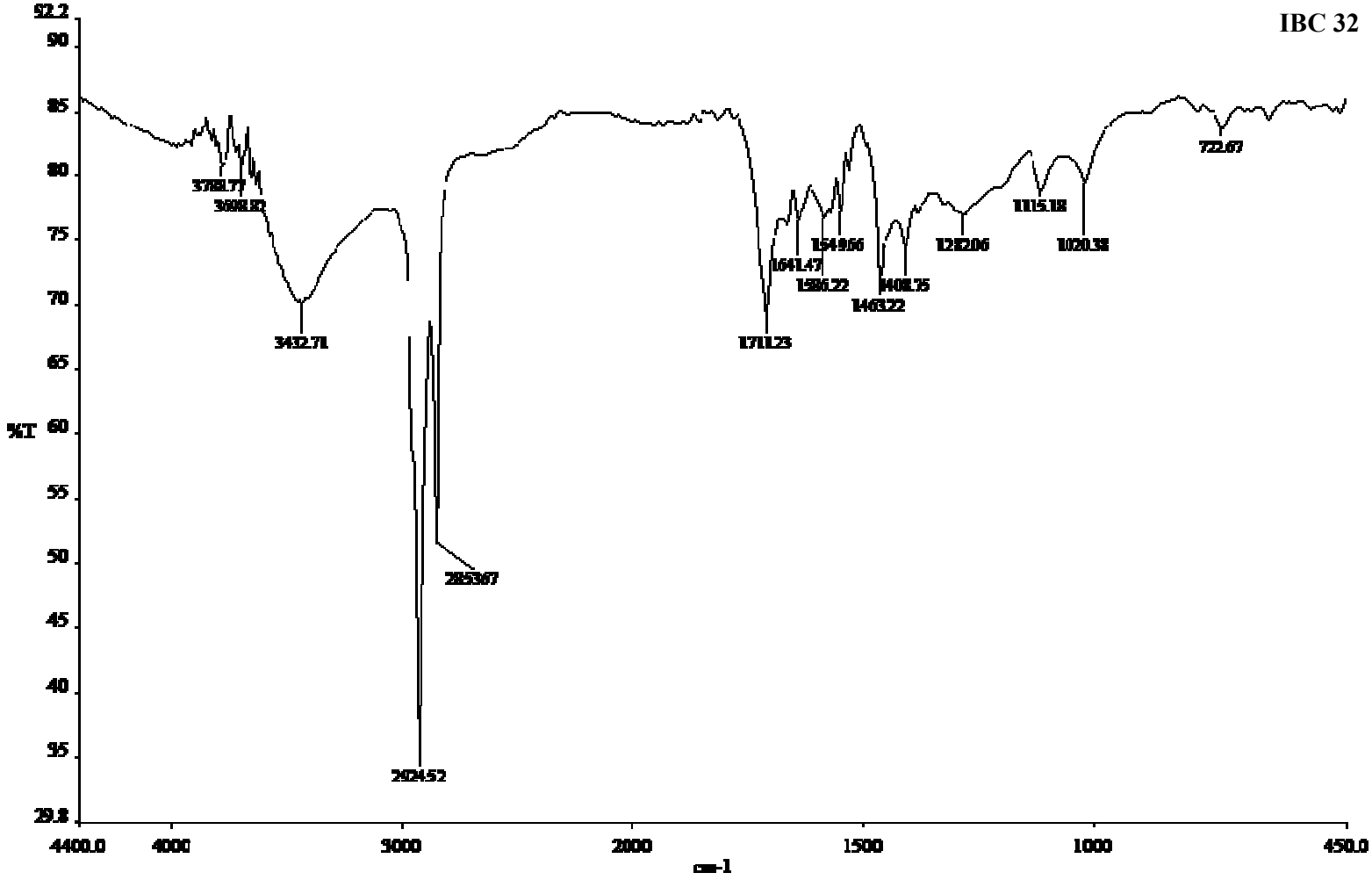


7,12-bis(1-hydroxyethyl)-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoic acid

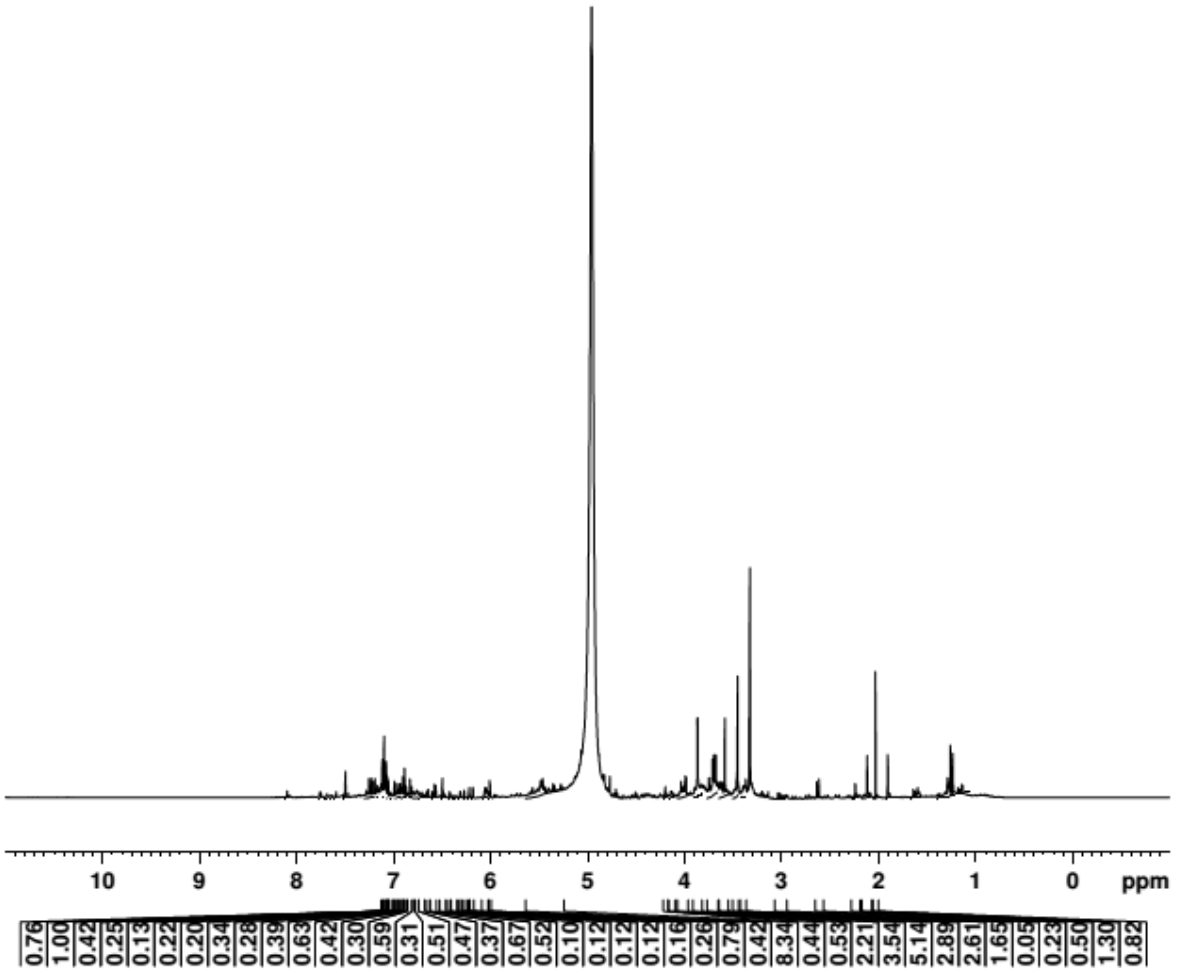
IR (KBr, ν_{max}) cm^{-1}	3698 (O-H stretching), 2924-2853 (C-H stretching), 1711 (C=O stretching), 1641-1549 (N-H bending), 1463 (C-C stretching), 1020 (C-N stretching).
^{1}H NMR (DMSO-D_6) δ	2.03 (s, 1H), 2.12 (d, 1H), 2.24 (s, 1H), 3.32-3.33 (m, 2H), 3.45 (s, 3H), 3.58 (s, 3H), 3.70 (t, 5H), 3.86 (s, 4H), 3.98-4.03 (m, 2H), 4.13 (t, 1H), 4.34 (S, 1H), 5.26-5.56 (m, 8H), 6.03-6.06 (m, 1H), 6.49 (t, 1H), 6.56-6.60 (m, 1H), 6.75-6.77 (m,1H), 6.82 (d, 1H), 6.90 (t, 1H), 7.49-7.52 (t,1H).
^{13}C NMR (DMSO-D_6) δ	14.37, 15.56, 18.42, 22.51, 24.93, 25.82, 28.95, 29.10, 29.29, 29.41, 29.90, 30.31, 31.59, 31.71, 31.99, 34.13, 35.38, 35.67, 42.63, 50.01, 58.58, 79.56, 114.48, 116.32, 132.11, 144.27, 175.02.
MASS SPECTROSCOPY	598.87 (**M^+**), 598(**M+1**), 108.00 (**B**).


IBC 32
Intensity
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
200
300
400
500
600
700
800
900
nm

IBC 32


92.2
90
85
80
75
70
65
60
55
50
45
40
35
29.8
%T
3788.77
3688.82
3432.71
2924.52
2855.67
1711.23
1641.47
1586.22
1549.66
1468.22
1408.75
1282.06
1115.18
1020.38
722.67
4400.0
4000
3000
2000
1500
1000
450.0
cm-1

IBC 32



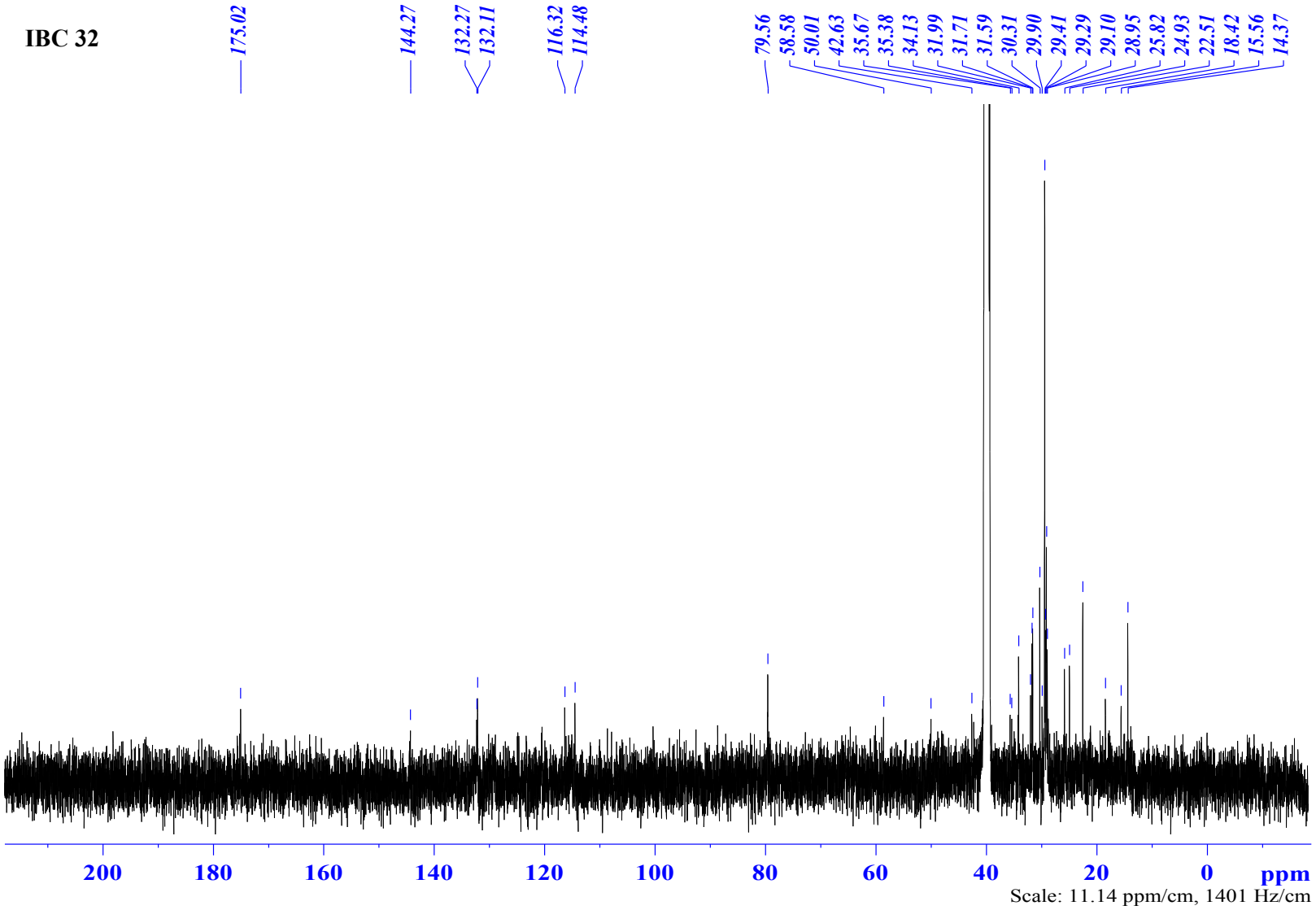
Current Data Parameters
NAME Mar07–2016
EXPNO 8
PROCNO 1

F2 – Acquisition Parameters
Date_ 20160307
Time 0.36
INSTRUM spect
PROBHD 5 mm PABBO BB–
PULPROG zg30
TD 32768
SOLVENT MeOD
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 101
DW 48.400 usec
DE 6.50 usec
TE 297.1 K
D1 1.00000000 sec
TD0 1

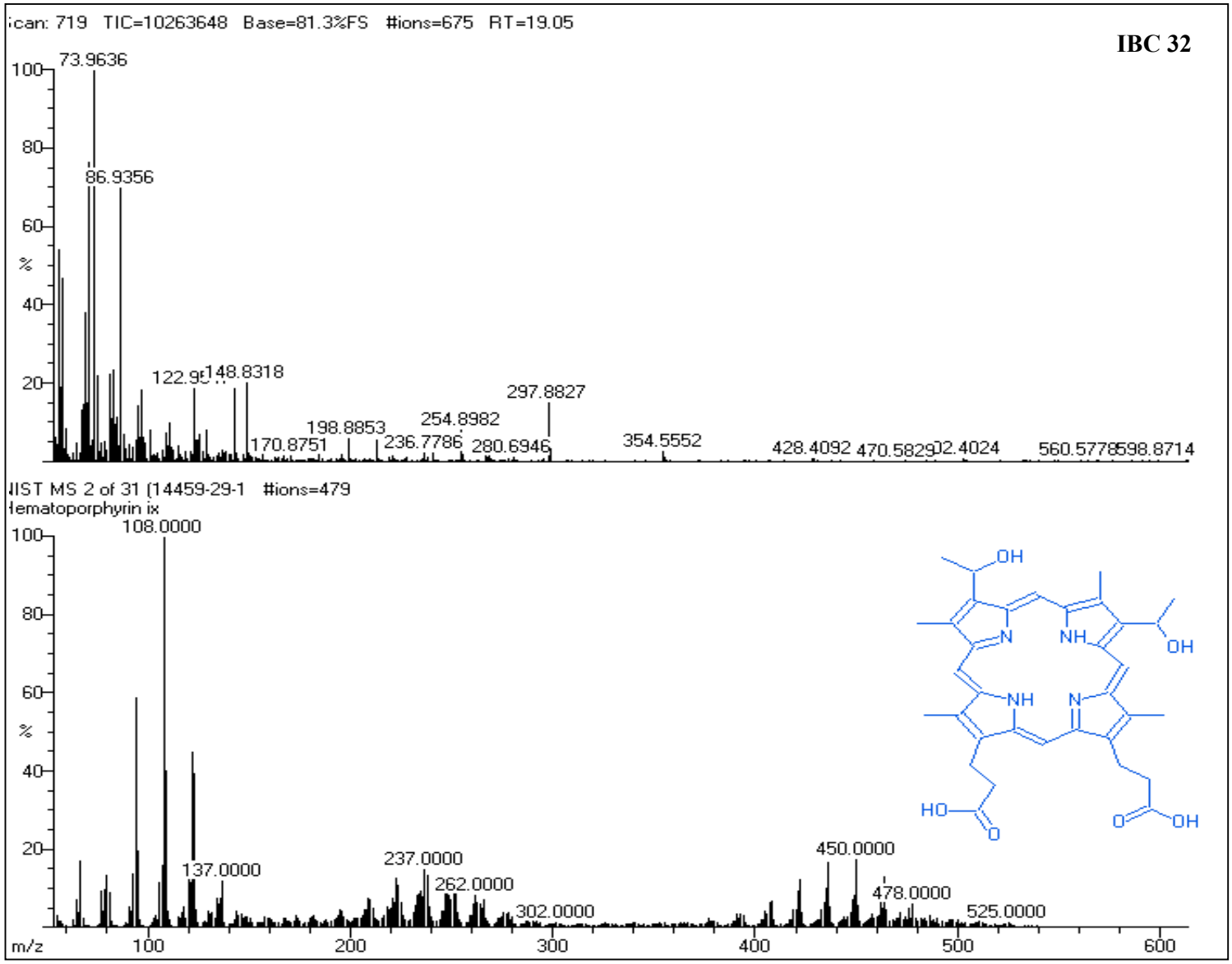
======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 – Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

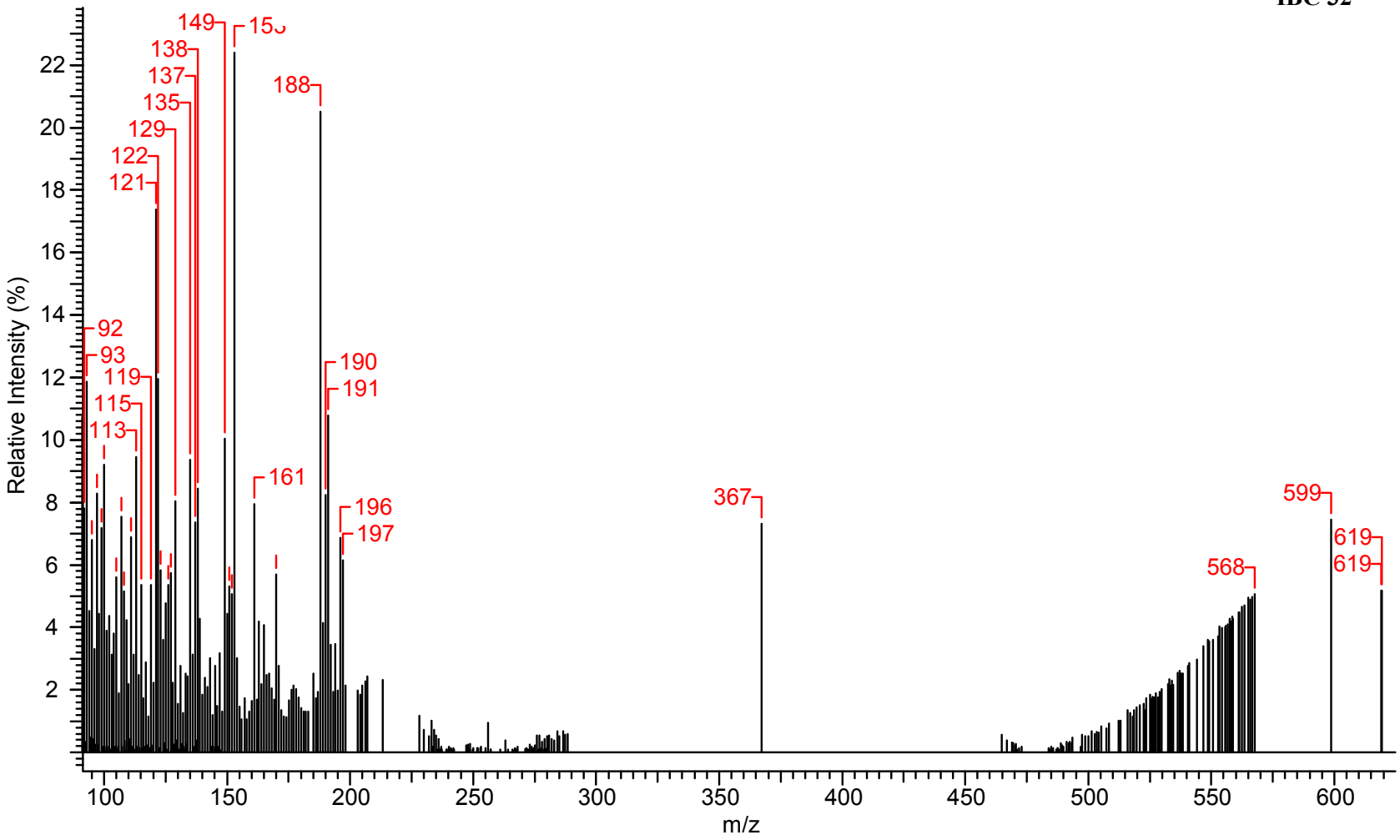
IBC 32


175.02
144.27
132.27
132.11
116.32
114.48
79.56
58.58
50.01
42.63
35.67
35.38
34.13
31.99
31.71
31.59
30.31
29.90
29.41
29.29
29.10
28.95
25.82
24.93
22.51
18.42
15.56
14.37
200
180
160
140
120
100
80
60
40
20
0
ppm
Scale: 11.14 ppm/cm, 1401 Hz/cm

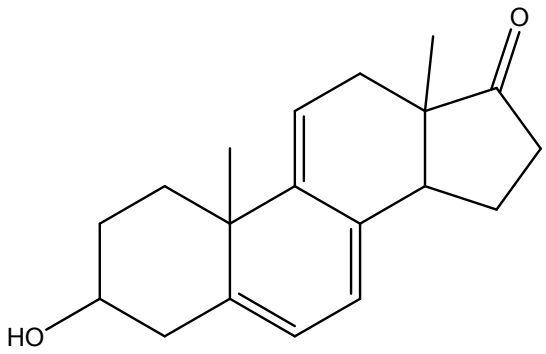
IBC 32


Scan: 719 TIC=10263648 Base=81.3%FS #ions=675 RT=19.05
73.9636
86.9356
148.8318
297.8827
254.8982
198.8853
236.7786
280.6946
170.8751
354.5552
428.4092
470.5829
560.5778
598.8714
%
MIST MS 2 of 31 (14459-29-1 #ions=479
Hematoporphyrin ix
108.0000
137.0000
237.0000
262.0000
302.0000
450.0000
478.0000
525.0000
m/z
OH
NH
N
HO
O

IBC 32

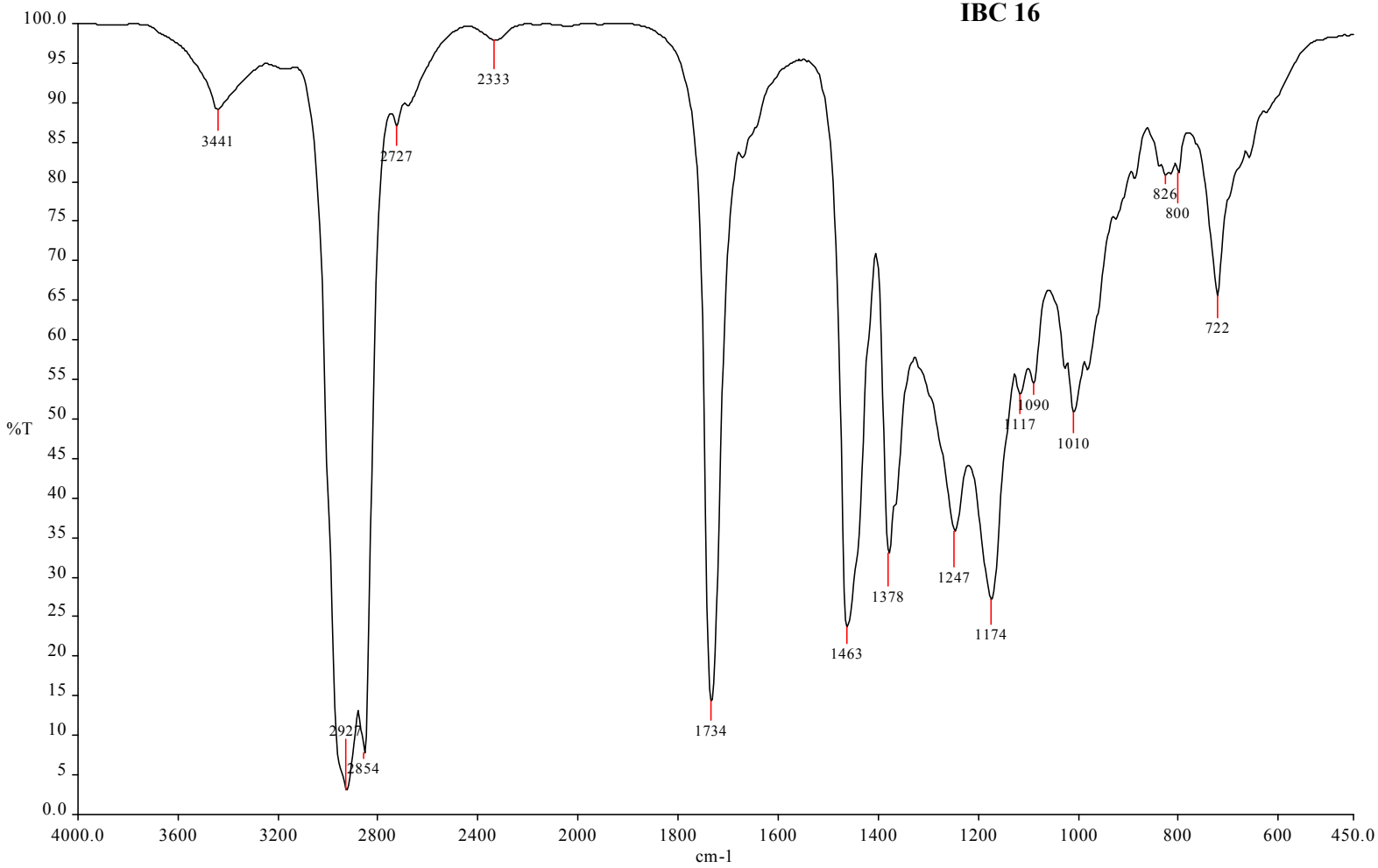

Relative Intensity (%)
m/z
149
153
138
137
135
129
122
121
188
92
93
119
115
113
190
191
161
196
197
367
599
619
619
568

IBC 16

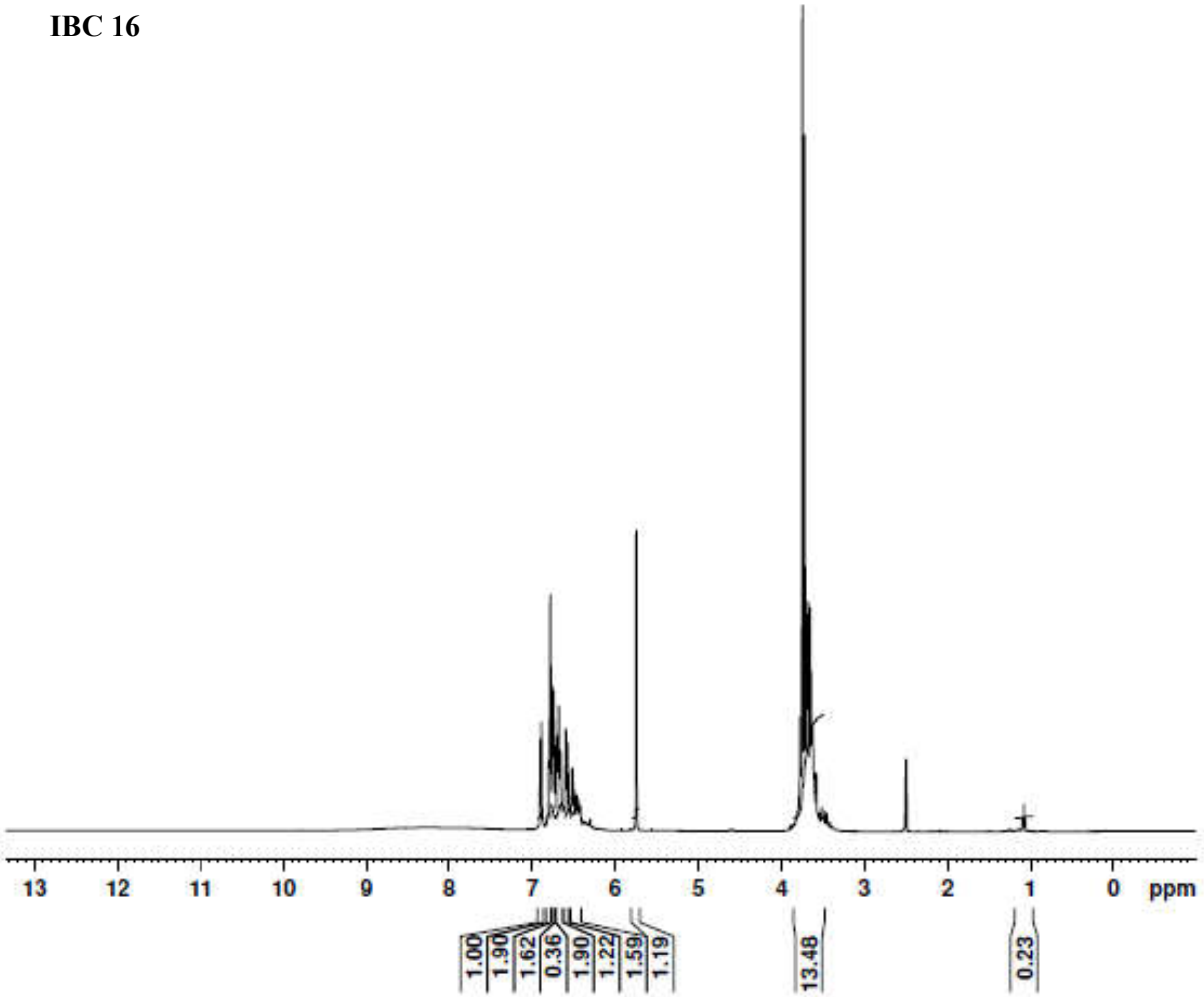


5,7,9(11)- androstatriene, 3-hydroxy-17-oxo

IR (KBr, ν_{max}) cm^{-1}	3441 (O-H stretching), 2927-2854 (C-H stretching super imposed O-H stretching), 2727 (C-H stretching), 1734 (C=O stretching for ketone), 1463(C=C stretching), 1378 (C-H def. in CH_3), 1247 (C-O stretching), 1010 (C-O stretching for alcohol).
^{1}H NMR (DMSO-D_6) δ	3.46-3.81 (m, 13H), 5.75 (s, 1H), 6.43-6.53 (m, 2H), 6.58-6.62 (m, 1H), 6.67- 6.70 (m, 2H), 6.74-6.76 (m, 2H), 6.80 (d, 2H), 6.90 (d, 1H).
^{13}C NMR (DMSO-D_6) δ	29.58, 39.48, 39.65, 39.81, 39.98, 40.15, 40.31, 40.48, 56.40, 108.52, 115.92, 122.00, 131.13, 149.23, 160.27
MASS SPECTROSCOPY	283.74 (**M^+**), 282.00(**M+1**), 148.67(**B**).


IBC 16
100.0
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0.0
%T
3441
2727
2333
2927
2854
1734
1463
1378
1247
1174
1117
1090
1010
826
800
722
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
450.0
cm-1

IBC 16



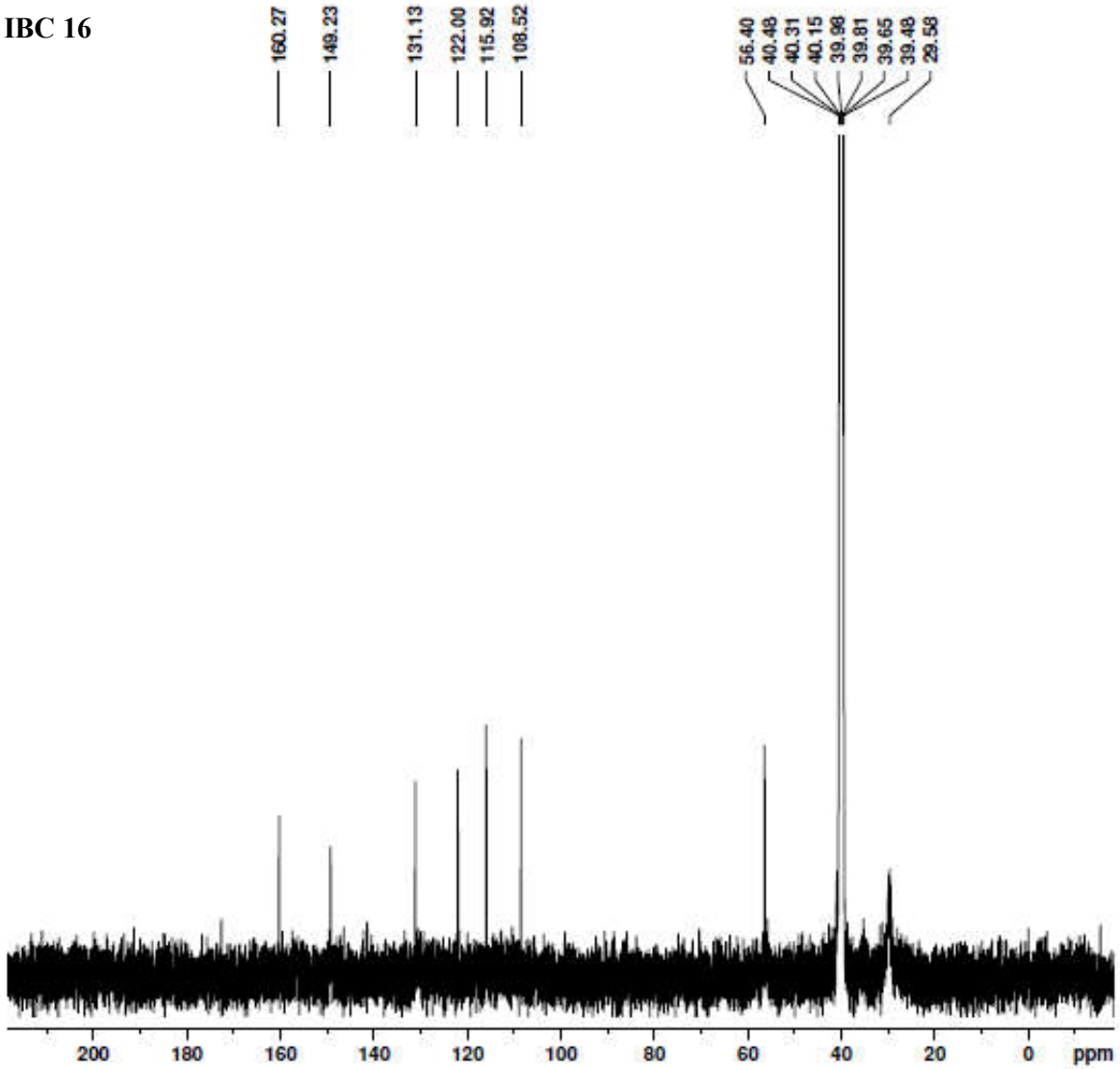
Current Data Parameters
NAME Feb12-2016
EXPNO 28
PROCNO 1

F2 - Acquisition Parameters
Date 20160212
Time 1.08
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 20.2
DW 48.400 usec
DE 6.50 usec
TE 297.5 K
D1 1.00000000 sec
TD0 1

====== CHANNEL f1 ======
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

IBC 16



Current Data Parameters
NAME Feb12-2016
EXPNO 29
PROCNO 1

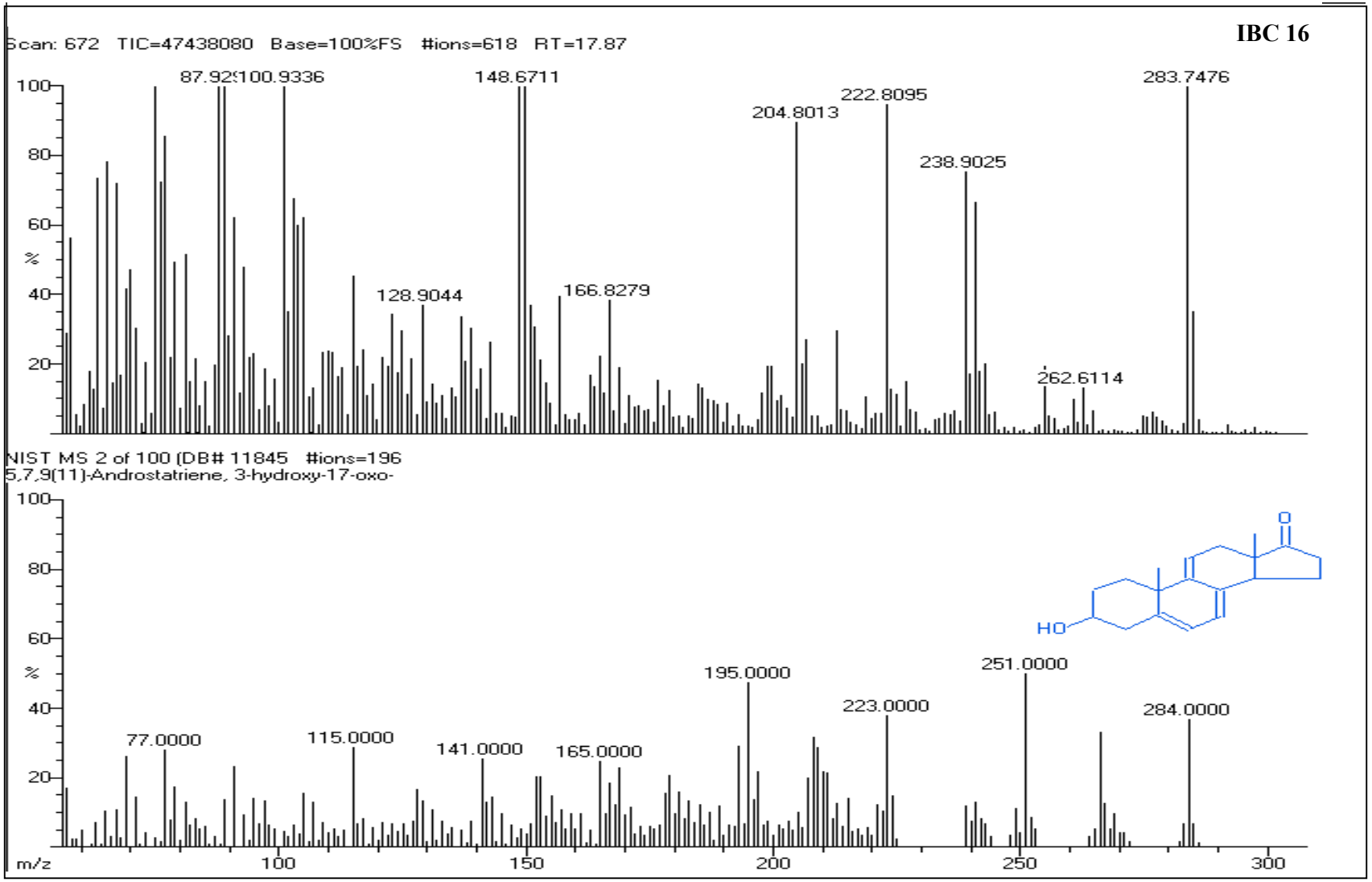
F2 - Acquisition Parameters
Date_ 20160212
Time 1.19
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1024
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 0.5505524 sec
RG 203
DW 16.800 usec
DE 6.50 usec
TE 298.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 7.80 usec
PL1 0.00 dB
PL1W 70.83519745 W
SFO1 125.7703643 MHz

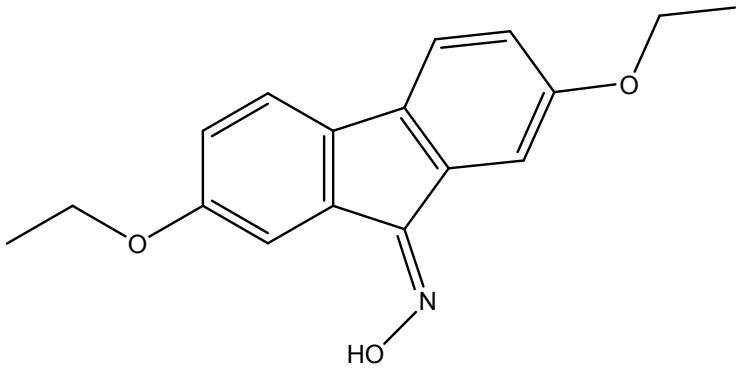
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.51 dB
PL13 18.00 dB
PL2W 23.53637505 W
PL12W 0.41757989 W
PL13W 0.37302643 W
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

IBC 16

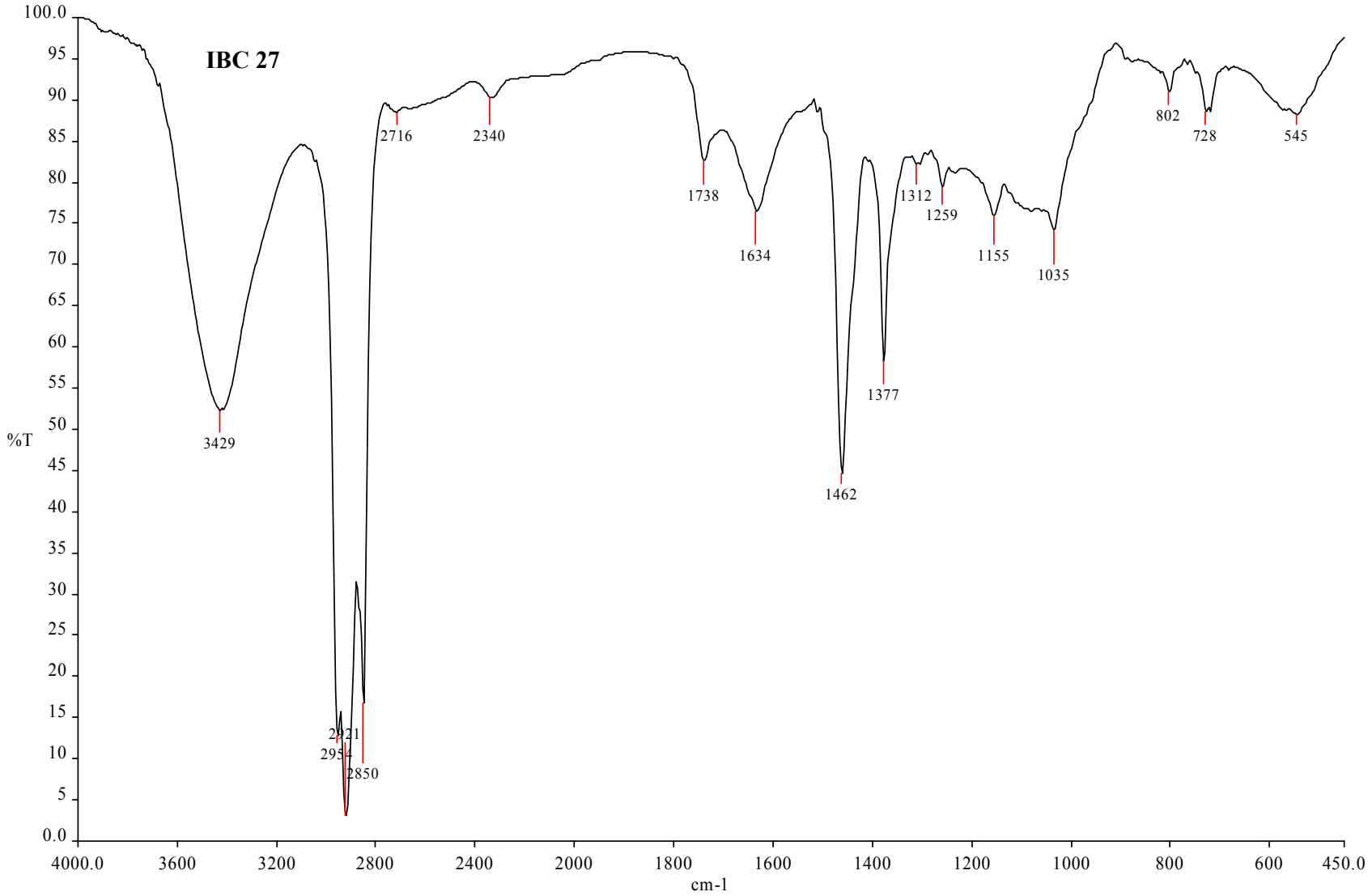

Scan: 672 TIC=47438080 Base=100%FS #ions=618 RT=17.87
87.9251
100.9336
148.6711
204.8013
222.8095
238.9025
283.7476
128.9044
166.8279
262.6114
NIST MS 2 of 100 (DB# 11845 #ions=196
5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo-
HO
O
77.0000
115.0000
141.0000
165.0000
195.0000
223.0000
251.0000
284.0000
%
m/z

IBC 27

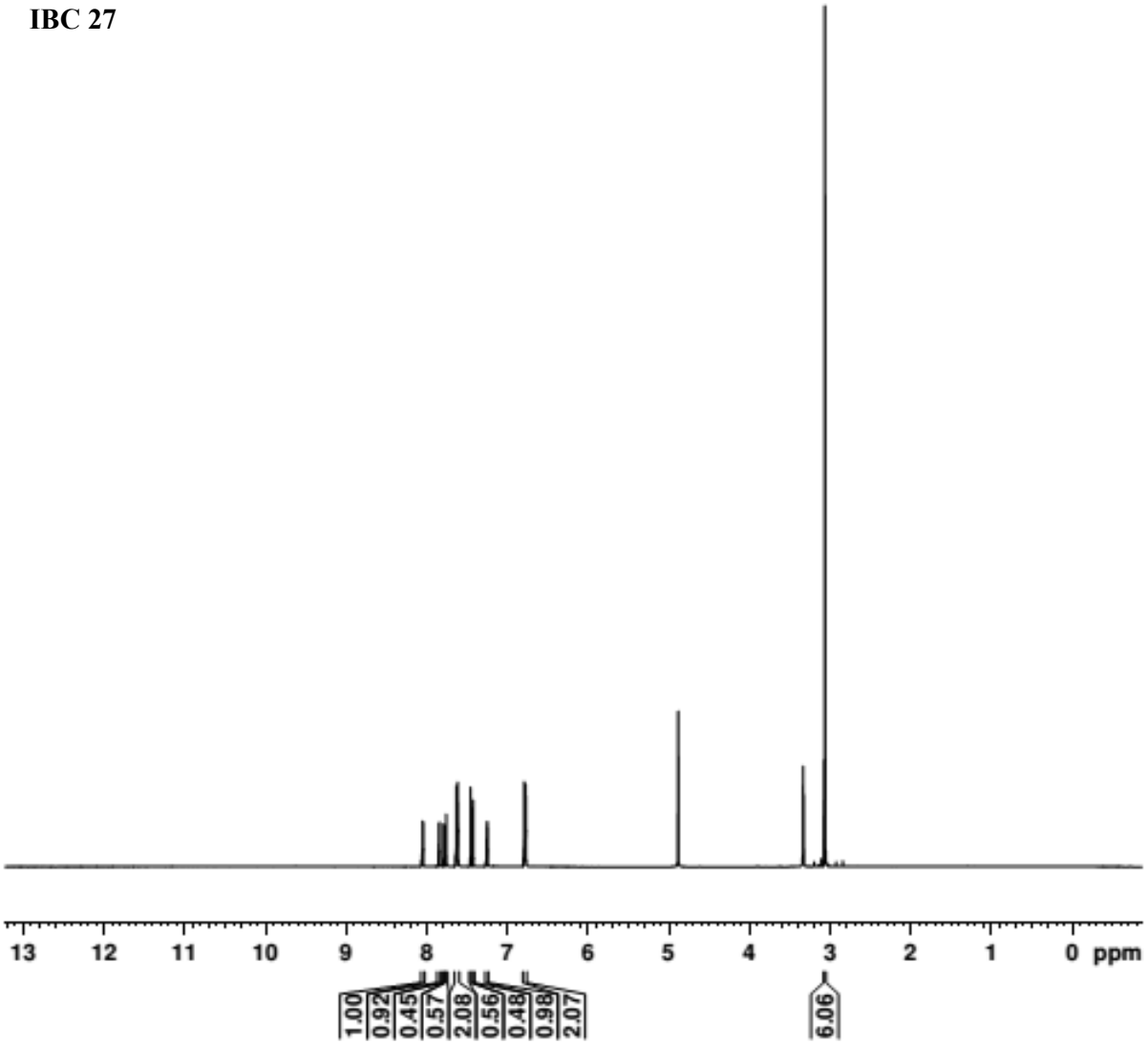


9-oximino-2,7-diethoxyfluorene

IR (KBr, ν_{max}) cm^{-1}	3429 (O-H stretching), 2954-2850 (C-H stretching super imposed O-H stretching), 2716 (C-H stretching), 2340 (C=N stretching), 1738 (C=O stretching for ketone), 1643-1462 (C=C stretching), 1377 (C-H def. in CH_3), 1259-1035 (C-O stretching).
^{1}H NMR (DMSO-D_6) δ	3.01 (s, 6H), 6.78 (d, 2H), 7.24-7.25 (m, 1H), 7.45 (s, 1H), 7.62 (d, 2H), 7.75 (s, 1H), 7.83-7.85 (m, 1H), 8.04-8.05 (m, 1H).
^{13}C NMR (DMSO-D_6) δ	39.48, 39.64, 39.81, 39.98, 40.15, 40.24, 40.31, 40.40, 40.48, 123.08, 125.42, 131.22, 131.46.
MASS SPECTROSCOPY	283.00 (**M^+**), 282.00(**M+1**), 151.78(**B**).

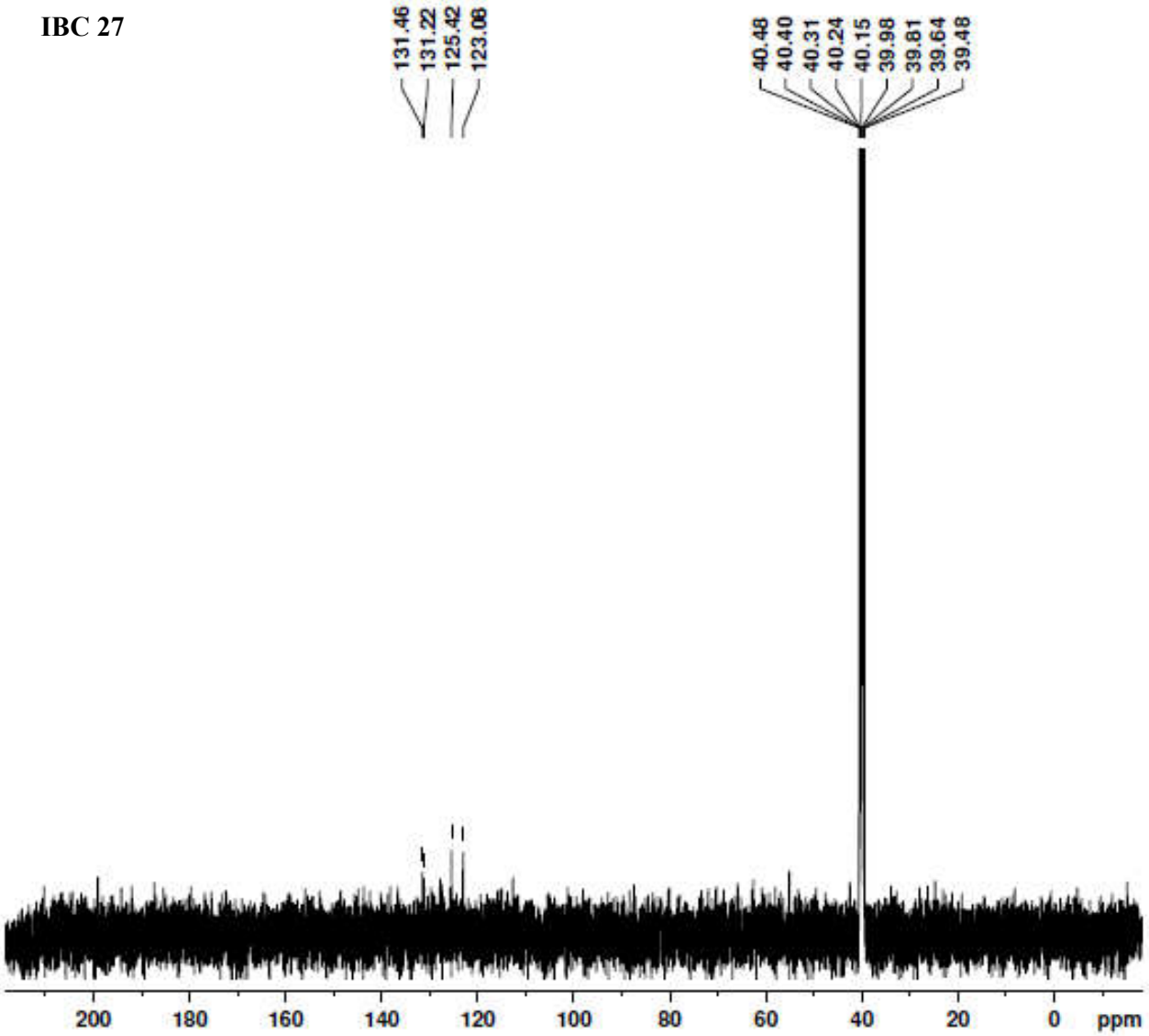

IBC 27
%T
cm-1
100.0
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0.0
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
450.0
3429
2921
2954
2850
2716
2340
1738
1634
1462
1377
1312
1259
1155
1035
802
728
545

IBC 27




```
Current Data Parameters
NAME          Mar09-2016
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_         20160308
Time             19.35
INSTRUM          spect
PROBHD  5 mm PABBO BB-
PULPROG          zg30
TD              32768
SOLVENT           MeOD
NS                  64
DS                   2
SWH          10330.578 Hz
FIDRES        0.315264 Hz
AQ           1.5860212 sec
RG                 203
DW              48.400 usec
DE               6.50 usec
TE               297.1 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1               1H
P1           10.65 usec
PL1           0.00 dB
PL1W      23.53637505 W
SFO1      500.1330885 MHz

F2 - Processing parameters
SI              32768
SF      500.1300000 MHz
WDW               EM
SSB                0
LB              0.30 Hz
GB                 0
PC              1.00
```

IBC 27



Current Data Parameters
NAME Feb12-2016
EXPNO 29
PROCNO 1

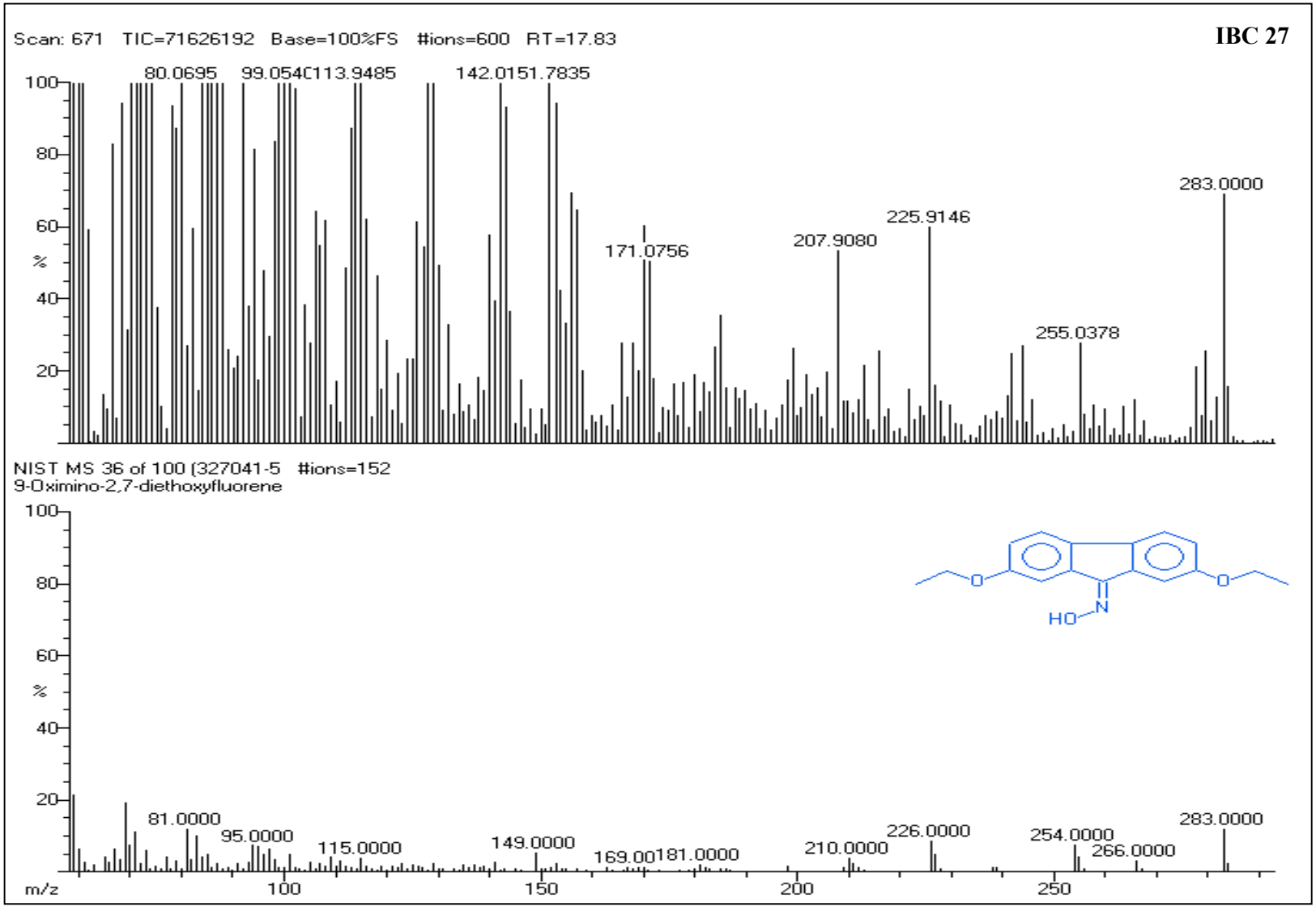
F2 - Acquisition Parameters
Date_ 20160212
Time 1.19
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1024
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 0.5505524 sec
RG 203
DW 16.800 usec
DE 6.50 usec
TE 298.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 7.80 usec
PL1 0.00 dB
PL1W 70.83519745 W
SFO1 125.7703643 MHz

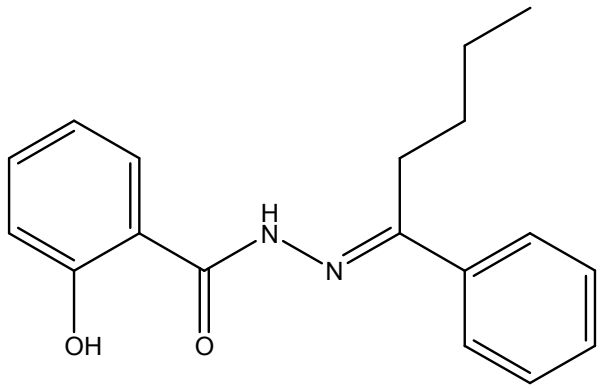
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.51 dB
PL13 18.00 dB
PL2W 23.53637505 W
PL12W 0.41757989 W
PL13W 0.37302643 W
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

IBC 27

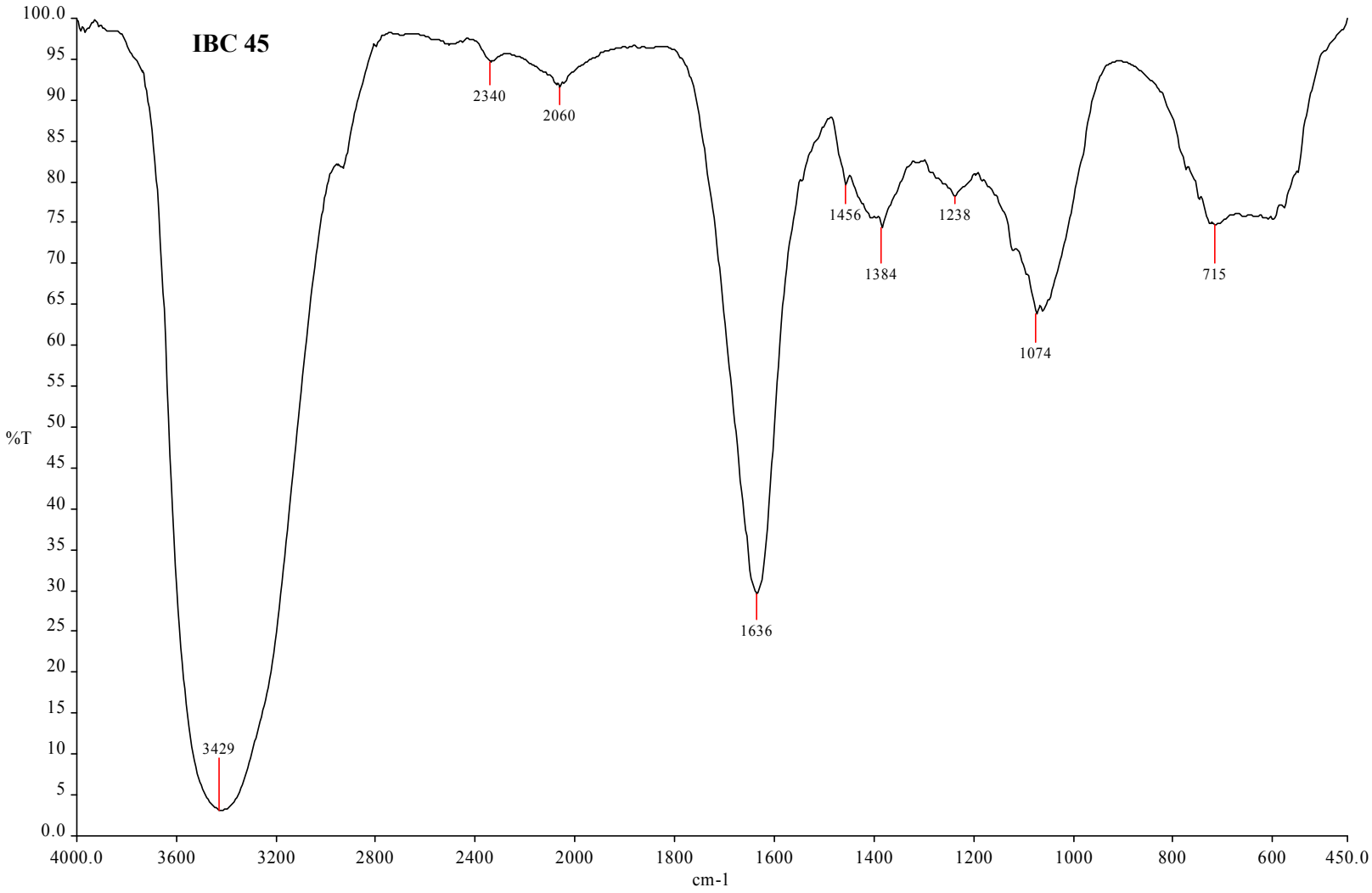

Scan: 671 TIC=71626192 Base=100%FS #ions=600 RT=17.83
80.0695
99.0540
113.9485
142.0151.7835
171.0756
207.9080
225.9146
255.0378
283.0000
100
80
60
%
40
20
NIST MS 36 of 100 (327041-5 #ions=152
9-Oximino-2,7-diethoxyfluorene
O
O
N
HO
81.0000
95.0000
115.0000
149.0000
169.00
181.0000
210.0000
226.0000
254.0000
266.0000
283.0000
m/z
100
150
200
250

IBC 45

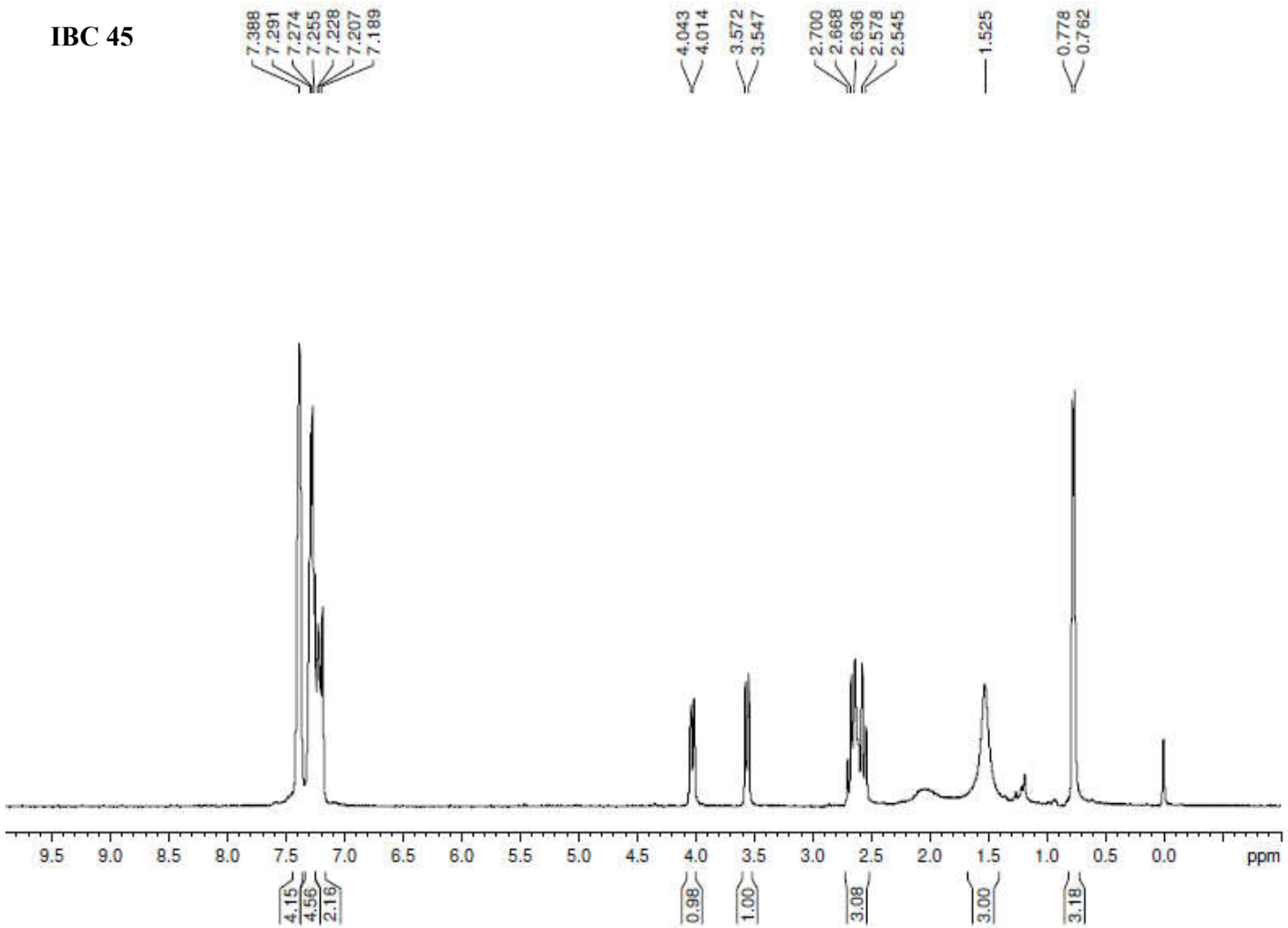


benzhydrazide,2-hydroxy-N2-(1-phenylpenylidene)

IR (KBr, ν_{max}) cm^{-1}	3429 (O-H stretching), 1636 (C=O stretching for ketone), 1456 (C=C stretching), 1384 (C-H def. in CH_3), 1238 (C-O stretching), 1074 (C-N stretching), 715 (characteristic mono substituted benzene).
^{1}H NMR (DMSO-D_6) δ	0.76 (d, 3H), 1.52 (s, 3H), 2.54-2.70 (m, 3H), 3.55 (d, 1H), 4.02 (d, 1H), 7.20 (t, 2H), 7.27 (t, 5H), 7.38 (s, 4H).
^{13}C NMR (DMSO-D_6) δ	47.09, 47.26, 47.43, 47.50, 47.51, 47.53, 47.54, 47.56, 47.60, 47.77, 47.82, 47.83, 47.85, 47. 87, 47.89, 47.94, 48.11.
MASS SPECTROSCOPY	295.77 (**M^+**), 294.22(**M+1**), 122.91(**B**).


IBC 45
100.0
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0.0
%T
3429
2340
2060
1636
1456
1384
1238
1074
715
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
450.0
cm-1

IBC 45



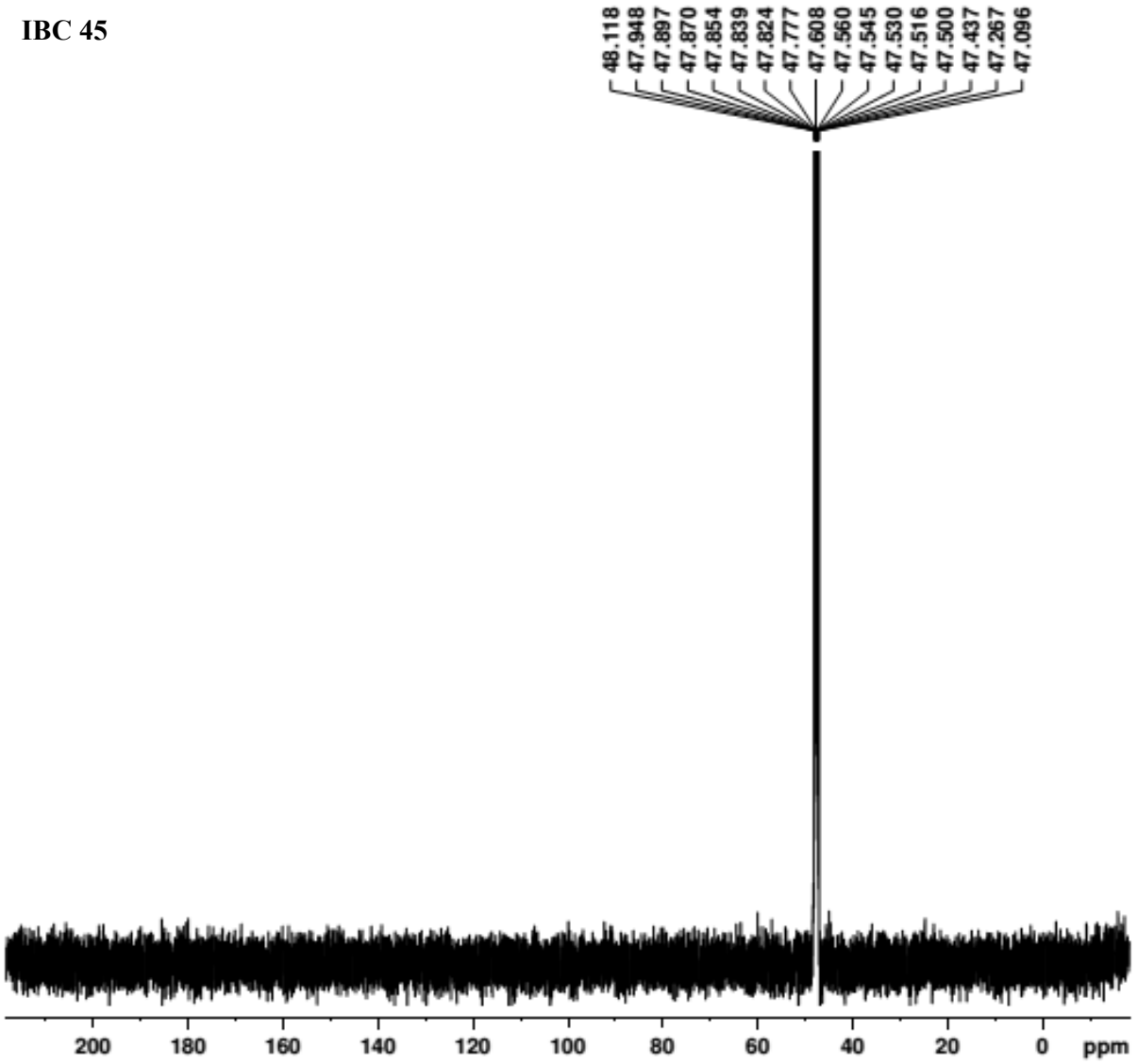
Current Data Parameters
NAME Feb12–2016
EXPNO 28
PROCNO 1

F2 – Acquisition Parameters
*Date_* 20160212
Time 1.08
INSTRUM spect
PROBHD 5 mm PABBO BB–
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 20.2
DW 48.400 usec
DE 6.50 usec
TE 297.5 K
D1 1.00000000 sec
TD0 1

====== CHANNEL f1 ======
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

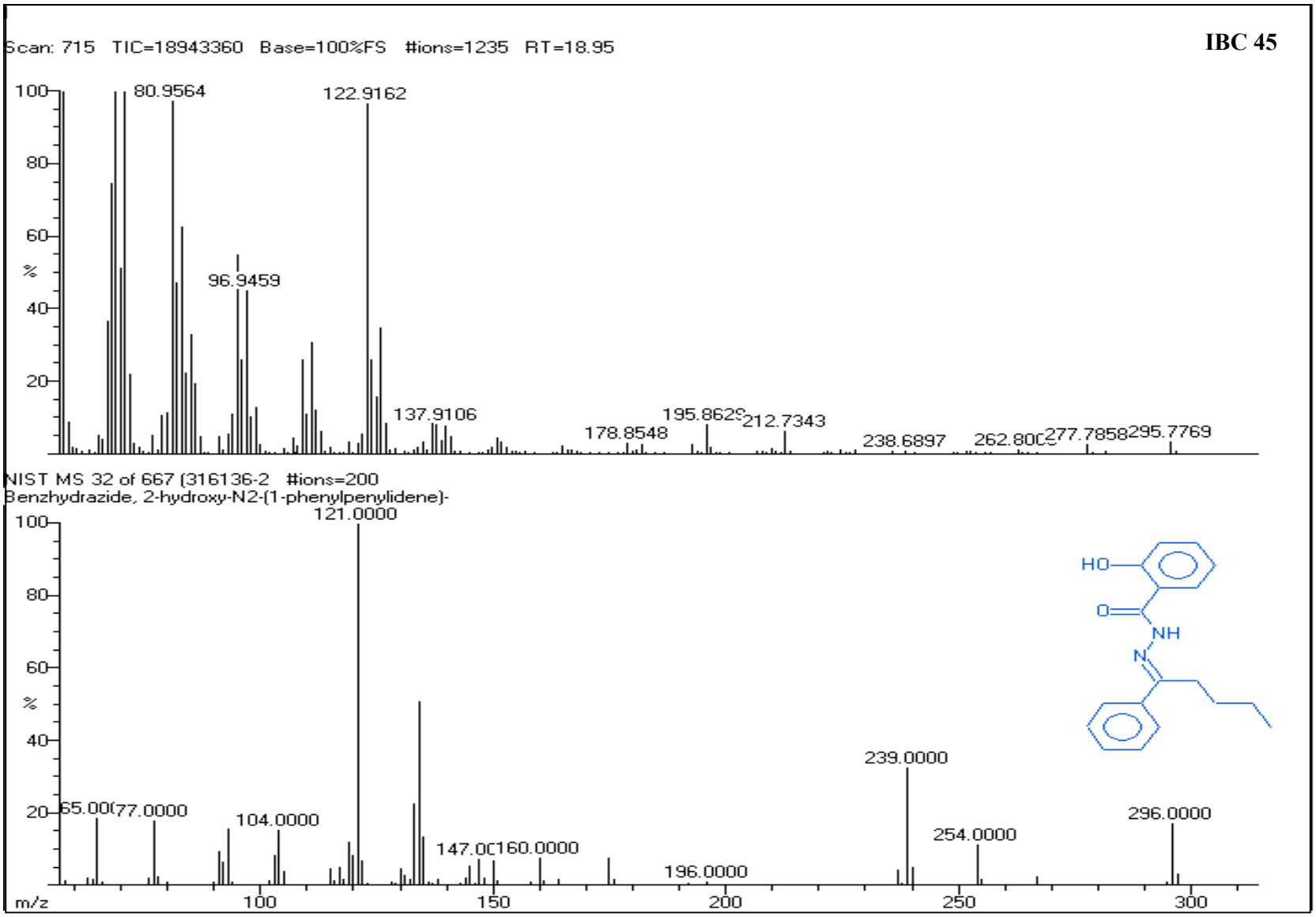
F2 – Processing parameters
SI 32768
SF 500.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

IBC 45




```
Current Data Parameters
NAME        Feb12-2016
EXPNO             31
PROCNO             1

F2 - Acquisition Parameters
Date_         20160212
Time              2.15
INSTRUM          spect
PROBHD  5 mm PABBO BB-
PULPROG        zgpg30
TD              32768
SOLVENT          MeOD
NS                512
DS                  4
SWH         29761.904 Hz
FIDRES       0.908261 Hz
AQ           0.5505524 sec
RG                203
DW             16.800 usec
DE              6.50 usec
TE              298.8 K
D1          2.00000000 sec
D11         0.03000000 sec
TD0                1

======== CHANNEL f1 ========
NUC1              13C
P1             7.80 usec
PL1            0.00 dB
PL1W       70.83519745 W
SFO1       125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2        waltz16
NUC2              1H
PCPD2         80.00 usec
PL2            0.00 dB
PL12          17.51 dB
PL13          18.00 dB
PL2W       23.53637505 W
PL12W       0.41757989 W
PL13W       0.37302643 W
SFO2       500.1320005 MHz

F2 - Processing parameters
SI             32768
SF         125.7577890 MHz
WDW               EM
SSB                0
LB              1.00 Hz
GB                 0
PC              1.40
```

IBC 45


Scan: 715 TIC=18943360 Base=100%FS #ions=1235 RT=18.95
100
80
60
%
40
20
80.9564
122.9162
96.9459
137.9106
178.8548
195.8625
212.7343
238.6897
262.8005
277.7858
295.7769
NIST MS 32 of 667 (316136-2 #ions=200
Benzhydrazide, 2-hydroxy-N2-(1-phenylpenylidene)-
121.0000
65.0000
77.0000
104.0000
147.0000
160.0000
196.0000
239.0000
254.0000
296.0000
m/z
100
150
200
250
300
HO
O
NH
N

8.3. DISCUSSION

Total ethanolic and hexane extracts were prepared from the leaves and stem of *Azima tetracantha* and the percentage yield was determined and tabulated. The ethanolic extract showed more percentage yield compared to other extracts.

Preliminary phytochemical analysis of the extracts was done for the detection of various phytochemical constituents. All the extracts were showed the presence of a variety of phytochemicals. Powders showed that, the presence of alkaloids, flavonoids, oils and steroids with various chemical reagents.

Ethanolic extracts of leaf and stem showed the presence of alkaloids, flavonoids and steroids, hexane extracts of leaf and stem showed the presence of flavonoids and steroids.

Fluorescence properties of the powdered leaf, stem and various extracts were studied and the drug showed no fluorescence.

TLC studies were carried out on the extracts by the use of different mobile phases and detecting agents, to identify the nature of the extracts for further chromatographic studies.

All the extracts (ethanol and hexane of leaf and stem) were evaluated for *invitro* anticancer activity using MCF-7 cell line. It showed that, the ethanolic extract possess anticancer activity in the ng range.

Based on MTT assay, the active extract was selected and performed Column chromatography using various solvents of increasing polarity. 45 fractions were

eluted and pooled together based on its R_f value obtained 15 fractions. The fractions were collected and dried under air current.

All the 15 fractions were screened for their *invitro* antioxidant activity using DPPH assay. The fraction with highest antioxidant effect on DPPH was selected for *invitro* anticancer study.

Fraction possessing isolated bioactive compounds such as IBC16, IBC27, IBC32 and IBC45 which displayed effective inhibition on DPPH in *invitro* anti-oxidant activity were subjected to spectral analysis and with the help of the spectra, the proposed structures were elucidated.

CHAPTER 9

PHARMACOLOGICAL STUDIES

Pharmacological screening is essential to evaluate the efficacy and potency of the plant drug. Isolated bioactive compound was selected for the acute toxicity studies in animal to determine the toxicity of the compound and anticancer screening in various cell lines using MTT assay based on the inhibition of cell lines.

9.1. MATERAIALS AND METHODS

9.1.1. ACUTE TOXICITY STUDY[123,124]

All the animal experiments have been carried out according to the internationally valid guidelines and they are approved by the "Institutional Animal Ethical Committee [IAEC] of Committee for the Purpose of Control and Supervision on Experimentation on Animals [CPCSEA]".

All the isolated bioactive compounds and extract which showed promising *in-vitro* antioxidant activity were subjected to *invivo* acute toxicity studies in order to find out the toxicity induced mortality and other behavioral changes.

The protocol for conducting the *in vivo* study in female adult Wister albino mice was approved by the Institutional Ethical Committee (ICE) of the Madras Medical College, Chennai - 600003, India. **Animal CPCSEA Approval Number: 05/243/CPCSEA, Dated: 9/3/16.**

Materials

- Acute toxicity studies were carried out by acute oral toxic class method (OECD guidelines, 423).

- The animals used for acute toxicity were Swiss Albino mice (20-30 gm) of female sex, n=3/group, with one untreated control group.

- The compounds showing *in-vitro* inhibitory activity on DPPH were selected for acute oral toxicity studies. The selected compound codes were IBC16, IBC 27, IBC 32, IBC 45 and ethanolic extract (EAT).

Experimental

Acute oral toxicity studies

The acute oral toxicity of the isolated bioactive compounds was performed by acute oral toxic class method (OECD guidelines, 423). In this method, the toxicity of the selected isolated bioactive compounds was tested by using a step wise procedure, each step using three mice of a single sex. Before the commencement of study, the animals were kept under fasting for a period of three to four hours. The animals are deprived of food (but not water should be withheld) during the period of study. Following the period of fasting, the animals were weighed and the isolated bioactive compounds were administered orally at a dose of 2000mg/kg/p.o. Animals were observed for any signs and symptoms of mortality and recorded for at least once during the first 30 min and from then for every 4 hrs it was observed in first 24 hrs and the animals are kept under observation for a period of 14 days. Careful observations were made at least twice a day for the effect on CNS, ANS, motor activity, salivation, skin coloration and other general signs of toxicity were also observed and recorded.

The animals were observed for the following gross observations

- Effect on cental nervous system- Stimulation, depression.
- Effect on respiration- Stimulation, depression, respiratory failure
- Effect on locomotor system- Increase in motor activity, reduction in motor activity.
- Effect on skin colour- Blanching, cyanosis, vasodilation
- Effect on excretion- Salivation, lacrimation, urination.
- Other effects- piloerection, tonic or clonic convulsioms, ataxia, death.

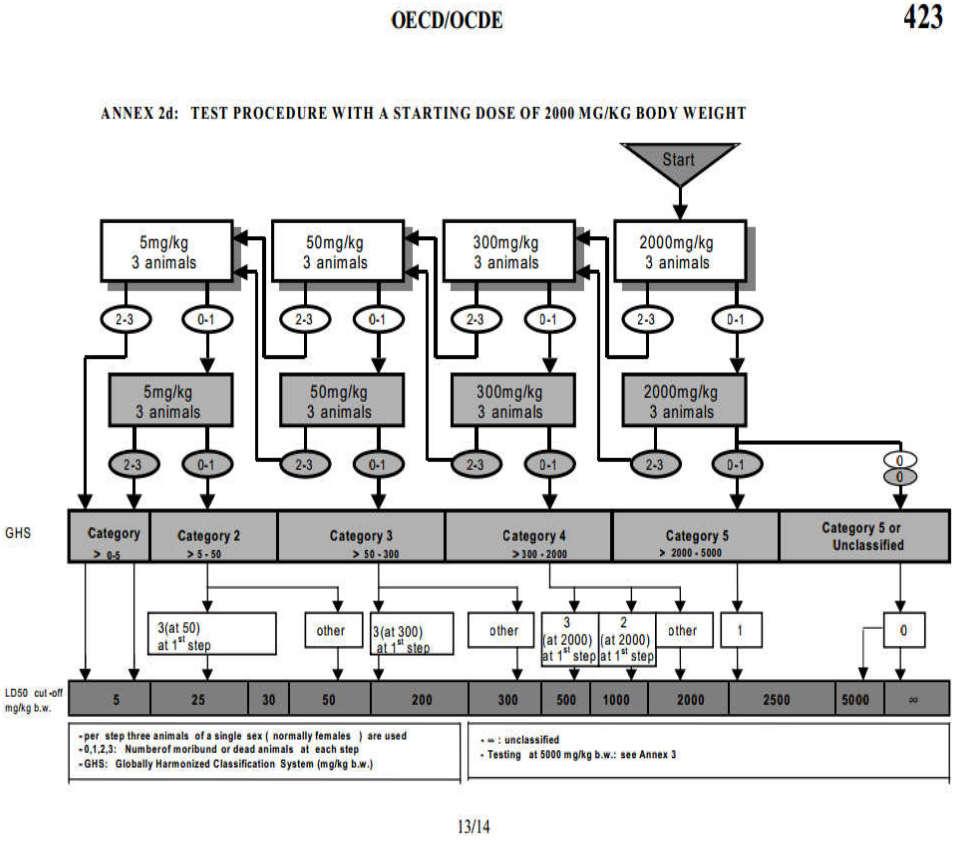


Fig 23: OECD guidelines

9.1.2. IN VITRO ANTICANCER ACTIVITY (MTT ASSAY)[113, 125]

Isolated bioactive compound used: IBC32

Cell line used: MCF-7, A549 and HeLa cell lines

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture

All the cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37◦C in a humidified atmosphere of 5% CO2. Cells were allowed to grow to confluence over 24 hrs before use.

Cell growth inhibition studies by MTT assay

Cell viability was measured with the conventional MTT reduction assay. Briefly, the cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 hrs, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations of test compound was added and incubated for 48 hrs. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37 ◦C for 4 hrs and then with DMSO at room temperature for 1 hr. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments.

Cell viability (%) = Mean OD/Control OD x 100

9.2. RESULTS

ACUTE ORAL TOXICITY

Table 21: Acute oral toxicity of isolated bioactive compounds and extract

Parameters	IBC16	IBC27	IBC32	IBC45	EAT
Aggressiveness	Absent	Absent	Absent	Absent	Absent
Alertness	Present	Present	Present	Present	Present
Convulsions	Absent	Absent	Absent	Absent	Absent
Corneal reflex	Absent	Absent	Absent	Absent	Absent
Gripping strength	Absent	Absent	Absent	Absent	Absent
Lacrimation	Absent	Absent	Absent	Absent	Absent
Pain response	Present	Present	Present	Present	Present
Pinna reflex	Present	Present	Present	Present	Present
Pupils	Normal	Normal	Normal	Normal	Normal
Respiration	Normal	Normal	Normal	Normal	Normal
Restlessness	Present	Present	Present	Present	Present
Righting reflex	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent	Absent
Skin colour	Normal	Normal	Normal	Normal	Normal
Touch response	Present	Present	Present	Present	Present
Tremors	Absent	Absent	Absent	Absent	Absent
Urination	Normal	Normal	Normal	Normal	Normal
Writhing	Absent	Absent	Absent	Absent	Absent
Mortality	Absent	Absent	Absent	Absent	Absent

The selected isolated bioactive compounds and extract **(IBC16, IBC27, IBC32, IBC45 and EAT)** had not shown any signs of toxicity up to 2000mg/kg body weight and was considered as safe (OECD-423 guidelines.

From the acute toxicity studies the data revealed that, all the isolated bioactive compounds and extract proved to be non-toxic at tested 2000mg/kg b.w. dose level and well tolerated by the experimental animals.

IN VITRO ANTICANCER ACTIVITY (MTT ASSAY)

The most effective isolated bioactive molecule was evaluated against various cell lines using MTT assay and reported the inhibition in **Table 22.**

Table 22: *In vitro* anticancer activity of IBC32 on various cell lines

Concentration (µg/ml)	% Inhibition of various cell lines		
	MCF-7	HeLa	A549
50	51.79	28.01	30.75
100	55.62	35.06	33.72
200	57.04	37.73	38.01
400	57.54	46.41	46.41
600	60.56	49.16	45.63
800	62.61	51.68	52.16

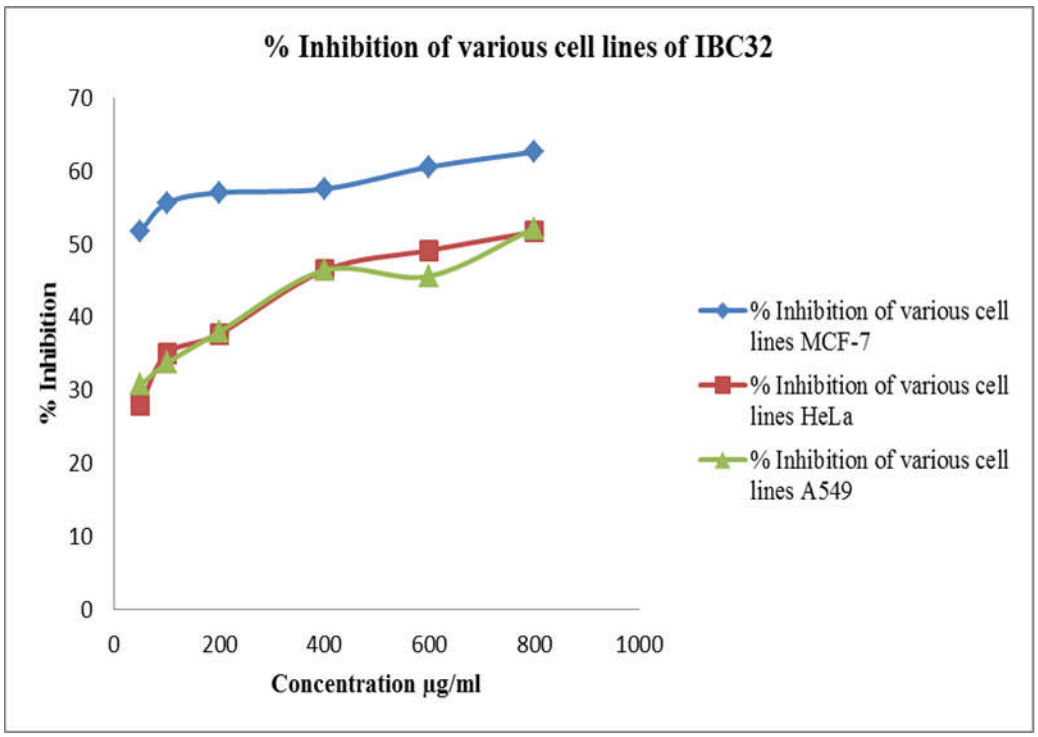


Fig 24: Percentage inhibition of various cell lines of IBC32

9.3. DISCUSSION

Acute toxicity studies of the isolated bioactive compounds (IBC16, IBC27, IBC32 and IBC45) and the corresponding extract revealed that, the drug was non-toxic upto the dose level of 2000mg/kg body weight.

Four compounds (**IBC16**, **IBC27, IBC32** and **IBC458)** which displayed effective inhibition on DPPH in *invitro* anti-oxidant activity were studied for their *in-vitro* anticancer potential using various cell lines. It was found that compound **IBC32** was active in *in-vitro* antioxidant assay, when compared to the other tested compounds.

Fraction **(IBC32)** showed highest activity on DPPH, further evaluated for the *invitro* anticancer potential on various cell lines such as MCF-7, HeLa and A549 cell lines using MTT assay. It was concluded that, the isolated bioactive compound most effective on MCF-7 cell line when compared to other cancer cell lines.

CHAPTER 10

DOCKING STUDIES

Docking is the technique which envisages the preferred orientation of one molecule to a second when bound to each other to form a stable complex in three dimensional spaces. In cell biology, the function of proteins is a result of its interaction (i.e. docking) with other proteins as well as other molecular components. Thus the results of the docking are exceptionally beneficial in finding drugs which are effective against particular disease. Knowledge of the favored orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring function.[126]

10.1. DOCKING PRINCIPLE

The two main components that are important for docking studies are:

- Secondary structure of our protein of interest.
- Library of ligands from suitable data base.

Docking tools are based on the search, algorithm and the scoring function. A search algorithm finds the best docking pose measured by the scoring function. A scoring function differentiates correct docking poses from incorrect ones.

The quality of any docking results depends on the reasonable starting structures of both the protein and the ligand. The protein and ligand structures require preparation before docking in order to achieve the best docking results.

Types of Docking[127]

Lock and Key or Rigid Docking- In rigid docking, both the internal geometry of the receptor and ligand is kept fixed and docking is performed.

Induced Fit or Flexible Docking- an enumeration on the rotations of one of the molecules (usually smaller one) is performed. Every rotation the surface cell occupancy and energy is calculated; later the most optimum pose is selected.

Molecular Docking by Argus Lab software

Docking analysis of bioactive compounds i.e. ligands was carried out by Argus lab docking software. Docking virtually screen a set of compounds and predict the strongest binding capacity based on various scoring function. It explores ways in which two molecules such as ligand and receptor (protein) fit together and docks to each other well. The molecule binding to a receptor inhibits its function and thus acts as drug.

Argus lab 4.0 distributed freely for windows platforms by planaria software. It is an introductory molecular modeling package with academics. Argus docking engine implementry in Argus lab approximates an exhaustive search method which is similar to DOCK and GLIDE. Flexible ligand docking is possible with Argus lab, where the ligand is described as torsion tree and grids are constructed that overlay the binding site. The accuracy of the Argus lab docking algorithm takes into account, the key features such as the nature of the binding site and the number of rotatable bonds to the ligand.

Molegro molecular viewer: Molegro molecular viewer is an application which helps in analyzing the energies and interaction of the binding site.

Protein and Preparation

A typical PDB (Protein Data Bank) structure file might be multimeric, which consists of heavy atoms, waters, cofactors, and metal ions. The structure usually has no information on bond orders, topologies, or formal atomic charges.

The following course of actions is necessary to make a protein perfect for docking study:

- Assigning ionization and tautomeric states of protein properly. (Side chains are reoriented when necessary and steric clashes are relieved).
- Deleting all water molecules (except those coordinated to metals/bridged water molecules, if water molecules are retained then hydrogen's should be added to them).
- Adjusting the protonation of the protein, as this is crucial in the metalloprotein receptor sites.
- Minimizing the protein to reorient side-chain hydroxyl groups and alleviating potential steric clashes present in the PDB structure.

Ligand Preparation

In order to get the best results, the ligands that are docked must be of good representations as they would appear in a protein-ligand complex. Most of the docking tools only modify the torsional internal coordinates of the ligand during docking; therefore the rest of the geometric parameters must be optimized beforehand.

The structures subjected to docking tool must meet the following requirements:

- They should be three-dimensional (3D).
- They should have realistic bond lengths and bond angles.
- They should each consist of a single molecule that has no covalent bonds to the receptor, with no accompanying fragments, such as counter ions and solvent molecules.
- They should have all their hydrogen's (filled valences).
- They should have an appropriate protonation state for physiological pH values.

Binding Site Analysis

Knowledge of the structure and function of protein binding sites is the cornerstone of structure- based drug design and this requires understanding of both the location and physical properties of the binding site. In addition, the identification of small-molecule binding sites as modulators of protein-protein interactions has gained increasing interest. Furthermore, even when a validated binding site has been significant, it is often noteworthy to find additional potential binding sites where appropriate targeting could result in diverse biological effects.

Scoring Methods

Scoring of docked poses is however considered as one of the major challenges in the area of molecular docking. The objective of the scoring procedure is the identification of the correct binding pose by its lowest energy value, and the ranking of protein-ligand complexes according to their binding affinities.[128-130]

BIOLOGICAL TARGET

There are various biosynthetic enzymes that are essential for the survival of the human and are considered as potential drug targets. Some of the targets are

- Human PGK in complex with L-ADP, 3PG And TSA Aluminium tetrafluoride (2Y3I)
- Cytochrome b5 core swab mutant (3OZZ)
- Top BP1 BRCT4/5 domain (3UEN)
- Human PGK1 3PG and tetrazosin (TZN ternary complex, 4O33)

2Y3I

2Y3I is a 2 chain structure with sequence from Homo sapiens. Defects in PGK1 are the cause of phosphoglycerate kinase 1 deficiency (PGK1D). It is a condition with a highly variable clinical phenotype that includes hemolytic anemia, rhabdomyolysis, myopathy and neurologic involvement. Patients can express one or more of these manifestations.[131-133]

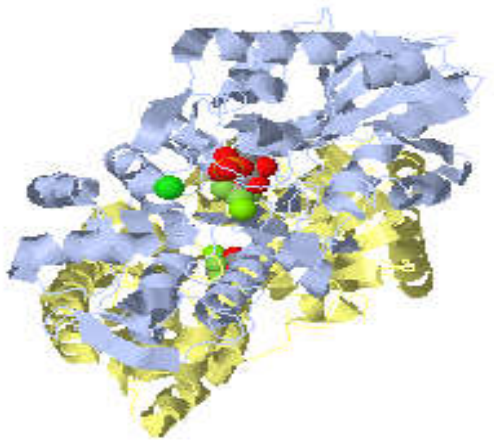


Fig 25: 3D Structure of 2Y3I

3OZZ

3OZZ is a 1 chain structure with sequence from Bos taurus. Cytochrome b5 is a membrane bound hemoprotein which function as an electron carrier for several membrane bound oxygenases.

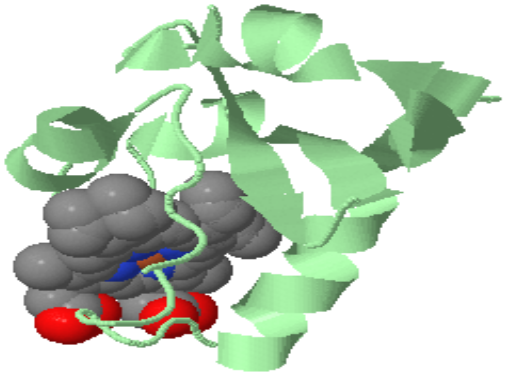


Fig 26: 3D Structure of 3OZZ

3UEN

3UEN is a 1 chain structure with sequence from Human. It is required for DNA replication. It plays a role in the rescue of stalled replication forks and checkpoint control. It binds double-stranded DNA breaks and nicks as well as single-stranded DNA. It recruits the SWI/SNF chromatin remodeling complex to E2F1-responsive promoters. It also down-regulates E2F1 activity and inhibits E2F1-dependent apoptosis during G1/S transition and after DNA damage.[131,134-137]

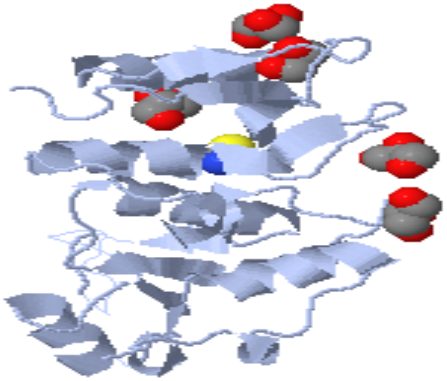


Fig 27: 3D Structure of 3UEN

4O33

4O33 is a 1 chain structure. Defects in PGK1 are the cause of phosphoglycerate kinase 1 deficiency (PGK1D). It is a condition with a highly variable clinical phenotype that includes hemolytic anemia, rhabdomyolysis, myopathy and neurologic involvement. Patients can express one or more of these manifestations.[138]

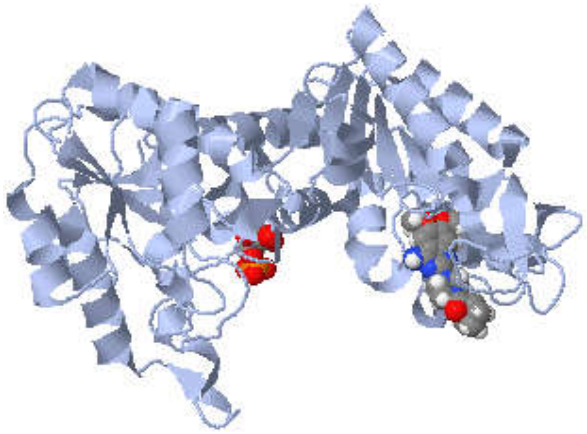


Fig 28: 3D Structure of 4O33

10.2. EXPERIMENTAL

Ligand preparation

- Draw the structure from chem. Sketch and save as MDL mol format.
- Import the ligand into workspace of Argus lab.
- Clean geometry → clean hybridization.
- Select the ligand, right click on the mouse → make a group from the residues → give name→ ligand→ OK.

Protein preparation

Step 1:

- Enter protein PDB-ID (2Y3I, 3OZZ, 3UEN, 4O33) in the protein data bank.
- Go to download files and select pdb as text file.
- Save the downloaded pdb text file to desktop.

Step 2:

- Open Argus lab file → open → Import pdb file from the desktop.
- 3D structure of the protein will appear in the workspace of Argus lab.
- Left side of the screen shows molecular tree view.
- Open pdb → open 'Residues' → open 'Misc'.
- From 'Misc' delete the inhibitor and hetero residues, do not delete cofactor.
- Open water press shift, select all water molecules and delete.
- Add hydrogen atoms.
- Go to calculation on toolbar → energy by UFF method → start.
- Save the prepared protein as *.agl file format in the desktop.

Docking Parameters

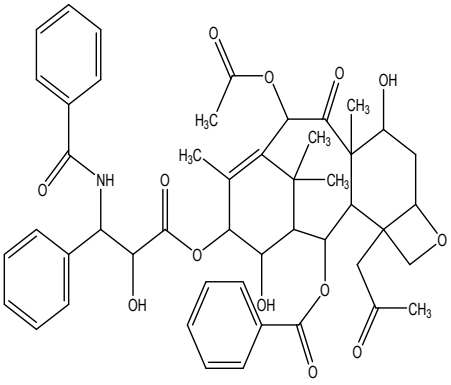
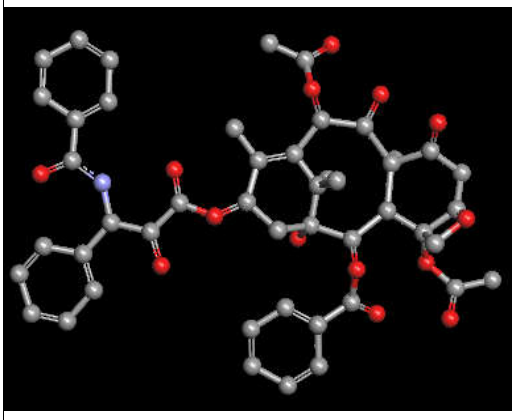
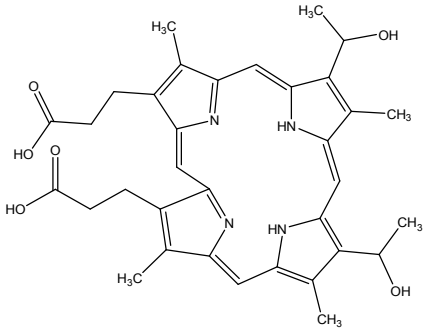
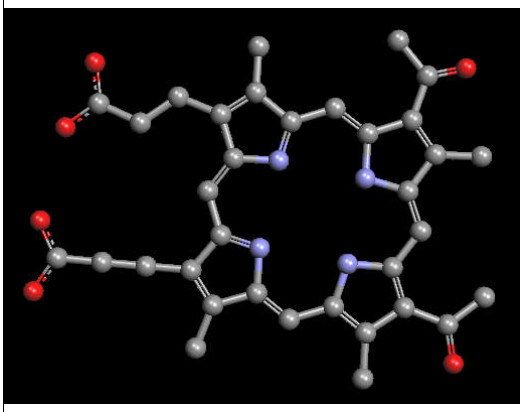
- Select calculation from the toolbar → Dock a ligand
- 'Argus Dock' as the Docking engine
- 'Dock' was selected as calculation type
- 'Flexible' for the ligand
- Ascore as the scoring function
- Calculation size
- Start docking
- Save the Docked protein Ligand complex as Brookhaven pdb files (*.pdb)

Visualization/ Interpretation of Docking

Molegro molecular viewer will help in analyzing the energies and interaction of the binding.

10.3. RESULTS

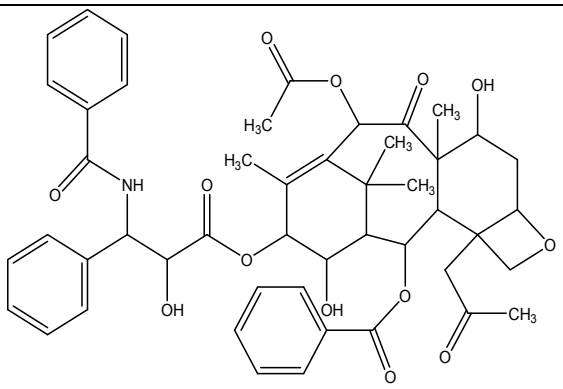
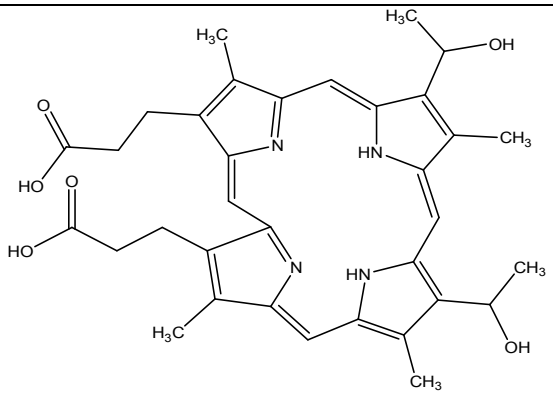
Table 23: 2D and energy minimized 3D structures of standard and IBC32

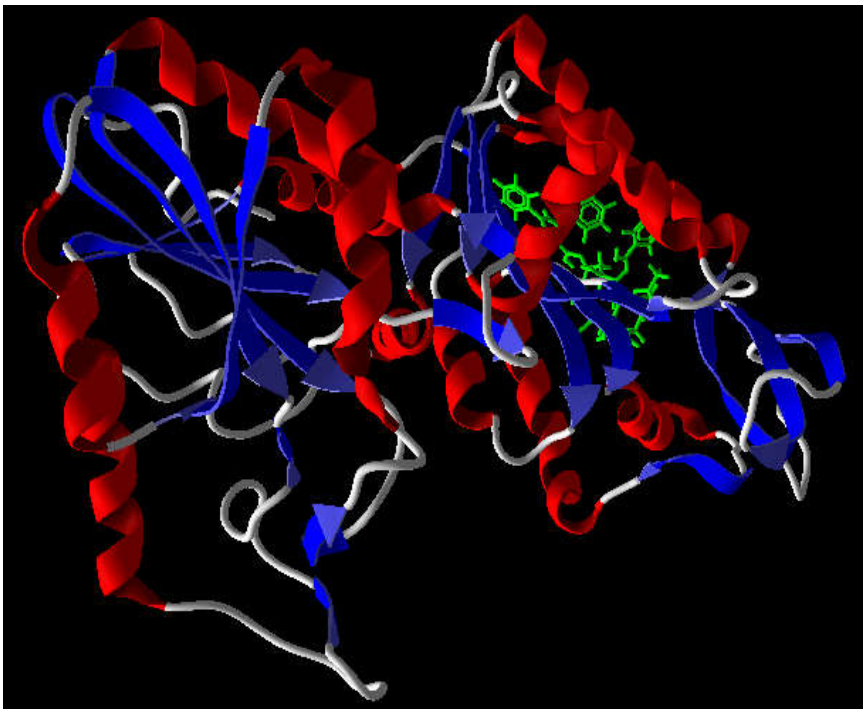
Ligand code	2D structure	Energy minimized 3D structure
PAC		
IBC32		

Docking scores of compounds against multiple target enzymes

The docking scores of the compounds ranges from -12.79 to-8.92 kcal/mol on all the target enzymes and were summarized in **Table 24, 25, 26 and 27.**

Table 24: Docking scores of compounds on PDB-ID 4O33

Ligand code	Structures	PDB-ID	Docking Scores (Kcal/mol)
PAC		4O33	-11.0293
IBC32			-11.0687



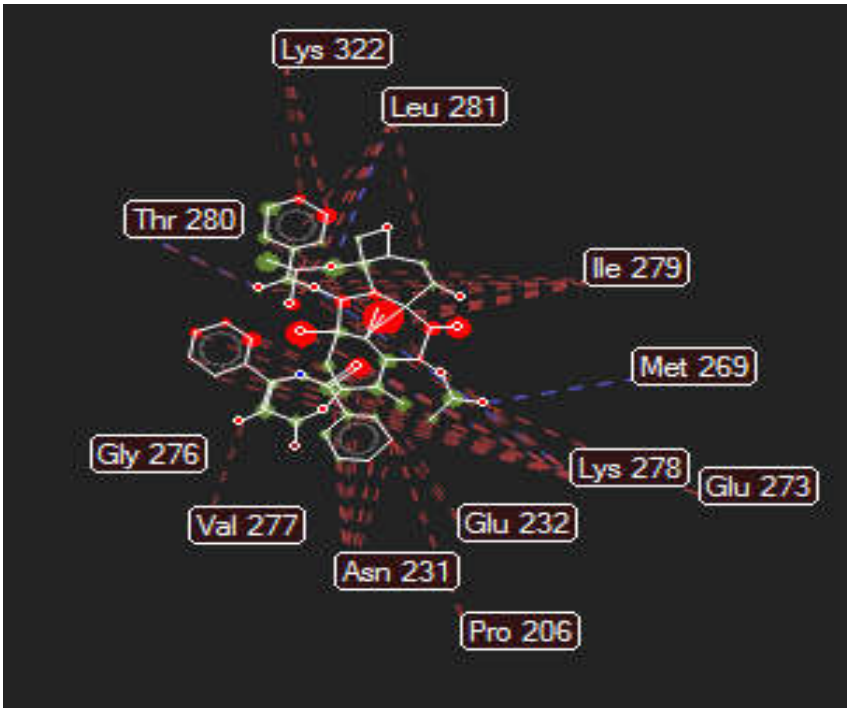
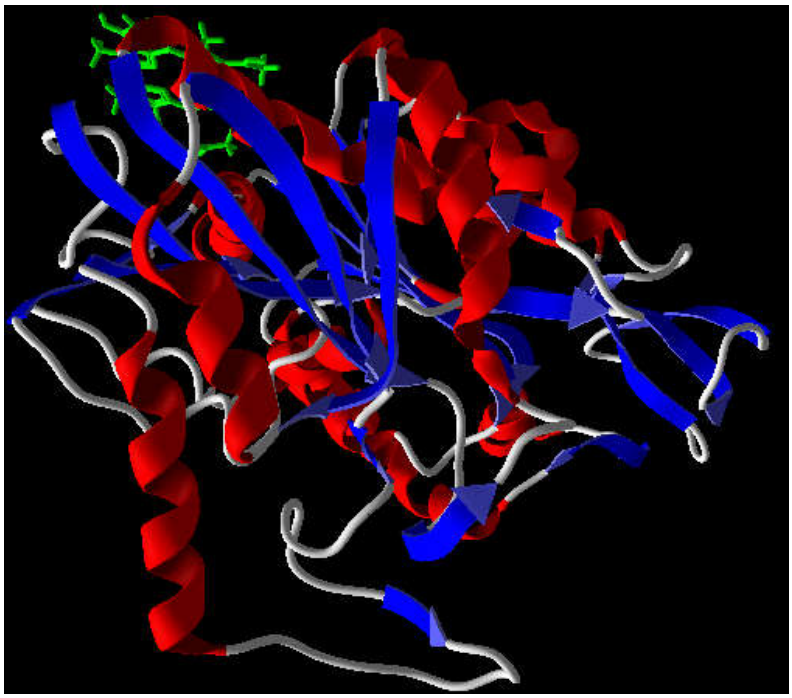


Fig 29: Paclitaxel docked with PDB-ID 4O33 and its interactions



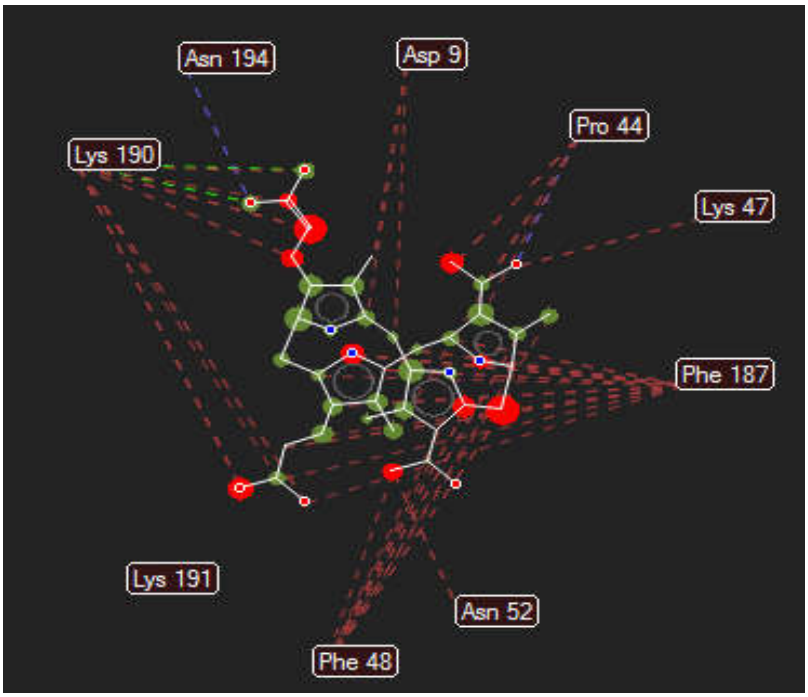
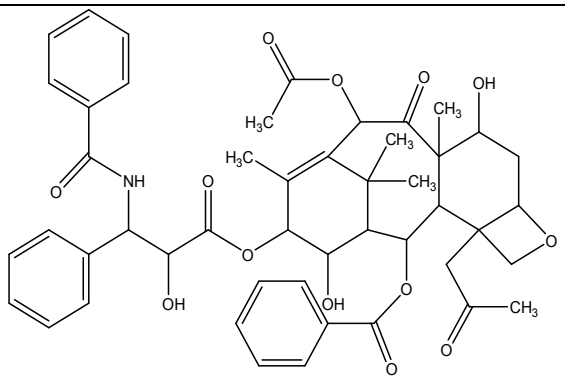
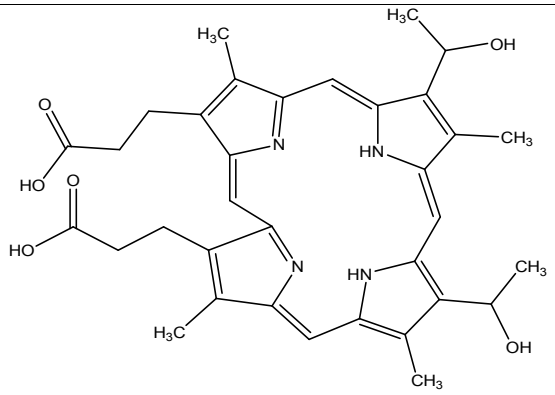
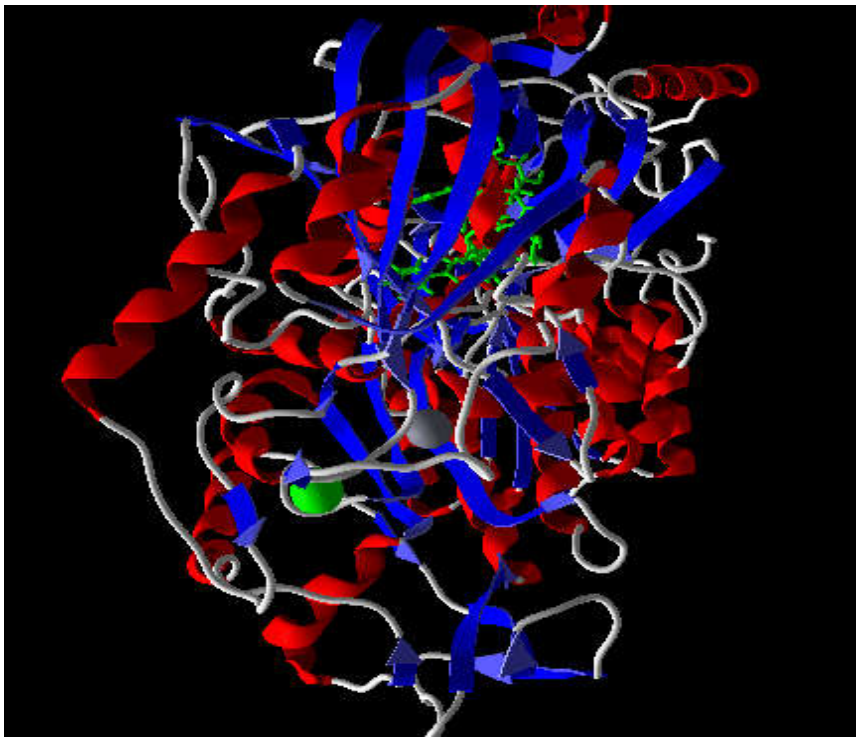


Fig 30: IBC32 docked with PDB-ID 4O33 and its interactions

Table 25: Docking scores of compounds on PDB-ID 2Y3I

Ligand code	Structures	PDB-ID	Docking Scores (kcal/mol)
PAC		2Y3I	-12.7911
IBC32			-10.7869



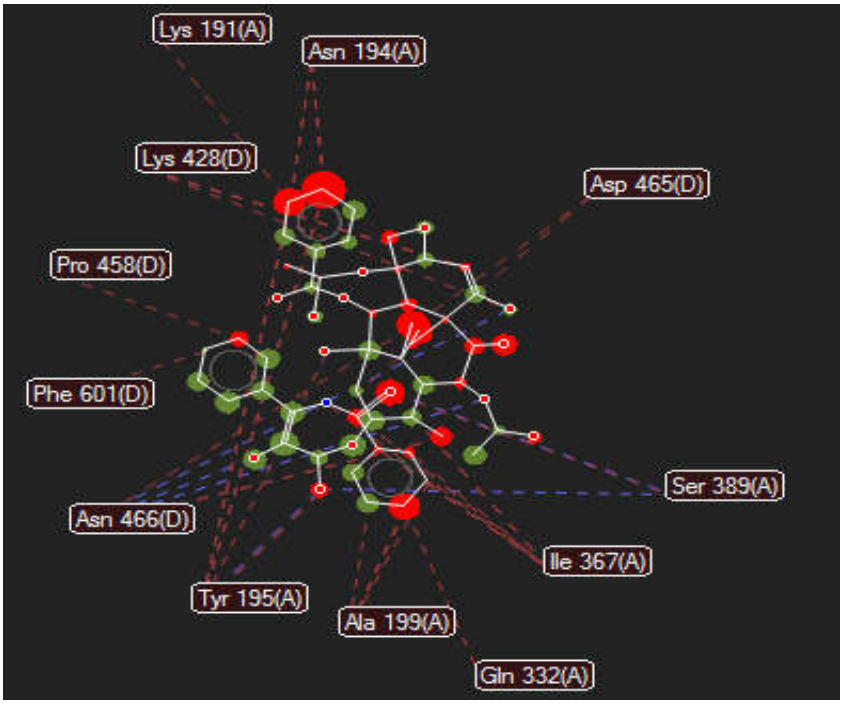
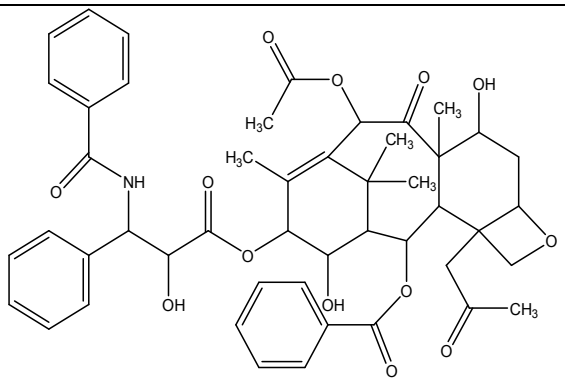
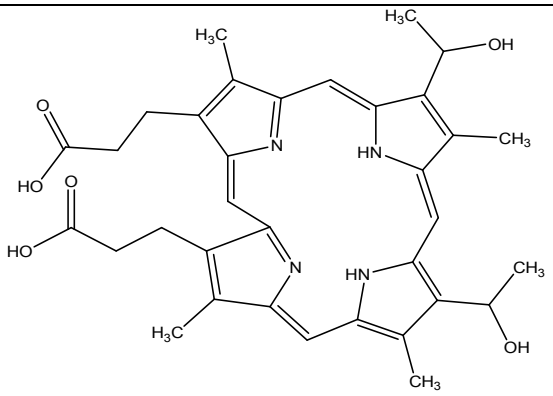


Fig 31: Paclitaxel docked with PDB-ID 2Y3I and its interactions

Fig 32: IBC32 docked with PDB-ID 2Y3I and its interactions

Table 26: Docking scores of compounds on PDB-ID 3UEN

Ligand code	Structures	PDB-ID	Docking Scores (kcal/mol)
PAC		3UEN	-11.1698
IBC32			-8.9262

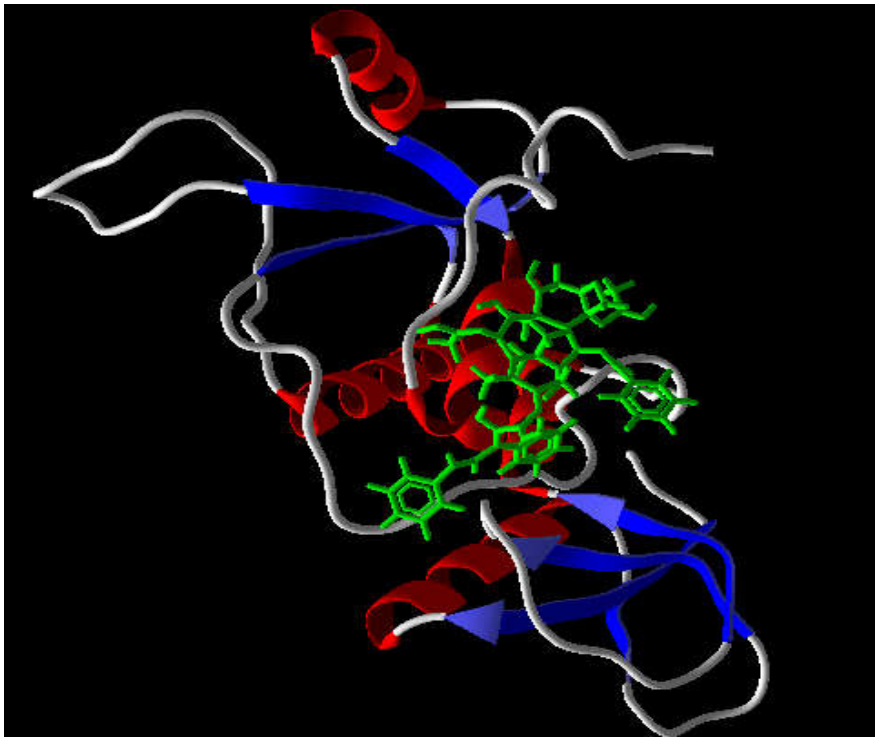
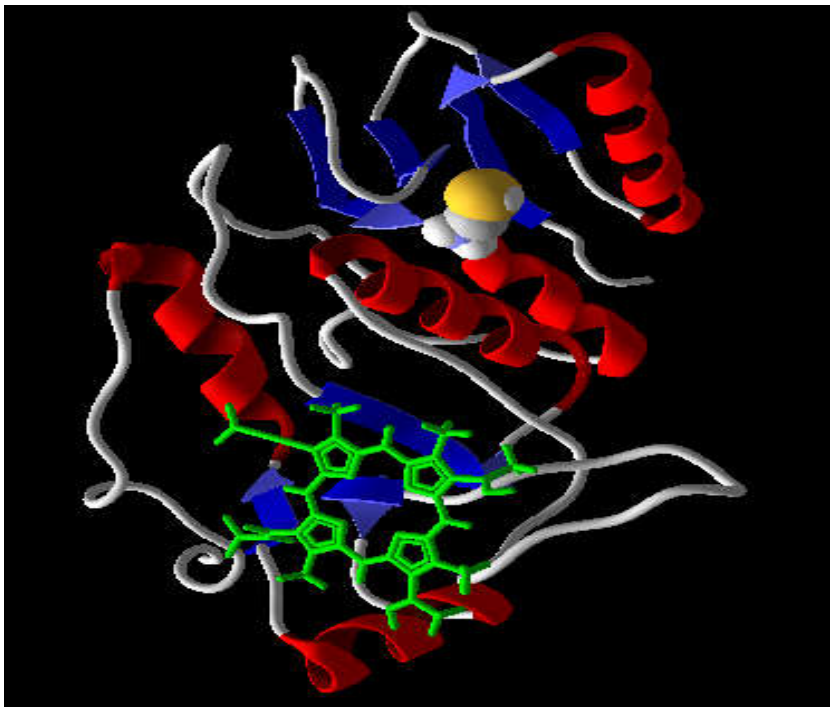


Fig 33: Paclitaxel docked with PDB-ID 3UEN and its interactions



7

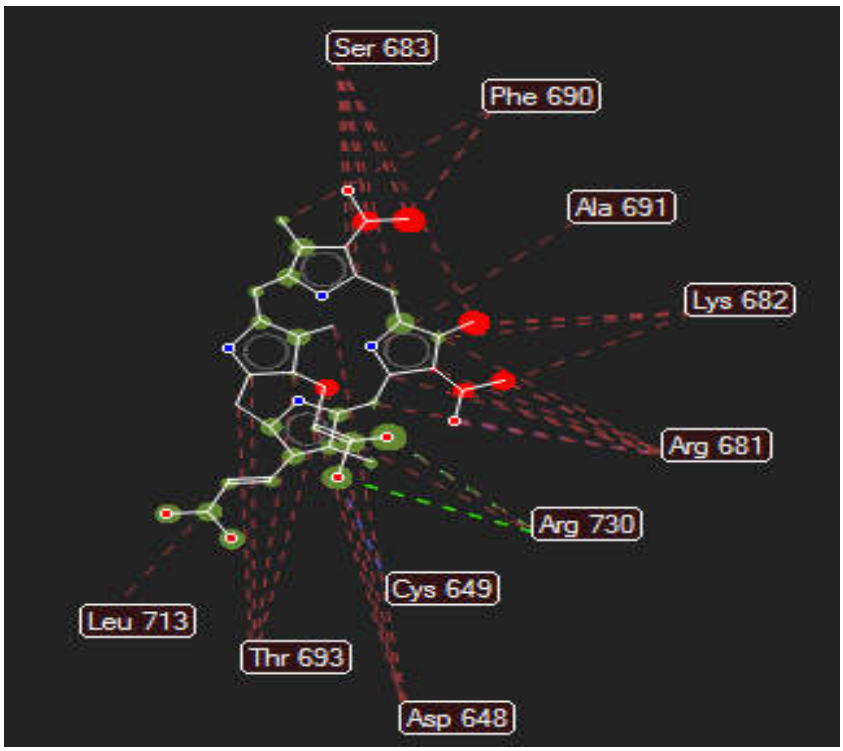
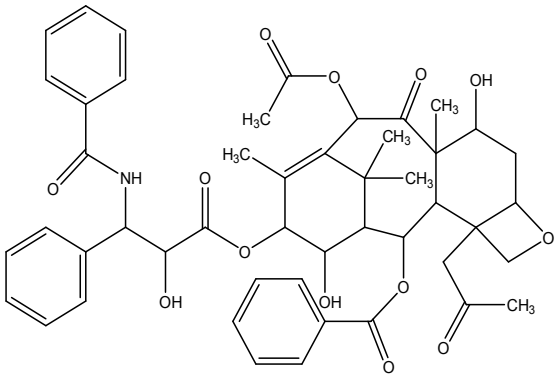
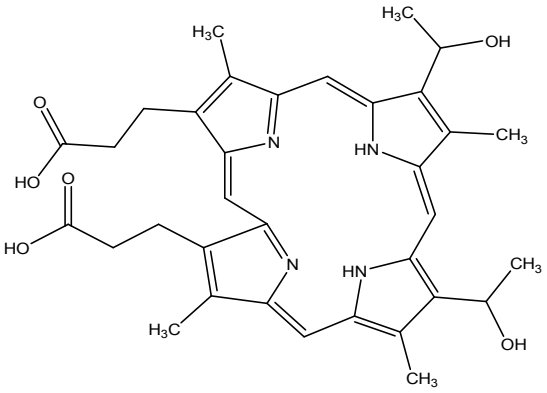


Fig 34: IBC32 docked with PDB-ID 3UEN and its interactions

Table 27: Docking scores of compounds on PDB-ID 3OZZ

Ligand code	Structures	PDB-ID	Docking Scores (kcal/mol)
PAC		3OZZ	-11.6924
IBC32			-10.0192

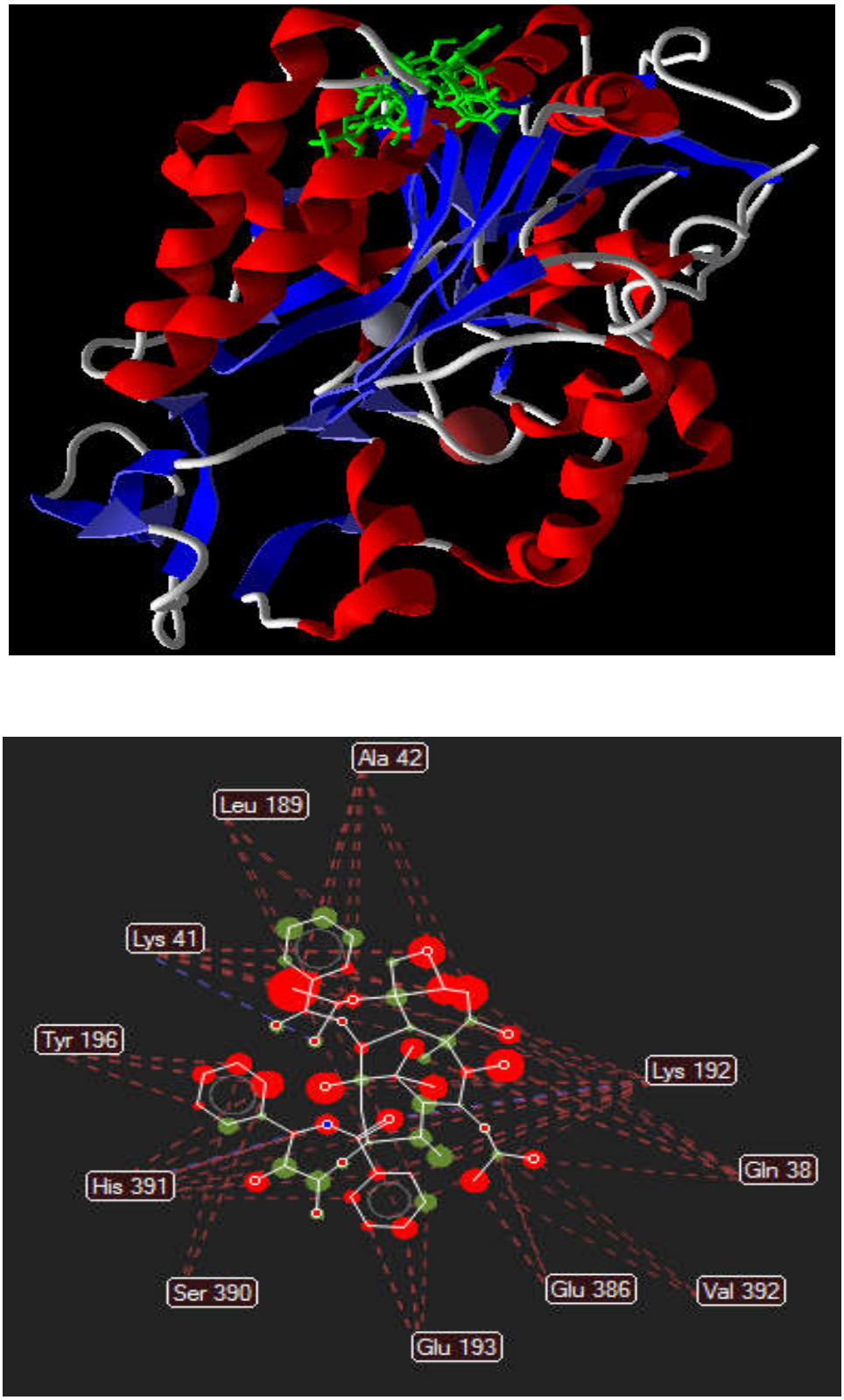


Fig 35: Paclitaxel docked with PDB-ID 3OZZ and its interactions

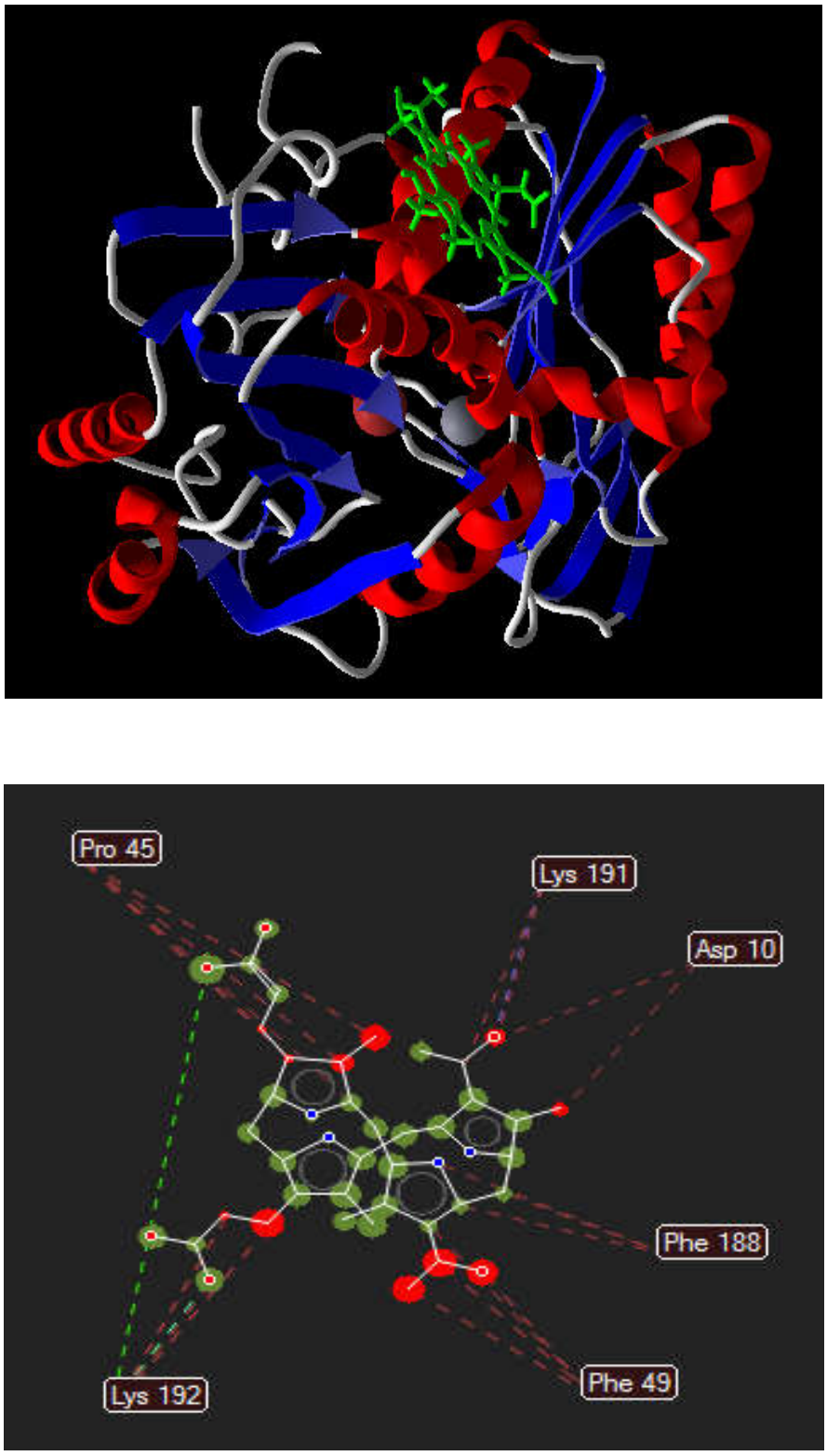


Fig 36: IBC32 docked with PDB-ID 3ZOZ and its interactions

10.4. DISCUSSION

CADD computational tools and softwares are used to simulate drug receptor interactions. Computational drug discovery helps scientists to get an insight into the drug- receptor interactions and also helps to reduce the time and cost.

The isolated bioactive compound was predicted to be effective against cancer through computational studies. This was achieved by the molecular docking studies against the target enzymes such as (PDB ID- 4O33, 2Y3I, 3UEN, 3ZOZ) of cancer. IBC 32 scored-11.0687kcal/cal when docked with PDB-ID 4O33 whereas the standard (Paclitaxel) scored -11.0293kcal/mol. Docking of IBC32 on other enzymes (PDB-ID 2Y3I, 3UEN and 3OZZ) also showed relatively nearer scores with that of standard.

CHAPTER 11

SUMMARY AND CONCLUSION

The thesis entitled "Evaluation of anticancer potential of leaves and stem of *Azima tetracantha* Lam" deals with pharmacognostical, phytochemical, toxicological and pharmacological investigations of *Azima tetracantha* Lam. A thorough literature survey revealed that *Azima tetracantha* highlighted for its valuable phytochemicals (azimine, azcarpaine and carpaine) and has an important role in antidiabetic, anticancer and hepatoprotective studies. This work was designed for the first time to notice isolation, characterization and docking of isolated bioactive compounds present in it and to establish its folklore claim on anticancer activity of the plant.

Recently, there is a progressive interest for natural antioxidant discovery for two valid reasons. 1. Epidemical and clinical evidences heighted the importance of fruits and vegetables in controlling chronic disease like cancer, 2. Phytochemicals are safer than synthetic chemicals.

The pharmacognostical evaluation of a species makes a valuable tool for the identification of the plant *Azima tetracantha.* Morphological study has provided a characteristic identity of the plant which was pale green colored, branchlets is terete or quadrangular.

The various distinguishing features observed through anatomical studies. The transverse section of the leaf showed dorsiventral nature and there was a distinction between upper and lower side of the leaf surface.

The powder microscopical examination of leaves exhibit the presence of parenchyma, sclerenchyma, trichomes, fibers, stomata, crystal sheath and xylem vessels and stem powder showed the presence of epidermis, fibres, pigments and xylem vessels.

Quantitative microscopic data can be helpful in the determination of the authenticity of the plant. Various physicochemical parameters such as ash values, extractive values, loss on drying and foreign organic matter were found to substantiate its standard.

The qualitative preliminary phytochemical screening was performed to detect the nature of phytoconstituents present in it. The ethanolic extract showed the presence of alkaloids, steroids, flavonoids etc. and its hexane extract showed the presence of flavonoids and alkaloids.

Fluorescence properties of the powdered leaf, stem and various extracts were studied and the drug showed no fluorescence.

Thin layer chromatography of various extracts was carried out for the identification of the nature of the extracts for further chromatography studies by the use of different mobile phases and detecting agents.

Any significant deviation in the percentage of any parameters reported in this work may indicate adulteration (or) substitution in the drug. In this mineral analysis, presence of calcium, ron, sodium and potassium added its nutritional value and may be utilized as nutritional supplements in mineral deficiency disorder. The pharmacognostical and phytochemical details evolved from *Azima tetracantha* would help to fix up its standard and its adulterant/ substitution from other related species.

The pharmacological studies revealed that the extracts showed significant anticancer property. In MTT assay method, ethanolic extract showed inhibition of MCF-7 cell line in the ng range. Hence the ethanolic extract was packed in column to scrutinize the phytoconstituents present in it.

45 fractions were eluted and TLC analysis was carried out. Based on R_f value, the samples are pooled together and get 15 fractions.

All the 15 fractions were screened for its *invitro* antioxidant activity using DPPH assay. The fraction with highest antioxidant effect on DPPH was selected for *invitro* anticancer study. Fraction possessing isolated bioactive compounds such as IBC16, IBC27, IBC32 and IBC45 which displayed effective inhibition of DPPH in *invitro* anti-oxidant activity were subjected to spectral analysis and with the help of the spectra, the proposed structures were elucidated.

The spectral data confirms the presence of various phytoconstituents in ethanolic extract and the chemical name of the compounds 1, 2, 3 and 4 were identified as 7,12-bis(1-hydroxyethyl)-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoic acid, 9-oximino-2,7-diethoxyfluorene, 5,7,9(11)- androstatriene, 3-hydroxy-17-oxo and benzhydrazide,2-hydroxy-N2-(1-phenylpenylidene) respectively.

All the compounds were structurally elucidated and the compound one was identified and confirmed as **"Hematoporphyrin".** This compound was isolated from *Azima tetracantha* for the first time.

In acute toxicity studies (OECD guidelines 423), the extract and isolated bioactive compounds did not produce any significant changes in body weight and were free of toxicity.

Four compounds (**IBC16**, **IBC27, IBC32** and **IBC45)** which displayed effective inhibition on DPPH in *invitro* anti-oxidant activity were studied for their *invitro* anticancer potential using various cell lines. It was found that compound **IBC32** was active in *invitro* antioxidant assay, when compared to the other tested compounds.

The isolated bioactive molecule **(IBC32)** showed highest activity on DPPH, further evaluated for the *invitro* anticancer potential on various cell lines such as MCF-7, HeLa and A549 cell lines using MTT assay. It was concluded that, the isolated bioactive compound most effective on MCF-7 cell line when compared to that of other cancer cell lines.

Finally, the docking study of the compound was performed by Argus lab 4.0 software and the result concluded that has good binding interaction with biomarker enzyme and proved for its anticancer activity in molecular modeling study. It was also interesting to notice that, the compound **IBC32** showed good docking scores when compared to standard paclitaxel.

This scientific study concluded that the plant *Azima tetracantha* and its isolated bioactive compound **(IBC32)** have potent anticancer activity. Further research work has to be carried out for the formulation of isolated bioactive compound. Finally, it has to be screened for preclinical and clinical studies against cancer.

CHAPTER 12

BIBILIOGRAPHY

1. Irfan AK, Atiya K. Herbal therapy for human diseases. Ukaaz Publications. 2007; p. 16.
2. Rang and Dales. Pharmacology, 6th edition. Elsevier, New York. 2010; 718-21.
3. Bolsover SR, Hymas JS, Jones S, Sephard EA, et al. From genes to cells. Wiley-Liss Inc. Publication. 1997; 70-1.
4. Defining cancer. National Cancer Institute. Retrieved 10 June 2014.
5. Kumar V, Abbas AK, Aster JC. Robbins basic pathology, 9th edition. Elsevier. 2013; 161-214.
6. Mohan H. Text book of pathology, 6th edition. Jaypee Brothers Publishers (P) Ltd. 2010; 192-235.
7. Kumar V, Abbas A, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, 8th edition. Elsevier. 2012; 259-330.
8. Hajdu SI, Thun MJ, Hannan LM, Jemal A. A note from history: landmarks in history of cancer, part 1. Cancer. 2011; 117 (5): 1097-102.
9. Paul of Aegina, 7th Century AD, quoted in Moss, Ralph W. "Galen on Cancer". Cancer Decisions. Michael Shimkin, Contrary to Nature, Washington, D.C.: Superintendent of Document. 2004; 79-720, p. 35.
10. Majno G, Joris I. Cells, tissues and disease: Principles of general pathology. Oxford University Press. 2004.
11. Eddy WH, Streltzov L, Williams J, Sciortino L. The effect of negative ionization on transplanted tumors. Cancer Research. 1951; 11 (4): p. 245.

12. www.who.int/cancer. Cancer World Health Organization NMH Fact sheet January 2010.
13. Macmillan cancer support. Curd-but at what cost? Long term consequences of cancer and its treatment. 2013. http://www.macmillan.org.uk.
14. Macmillan cancer support. The road to recovery: Getting back to work. 2007. http://www.macmillan.org.uk.
15. http://www.nationalbreastcancer.org/what-is-cancer. Retrieved on October 6, 2015.
16. Sudhakar A. History of cancer, ancient and modern treatment methods. Journal of Cancer Science and Therapy. 2009; 2 (i-iv): 2.
17. Varricchio CG. A cancer source book for nurses. Bosten: Jones and Bartlett Publishers. 2004; p. 229.
18. http://www.cancerresearchuk.org/about-cancer/what-is-cancer. Retrieved on October 6, 2015.
19. Jemal A, Bray F, Center MM, Ferlay J, et al. Global cancer statistics. CA: A Cancer Journal for Clinicians. 2011; 61(2): 69-90.
20. Lozano R, Mohsen N, Foreman K, Lim S, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2013; 380 (9859): 2095-128.
21. WHO (October 2010). Cancer. World Health Organization. Retrieved 5 January 2011.
22. Croce CM. Oncogenes and cancer. New England Journal of Medicine. 2008; 358(5): 502-11.

23. Knudson AG. Two genetic hits (more or less) to cancer. Nature Reviews Cancer. 2001; 1(2): 157-62.

24. http://www.cancer.org/cancer/cancerbasics/signs-and-symptoms-of-cancer. Retrieved on January 4, 2016.

25. Kufe DW, Pollock RE, Weichselbaum RR, Bast RC, et al. Holland-Frei cancer medicine. 6th edition. BC Decker. 2003.

26. Ebadi M. Pharmacodynamic basis of herbal medicine, 2nd edition. Taylor and Francis. 2009.

27. Bomford CK, Kunkler IH, Walter J. Walter and Miller's Textbook of Radiation therapy 6th edition. P. 311.

28. Waldmann TA. Immunotherapy: Past, present and future. Nature Medicine. 2003; 9 (3): 269-77.

29. https://en.wikipedia.org/wiki/Management_of_cancer. Retrieved on January 4, 2016.

30. Lind MJ. Principles of cytotoxic chemotherapy. Medicine. 2008; 36 (1): 19-23.

31. National Cancer Institute (Dec 2012). Targeted Cancer Therapies. www.cancer.gov. Retrieved 9 March 2016.

32. Nastoupil LJ, Rose AC, Flowers CR. Diffuse large B-cell lymphoma: Current treatment approaches. Oncology. 2012; 26 (5): 488.

33. Freedman A. Follicular lymphoma: 2012 update on diagnosis and management. American journal of hematology. 2012; 87 (10): 988-95.

34. Rampling R, James A, Papanastassiou V. The present and future management of malignant brain tumours: surgery, radiotherapy, chemotherapy. Journal of Neurology, Neurosurgery & Psychiatry. 2004; 75 (suppl 2): ii24-30.

35. Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. Lancet. 2010; 375 (9715): 673-85.

36. http://cancerlive.net/cancer-treatment/advantages-and-disadvantages-of-cancer-treatment/. Retrived on March 8, 2016.

37. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. Journal of Natural Products. 1997; 60: 52-60.

38. Cragg GM, Newman DJ. Discovery and development of antineoplastic agents from natural sources. Cancer Invest. 1999; 17: 153-63.

39. Wani MC, Taylor HL, Wall ME, Coggon P, et al. Plant antitumour agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumour agent from *Taxus brevifolia*. Journal of American Chemical Society. 1971; 93: 2325-27.

40. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Current Medicinal Chemistry. 2001; 8:1467-86.

41. http://herbs.lovetoknow.com/Advantages_and_Disadvantages_of_Herbal_Medicine retrived on March 8, 2016.

42. Balakrishnan M, Dhanapal R, Vamsi LM, Chandra Sekhar KB. Studies on pharmacognostical specifications of *Azima tetracantha* Lam. International Journal of Phytopharmacology. 2010; 1(1): 35-42.

43. Vinoth B, Manivasagaperumal R. Antimicrobial activity of different extracts of *Azima tetracantha* root. International Journal of Pharma and Bio Sciences. 2015; 6 (2): 613-20.

44. Gowthami M, Tamil Selvi S, Senthil Kumar G, Panneerselvam A. Phytochemical analysis and antibacterial properties of leaf extract of *Azima tetracantha* (Lam.). Asian Journal of Plant Science and Research. 2012; 2 (2): 110-14.

45. Muthuswamy P, Elakkiya S, Manjupriya K, Deepa K, et al. Preliminary phytochemical and *in vitro* antioxidant perspectives of the leaf extracts of *Azima tetracantha* lam (Family: Salvadoraceae). International Journal of Pharma and Bio Sciences. 2012; 3 (1): 50-8.
46. Gayathri G, Bindu RN, Babu V. GC-MS Analysis of *Azima tetracantha* (Lam). Journal of Pharmacy Research. 2012; 5 (7): 3746-7.
47. Bennett RN, Mellon FA, Rosa EA, Perkins L, et al. Profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of *Azima tetracantha* L.(Salvadoraceae). Journal of agricultural and food chemistry. 2004; 52(19): 5856-62.
48. Abirami R, Mugammad ilyas MH, Prem kumar K, Nargis BT, et al. Chemical composition of the hexane extract of leaves of *Azima tetracantha* (Lam). International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7 (5): 340-4.
49. Vinoth B, Gomathinayagam M, Manivasagaperumal R. Screening of phytochemical and antimicrobial activities of *Azima tetracantha* Lam. leaf extracts. International Journal of Pharma Research & Review. 2014; 3 (10): 1-7.
50. Hema TA, Shiny M, Parvathy J. Antimicrobial activity of leaves of *Azima tetracantha* against clinical pathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4 (4): 317-9.
51. Gayathri G, Bindu RN, Babu V. *Invitro* antimicrobial and antioxidant studies on leaves of *Azima tetracantha* (Lam.). International Journal of Current Research 2011; 3 (12): 87-90.

52. Duraipandiyan V, Gnanasekar M, Ignacimuthu S. Antifungal activity of triterpenoid isolated from *Azima tetracantha* leaves. Folia Histochemica Et Cytobiologica. 2010; 48 (2): 311-3.
53. Maruthi TE, Ramesh CK, Mahmood R. Evaluation of anthelmintic and antimicrobial activities of *Azima tetracantha* Lam. International Journal of Pharmaceutical Sciences. 2010; 2 (1): 375-81.
54. Vinoth B, Manivasagaperumal R, Prakash P. Free radical scavenging potential of different extracts from *A. tetracantha* Lam. International Journal of Research in Ayurveda Pharmacy. 2015; 6 (1): 131-7.
55. Hepsibha BT, Sathiya S, Babu CS, Premalakshmi V, et a. *In vitro* studies on antioxidant and free radical scavenging activities of *Azima tetracantha* Lam leaf extracts. Indian Journal of Science and Technology. 2010; 3 (5): 571-7.
56. Antonisamy P, Duraipandiyan V, Ignacimuthu S. Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. in mouse and rat models. Journal of Pharmacy and Pharmacology. 2011; 63, 1070-7.
57. Ismail TS, Gopalakrishnan S, Begum VH, Elango V. Anti-inflammatory activity of *Salacia oblonga* Wall. and *Azima tetracantha* Lam. Journal of Ethnopharmacology. 1997; *56* (2): 145-52.
58. Konda VR, Arunachalam R, Eerike M, Rao R, et al. Nephroprotective effect of ethanolic extract of *Azima tetracantha* root in glycerol induced acute renal failure in Wistar albino rats. Journal of Traditional and Complementary Medicine. 2015; 1-8.

59. Manikandaselvi S, Ramya D, Ravikumar R, Thinagarbabu R. Evaluation of antinephrotoxic potential of *Azima tetracantha* Lam. and *Tribulus terrestris* linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4, 566-8.
60. Soumya A, Nagarajan V. Hepatoprotective effect of *Azima tetracantha* Lam. on ferrous sulphate induced toxicity in albino rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014; 5 (1): 380-7.
61. Sunil C, Duraipandiyan V, Ignacimuthu S, Al-Dhabi NA. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from *Azima tetracantha* Lam. leaves. Food Chemistry. 2013; 139 (1–4): 860-5.
62. Sambasivam M, Ravikumar R, Thinagarbabu R, Davidraj C, et al. Hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate-induced toxicity in rat. Bangladesh Journal of Pharmacology. 2013; 8 (3): 357-60.
63. Balakrishnan M, Dhanapal R, Vamsi MLM, Sekhar KC. Hepatoprotective activity of *Azima tetracantha* Lam. against Carbon tetra Chloride (CCl4)-induced hepatotoxicity in Wistar male albino rats. International Journal of Biological & Pharmaceutical Research. 2012; 3 (3): 366-72.
64. Nargis BT, Muhammad Ilyas MH, Vijaya Anand A. Hepatoprotective activity of *Azima tetracantha* Lam in experimental animals. Journal of Pharmacy Research. 2011; 4 (7): 2359-60.
65. Arthika S, Shanthammal Y, Sheryl Igal N, Elankini P, et al. Hepatoprotective activity of the ethanoilc extract of *Azima tetracantha* against paracetamol-induced hepatotoxicity in wistar albino rats, Journal of Advances in Pharmacy and Healthcare Research. 2011; 14-22.

66. Ekbote RM, Mahmood C, Thippeswamy R. Hapatoprotective and antioxidant effect of *Azima tetracantha* Lam, leaves extracts against CCL4 induced liver injury in rats. Indian Journal of Natural Product Research. 2010; 1: 493-9.
67. Antonisamy P, Duraipandiyan V, Aravinthan A, Al-Dhabi NA, et al. Protective effects of friedelin isolated from *Azima tetracantha* Lam. against ethanol-induced gastric ulcer in rats and possible underlying mechanisms. European Journal of Pharmacology. 2015; 750: 167-75.
68. Muthusamy P, Suresh AJ, Balamurugan G. Antiulcer activity of Azima tetracantha: a biochemical study. Research Journal of Pharmacy and Technology. 2009; 2 (2): 344-8.
69. Nargis BT, Muhammad IMH, Kalavathy S, Vijaya AA, et al. Effects of ethanolic leaf extract of *Azima tetracantha* Lam. on Enrlich Ascites Carcinoma tumour bearing mice. Research Journal of Medicine & Medical Sciences. 2009; 4 (2): 351-4.
70. Begum HV, Dhanalakshmi M, Muthukumaran P. *In vivo* evaluation of antidiarrhoeal activity of the leaves of *Azima tetracantha* Lam. International Journal of Nutrition and Metabolism. 2013; 5(8): 140-4.
71. Nargis BT, Muhammad IMH, Burkanudeen A, Kalavathy S, et al. Hypoglycemic and antihyperlipidemic activity of ethanolic leaf extract of *Azima tetracantha* lam. on Alloxan-induced diabetic rats. Journal of Cell and Tissue Research. 2009; 9(1): 1681-5.
72. Khare CP. Indian medicinal plants, An Illustrated Dictionary 2007, 76.
73. www.indiabiodiversity.org/species/show/264016. Retrieved on 25 August 2015.
74. http://en.wikipedia.org/wiki/*Azima_tetracantha*. Retrieved on 25 August 2015.

75. Schmelzer GH, Gurib- Fakim A. Medicinal plants 1. Plant Resources of Tropical Africa 2008, 11 (1): 109- 110.

76. Senthamarai R, Kavimani S, Jayakar B, Jayalakshmi A, et al. Diuretic activity of *Azima tetracantha*. Indian Drugs. 1996; 33 (9):478-9.

77. The Wealth of India raw materials Vol - I: A Revised Edition, 512.

78. Nandgude TD, Bhojwani AP and Krisnhakinage. Analgesic activity of various extracts of leaves of *Azima tetracantha* Lam. International Journal of Green Pharmacy, 2007; 1(1): 37-8.

79. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. Ethnomedicine of Dharwad District in Karnataka, India: Plant used in oral health care. Journal of Ethnopharmacology. 2004; 94(2-3): 261-6.

80. Nadkarni KM. Indian Meteria Medica, 3rd edition, Popular Prakhasan, Bombay 1976; 1: 45-52.

81. Kokate CK, Purohit AP, Gokahle SB. Pharmacognosy, 24th edition. Vallabh Prakashan, Pune. 2003.

82. Deore SL, Khadabadi SS, Baviskar BA. Pharmacognosy and phytochemistry: A comprehensive approach. PharmaMed Press. 2014.

83. Kokate CK. Practical Pharmacognosy. 4th ed., M.K. Jain for Vallabh Prakashan. 2007.

84. Iyengar MA. Pharmacognosy of powdered crude drugs., 10th edition. Iyengar series, Manipal Technologies Limited, Manipal. 2011.

85. Easu K. Plant Anatomy John Wiley and sons. New York. 1964; 767.

86. Iyengar MA, Nayak SCK. Anatomy of crude drugs. Iyengar Series, Manipal Press limited, Manipal. 2012.

87. Gamble JS. Flora of the Presidency of Madras. Botanical Survey of India, Calcutta, India 1935; Vol. I, II, & III.

88. Henry AN, Kumari GR, Chitra, V. Flora of Tamilnadu, Botanical Survey of India, Southern Circle, Coimbatore, India. 1987; 3: 258.

89. Johansen DA. Plant Microtechnique. Mc Graw Hill Book Co., New York. 1940; 523.

90. Mathew KM. The Flora of Tamil Nadu Karnatic. Polypetalae. 1983; 1: 688.

91. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons Clarendon Press, Oxford. 1950; Vol.I &II.

92. O'Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell walls by toluidine blue-O.Protoplasma.1964; 59: 364-373.

93. Indian pharmacopoeia, 4th edition, Government of India , Ministry of Health and Welfare, Controller of Publications, New Delhi. 1996: Vol- II: A53- A54.

94. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright- Scientechnica, Bristol. 1975:36- 45.

95. Who Regional Office for the Western Pacific Research guidelines for evaluating the safety efficacy of herbal medicines, Manila, WHO 1993.

96. Kokate CK, Purohit AP, Gokhale SB. Analytical pharmacognosy, 23rd edition. Nirali prakashnan, Pune. 2003; 111-2.

97. Pharmacopoeia of India. Published by the Manager of publication, Delhi. 1970; 651.

98. Quality Control Methods for Medicinal Plant Materials . WHO Geneva, Indian edition 2004; 28-37.

99. The Ayurvedic Pharmacopoeia of India. Vol. 1,2,3,4 and 5. New Delhi: The controller of publications, Ministry of Health Walfare, Govt. of India. 2002.

100. Lira, Sergio, Peter B, Laurence S, et al. The use of inductively coupled plasma-optical emission spectroscopy in the determination of heavy metals in crospovidone and povidone as a replacement for the concomitant visual comparison test. Pharmacopoeial Forum. 2008; 34 (6).

101. Archa V, Narneet P, Avinash C. Physiochemical analysis of ash of some medicinal plants growing in Uttarkhand. Nature and Science. 2010; 8(6); 88-91.

102. John TA, Rachel M, John TR. Phytochemistry of medicinal plants. Springer Science and Business Media. 2013.

103. Jigna P, Sumitra VC. *In-vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plant. Turkish Journal of Biology. 2007; 31: 53-8.

104. Makut MD. Phytochemical screening and antimicrobial activity of the ethyl acetate and methanol extracts of the leaf and bark of *Khaya senegalensi*. African Journal of Biotechnology. 2008; 7 (9): 1216-9.

105. Tyler VE, Brady LR, Robbers JE. Pharmacognosy, 9th edition, Philadelphia USA: Lea and Febrigoree. 1998; 78.

106. Raaman N. Qualitative phytochemical screening. Phytochemical techniques. New India Publishing Agency, New Delhi. 2000; 19-24.

107. Kokate CK, Purohit AR, Gokhale SB. Pathway to screen phytochemical nature of natural drugs. Pharmacognosy, 42 nd edition. Nirali publication, Pune. 2008; A1-A6.

108. Evans WC, Trease. Texbook of pharmcognosy, 13th edition. English language book society, England. 1998; 289-388.

109. Chase CR, Pratt RJ. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Am. Pharmacol. Assoc. 1949; 38: 32.

110. Harbone JB. Phytochemical methods, 2nd edition. Chapman and Hall, London. 1984; 1-10.

111. Connors KA. Fluorescence spectroscopy: A textbook of pharmaceutical analysis, 3rd edition. John Wiley and sons (ASIA) Pvt. Ltd, Singapore. 1982; 235.

112. Lala PK. Lab manuals of pharmacognosy, 5th edition. CSI publishers and distributors, Calcutta. 1993.

113. Evelyn MLP, Fernando WCA, Ivone AS, Isla VGAB, et al. Pharmacological screening and acute toxicity of bark roots of *Guettarda platypoda.* Brazilian Journal of Pharmacognosy. 2012.

114. Peter JH, Amala R. Laboratory hand book for the fractionation of natural extracts. Springer International. 2011; 82.

115. Indrayan AK, Sharma S, Durgapal D, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Current Science 89. 2005; 1252-5.

116. Machlin LJ. Vitamin E: A comprehensive treatise. New York, Dekkar. 1980.

117. Von Gadow A, Joubert E, Hansmann CF. Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (Aspalathon linearis), α-tocopherol, BHT and BHA. Journal of Agricultural and Food Chemistry, 1997; 45: 632-8.

118. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. Journal of Agricultural and Food Chemistry, 1994; 42: 629-32.

119. Scott AI. Interpretation of ultraviolet spectra of natural products. Pergamon Press, Oxford. 1964.

120. Nakanishi K, Solomon PH. Infrared absorption spectroscopy-practical, 2nd edition. Holden-Day, San Francisco. 1977.

121. Nakanishi K (Ed.). One-dimensional and two-dimensional NMR spectra by modern pulse technique. Kodansha, Tokyo; University Science Books, Mill Valley, California. 1990.

122. McLafferty FW, Stauffer DB. Registry of mass spectral data. Wiley, New York. 1989.

123. OECD. Guideline for testing of chemicals 423 (online); 2012[cited on 2010 Feb 11] Available from URL:http://icvvam. Nih.Gov/suppdocs/ OECD/OECD GL 423.

124. OECD (The organization of economic co-opreation development), The olson, betton HG, Robinson D, Thomas K, Monro A, kolaja G, Lilly P, Sanders J.2001.

125. Abondanza TS, Oliveria CR, Barbosa CMV. Bcl-2 expression and apoptosis induction in human HL-60 leukaemic cells treated with a novel organotellurium compound RT-04. Food Toxicol. (2008) 46: 2540-5.

126. Yamane K, Tsuruo T. Conserved BRCT regions of TopBP1 and of the tumor suppressor BRCA1 bind strand breaks and termini of DNA. Oncogene. 1999; 18 (37): 5194-203.

127. Cohen-Solal M, Valentin C, Plassa F, Guillemin G, et al. Identification of new mutations in two phosphoglycerate kinase (PGK) variants expressing different clinical syndromes: PGK Creteil and PGK Amiens. Blood. 1994; 84 (3): 898-903.

128. Ookawara T, Dave V, Willems P, Martin JJ, et al. Retarded and aberrant splicings caused by single exon mutation in a phosphoglycerate kinase variant. Arch Biochem Biophys. 1996; 327 (1): 35-40.

129. Makiniemi M, Hillukkala T, Tuusa J, Reini K, et al. BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. J Biol Chem. 2001; 276 (32): 30399-406.

130. Honda Y, Tojo M, Matsuzaki K, Anan T, et al. Cooperation of HECT-domain ubiquitin ligase hHYD and DNA topoisomerase II-binding protein for DNA damage response. J Biol Chem. 2002; 277 (5): 3599-605.

131. Liu K, Lin FT, Ruppert JM, Lin WC. Regulation of E2F1 by BRCT domain-containing protein TopBP1. Mol Cell Biol. 2003; 23 (9): 3287-304.

132. Liu K, Luo Y, Lin FT, Lin WC. TopBP1 recruits Brg1/Brm to repress E2F1-induced apoptosis, a novel pRb-independent and E2F1-specific control for cell survival. Genes Dev. 2004; 18 (6): 673-86.

133. Maeda M, Yoshida A. Molecular defect of a phosphoglycerate kinase variant (PGK-Matsue) associated with hemolytic anemia: Leu----Pro substitution caused by T/A----C/G transition in exon 3. Blood. 1991; 77 (6): 1348-52.

134. Valentin C, Birgens H, Craescu CT, Brodum-Nielsen K, et al. A phosphoglycerate kinase mutant (PGK Herlev; D285V) in a Danish patient with isolated chronic hemolytic anemia: mechanism of mutation and structure-function relationships. Hum Mutat. 1998; 12 (4): 280-7.

135. Maeda M, Bawle EV, Kulkarni R, Beutler E, et al. Molecular abnormalities of a phosphoglycerate kinase variant generated by spontaneous mutation. Blood. 1992; 79 (10): 2759-62.

136. Fujii H, Kanno H, Hirono A, Shiomura T, et al. A single amino acid substitution (157 Gly----Val) in a phosphoglycerate kinase variant (PGK Shizuoka) associated with chronic hemolysis and myoglobinuria. Blood. 1992; 79 (6): 1582-5.

137. Fujii H, Chen SH, Akatsuka J, Miwa S, et al. Use of cultured lymphoblastoid cells for the study of abnormal enzymes: molecular abnormality of a phosphoglycerate kinase variant associated with hemolytic anemia. Proceedings of the National Academy of Sciences, USA. 1981; 78 (4): 2587-90.

138. Chen X, Zhao C, Li X, Wang T, et al. Terazosin activates Pgk1 and Hsp90 to promote stress resistance. Nature Chemical Biology. 2014.

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी,एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2016/Tech. /178

दिनांक/Date: 5th February 2016

सेवा में / To

Ms. S Nandhini
II Year M. Pharm.
Department of Pharmacognocy College of Pharmacy
Madras Medical College
Chennai – 600 003

महोदया/Madam,

The plant specimen sent by you for identification is identified as *Azima tetracantha* Lam. - SALVADORACEAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

GVSmurthy 5/2/16

(डॉ. जी.वी.एस. मूर्ति /Dr. G.V.S. Murthy)
वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष /
Scientist 'F' & Head of Office

वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष
Scientist 'F' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयंबत्तूर / Coimbatore - 641 003

CERTIFICATE

This is to certify that Ms. **S. NANDHINI**, M.Pharm II year, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai – 600003 had submitted her protocol (Part B Application) 19|243|CPCSEA for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai – 600003.

TITLE: EVALUATION OF ANTICANCER POTENTIAL OF LEAVES AND STEM OF *Azima tetracantha* Lam.

The Animal Ethical Clearance Committee experts screened her proposal No: 19|243|CPCSEA and have given clearance in the meeting held on 10|08|2015 at Dean's Chamber in Madras Medical College, Chennai – 600003. Her study involves only Swiss Albino mice.

Signature

9|3|2016.

Dr. S.K. SEENIVELAN, B.V.Sc.,
Reg. No: 2175
SPECIAL VETERINARY OFFICER
ANIMAL EXPERIMENTAL LABORATORY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003.

List of papers published in the Present work

1. "Pharmacognosy of *Azima tetracantha* Lam.: A review", by **S. Nandhini,** R. Radha in **International Journal of Ayurvedha and Pharma Research.** 2015; 3 (12):13-19.
2. "*Invitro* anticancer activity of leaves and stem of *Azima tetracantha* Lam.", by **S. Nandhini,** R. Radha, P. Muthusamy in **World Journal of Pharmacy and Pharmaceuticl Sciences.** 2016; 5 (3): 1242-1246.

List of papers accepted in the Present work

1. "Pharmacognostical studies of *Azima tetracantha* Lam. (Salvadoraceae)", by S. Nandhini, R. Radha, R. Vijayabharathi in **World Journal of Pharmaceuticl Sciences.** Received: 22-02-2016 / Revised: 09-03-2016 / Accepted: xx-xx-2016.
2. "Bioactivity guided isolation of crude ethanol extract *Azima tetracantha* leaves", by S. Nandhini, R. Radha, N. Jayshree in **World Journal of Pharmacy and Pharmaceuticl Sciences.** DOI: 10.20959/wjpps20164-6378.

List of papers published in other work

1. "Preliminary phytochemical and invitro antibacterial evaluation of Ziziphus oenoplia (L) Mill., stem bark extracts by **S. Nandhini,** R. Radha in **International Journal of Pharmacy and Integrated Life Sciences.** 2016; 4 (3): 01-05.

2. "Preliminary phytochemical and antidiabeticactivity of Cassia sophera Linn. by **S. Nandhini,** S. Geethalakshmi, S. Selvam, R. Radha, A. Jerad Suresh, P. Muthusamy in**Journal of Pharamacognosy and Phytochemistry.** 2016;5(1):87-91

ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Review Article

PHARMACOGNOSY OF *AZIMA TETRACANTHA* LAM.: A REVIEW

Sundaresan Nandhini[1]*, Ramalingam Radha[2]

*[1]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

[2]Professor & Head of the Department, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

ABSTRACT

Plants have played a central role in the prevention and treatment of diseases since prehistoric times. In recent years, there has been growing interest in the study of medicinal plants and their extensive use in different countries. However, today it is essential to pay for the scientific proof as to whether it is rational to use a plant or its active principles. Hence the present communication constitutes a review with adequate information on the medicinal properties, ethno medicinal uses, phytochemistry, and pharmacological activities of an Indian medicinal plant, *Azima tetracantha* Lam belonging to the family of Salvadoraceae. A wide range of phytochemical constituents have been isolated from *A. tetracantha* Lam which possesses activities like as stimulant, expectorant, antispasmodic, analgesic, anti-inflammatory, anti-ulcer, anti-diarrhoeal, anti-microbial, hepatoprotective, nephroprotective, hypoglycemic and hyperlipidemic activities. Hence, extracts of *Azima tetracantha* could form one of the best options for developing novel natural medicine.

KEYWORDS: *Azima tetracantha* Lam, Salvadoraceae, Sung-ilai, Azimine etc.

INTRODUCTION

In India treating specific ailments by the use of the different parts of several medicinal plants has been in vogue from ancient times. The indigenous system of medicines namely Ayurvedic, Siddha and Unani has been in subsistence for several centuries. Some drugs from Ayurveda approaching modern diseases, have already reached the market place[1]. It is estimated that nearly 70000 plant species have been used for medicinal purposes. India recognizes more than 2500 plant species having medicinal value, Sri Lanka around 1400 and Nepal around 700[2]. About 40% of doctors' especially in India and in China have reverted to increasing use of indigenous drugs and natural medicines[3,4]. The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicines for their primary health care needs.

Azima tetracantha Lam (Family: Salvadoraceae) plays a major role in the medicinal properties. The plant parts of *Azima tetracantha* such as roots, leaves, fruits and stems are used traditionally to treat various ailments and possesses activities like stimulant, expectorant, antispasmodic, analgesic, anti-inflammatory, anti-ulcer, anti-diarrhoeal, anti-microbial, hepatoprotective, nephroprotective, hypoglycemic and hyperlipidemic activities[5]. This review provides the botany, morphological character, geographical distribution, medicinal values, physicochemical characters, phytochemical characters and pharmacological activities of *Azima tetracantha*.

Synonyms

Synonyms of *Azima tetracantha* includes *Monetia barlerioides* L'Herit., *Azima nova* J. F. Gmel., *Kandena spinosa* Rafin., *Monetia angustifolia* Boj. Ex A. DC., *Monetia tetracantha* (Lam.) Salisb. It is also called as "kundali" in Ayurveda, "mulchangan" in Siddha[6].

Scientific Classification[7]

The scientific classification of *A. tetracantha* is demonstrated as follows:

Kingdom: Plantae, Phylum: Tracheophyta, Class: Magnoliopsida, Order: Capparales, Family: Salvadoraceae, Genus: Azima, Species: *Azima tetracantha* lam.

Vernacular Names[8]

The vernacular names of *A. tetracantha* are described as follows:

Sanskrit: Kundali, Hindi: Kanta- gur-kamai, Malayalam: Essanku, Sankukuppi, English: Bee sting bush, Fire thorn, Needle bush, Tamil: Sung-ilai, Ichanka

Description[9]

Azima tetracantha is a perennial shrub growing upto 3m in hot, dry riverine scrub, particularly on alluvial or saline soil. The plant is dioecious, erect shrub with (1–)2 spines 0.5–5 cm long in each leaf axil, sometimes scandent with stems up to 8 m long; branchlets are terete or quadrangular, glabrous to densely hairy. The leaves of the plant are elliptical in shape and are rigid, pale green colored. The flowers are

small, greenish white (or) yellow colored, unisexual in axillary fascicles. Fruits are globular, white shiny. Seeds are compressed, circular. It occurs naturally in central, eastern and southern Africa as well as in the Indian Ocean Islands, and extends through Arabia to tropical Asia.

Chemical Constituents[10]

Table1: The chemical constituents present in *Azima tetracantha*

Class	Chemical Constituents
Glucosinolates and glucosinolate derived compounds	3- indolylmethylglucosinolate N- hydroxyl- 3- indolylmethyl- glucosinolate N- methoxy- 3- indolylmethyl- glucosinolate Neoascorbigen
Dimeric piperidine alkaloids	Azimine Azcarpaine Carpaine

Medicinal Importance of *A. tetracantha*

Azima tetracantha is a potent diuretic to treat rheumatism, dropsy, dyspepsia, chronic diarrhorea; it is used as stimulant tonic after child birth. *A. tetracantha* is used to treat cough, phthisis, asthma, small pox and diarrhorea. Rheumatism has been cured by its leaves, root and root bark[5].

Traditional Uses

Traditionally, root used as diuretic. In Siddha, root is used in the treatment for dropsy and rheumatism. Leaves are used as stimulant, expectorant and antispasmodic. It is also used in cough and asthma. Bark is used as antiperiodic, astringent and expectorant. In western India, juice of the leaves is applied as ear drops against earache and crushed leaves are placed on painful teeth. In India and Sri Lanka the root, root bark and leaves were administered with food as a remedy for rheumatism, dropsy, dyspepsia, chronic diarrhea and is considered as stimulant tonic and given to pubertal women immediately after confinement[11,12]. Locally, the traditional healers from Tirunelveli district of Tamilnadu use the root bark (paste with buttermilk) as potent remedy for jaundice.

MACROSCOPIC CHARACTERS[13]

Table 2: Preliminary macroscopical characters of *Azima tetracantha*

Azima tetracantha		Characters
Leaf structures	Nature	Decussately opposite
	Shape	Blade elliptical-oblong to ovate-oblong or orbicular
	Dimensions	1.5–5.5 cm × 0.5–4.5 cm
	Stipules	Absent/ rudimentary
	Leaf margin	Simple and entire
	Leaf apex	Mucronate
	Leaf base	Pinnately veined with one pair of lateral veins
Flower structures	Petals shape	Linear oblong to oblong
	Length	2- 4 mm
	Lobes	Triangular
	Male flowers	Stamens inserted at the base
	Female flowers	Staminoids and superior ovary
Fruit structures	Nature	Globose berry
	Dimension	0.5- 1cm diameter

MICROSCOPIC CHARACTERS[13]

Table 3: Microscopical characters of *Azima tetracantha*

Azima tetracantha		Characters
Leaf structures	Transverse section	Dorsiventral nature
	Midrib	Flat and hemispherical
	Cuticle	Thin, rectangular and prominent
	Vascular bundle	Single and abaxial arc shaped phloem
	Sclerenchyma	Absent
	Lamina	230mm thick
	Trichomes	Absent
	Abaxial epidermis	Stomatiferrous
	Epidermal tissues	Stomata and epidermal cells
	Stomata	Anisocytic

	Petiole (basal and upper part)	1.15µm diameter, circular
Stem structures	Young stem	1.5mm thick, consists of a distinct continuous epidermis, cortex, vascular cylinder and pith
	Epidermal cells	Squarish or rectangular
	Cuticle	Thick
	Stomata	Frequently seen
	Cortex	150mm width, consists of chlorenchyma and parenchyma
	Pith	Wide, homogenous and parenchymatous.
	Vascular cylinder	29 discrete vascular bundles
Root bark structures	Periderm	No deep fissures and contains homogenous phellan cells
	Pseudocortex	Inner to the periderm, is a wide parenchymatous zone
	Secondary phloem	It consists of Collapsed and Non- collapsed phloem

PHARMACOLOGICAL ACTIVITIES

Antimicrobial activity of *Azima tetracantha* Lam

Antimicrobial activity of different extracts of *Azima tetracantha* root was carried out by Vinoth and Manivasagaperumal, in 2015 against human pathogenic bacterial and fungal strains using disc diffusion method. The study concluded that methanolic root extract of *Azima tetracantha* had a potential antimicrobial activity against all the microorganisms tested[14]. The *invitro* antimicrobial activity of *Azima tetracantha* leaves was studied by Vinoth *et al*., in 2014 against various human bacterial and fungal pathogens using disc diffusion method. Phytochemical analysis of *Azima tetracantha* leaf extracts revealed that the extracts justify the presence of secondary metabolites and their liability for the activity[15]. Antibacterial activity of phytocompound separation from alkaloids, flavonoids and sterol were tested against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E.coli* by Gowthami *et al*., in 2012. The sterols compound exhibited maximum activity when compared with alkaloids and flavonoids[16]. The antimicrobial potential of leaves of *Azima tetracantha* was checked against the clinical pathogens by Hema *et al*., in 2012 using agar well diffusion method. Antimicrobial activities of five solvent extracts (ethanol, methanol, acetone, chloroform and distilled water) were tested against seven clinical pathogens such as *Staphylococcus aureus* (Pus), *Klebsiella* sp. (Sputum), *Escherichia coli* (Urine), *Pseudomonas* sp. (Pus), *Enterococci* sp. (Urine), *Serratia* sp. (Sputum) and *Proteus* sp. (Sputum). Among the five solvent extracts tested, ethanolic extracts of *Azima* showed superior activity against the pathogenic organisms[17]. The study based on the evaluation of the antifungal activity of *Azima tetracantha* extracts and isolated compound (friedelin) against fungi was carried out by Duraipandiyan *et al*., in 2010 using the micro dilution method. The study concluded that extracts have antifungal activity and strongly suggests that isolated compound friedelin as an antifungal agent[18]. The study designed by Maruthi *et al.,* in 2010 evaluated the anthelmintic and antimicrobial activities of *Azima tetracantha*. The results revealed that, alcoholic extract was found to possess antibacterial and antifungal activities while chloroform extract showed only antibacterial activity[19].

Antioxidant and free radical scavenging activities of *Azima tetracantha* Lam

Phenolic compounds are classic active oxygen scavengers in plants and are acknowledged to contribute directly to antioxidant action. The hydroxyl groups of the phenolic compounds confer the scavenging ability of the plant (Yildrim)[20]. The different extracts of *A. tetracantha* root were studied for antioxidant potential by Vinoth *et al.,* in 2015 by using different *invitro* assays such as inhibition of DPPH, ABTS, hydroxyl radical and superoxide anions. The total phenolic contents and ferric reducing antioxidant power of the extracts were also determined by using standard phytochemical reaction methods. The results revealed that the different extracts of *A. tetracantha* root showed a good dose dependent free radical scavenging activity in all the models. Ferric reducing antioxidant power was found to be increased with increase in extracts concentrations[21]. Muthuswamy et al., in 2012 performed an antioxidant study of methanol and ethyl acetate leaf extract of *Azima tetracantha* Lam. The study designed against the inhibition of the LPO, DPPH, superoxide anion, hydroxyl radical. The result indicated that the ethyl acetate extract posses moderate activity when compared with that of the standards[22]. In an antioxidant study of leaf extracts of *Azima tetracantha* Lam done by Gayathri G *et al.,* in 2011, the reducing capacity improved with the increasing concentration of the extract. This showed that the antioxidant compounds can react with free radical to convert them to more stable products and thereby terminate radical chain reactions. The report concluded that the leaves of *A. tetracantha* were proved to be good source of natural phenolic compounds[23]. The study designed by Thendral Hepsibha *et al.,* in 2010 evaluated antioxidant and free radical scavenging activities of *Azima tetracantha*. Lam. leaf extracts. The result suggested that the methanolic extract of the *A. tetracantha* leaves showed better free radical capacity against different reactive oxygen /nitrogen species, among other extracts although with different efficiencies. The study concluded that the high content of antioxidants like phenolic compounds, flavonoids and vitamins found in these extracts, may impart health benefits by combating the free radicals in synergistic manner along with other compounds and thus constitute part of the basis for the ethno pharmacological claim[24].

Anti- inflammatory, Analgesic and Antipyretic effects from *Azima tetracantha* Lam

The study designed by Antonisamy *et al*., in 2011 evaluated the anti-inflammatory, analgesic and anti-pyretic effects of friedelin. The effects of friedelin on inflammation were studied by using carrageenan-induced hind paw oedema, croton oil-induced ear oedema, acetic acid-induced vascular permeability, cotton pellet-induced granuloma and adjuvant-induced arthritis. The analgesic effect of friedelin was evaluated using the acetic acid-induced abdominal constriction response, formalin induced paw licking response and the hot-plate test. The antipyretic effect of friedelin was evaluated using the yeast induced hyperthermia test in rats. In the acute phase of inflammation, maximum inhibitions were prominent with friedelin in carrageenan-induced paw oedema and croton oil-induced ear oedema. Administration of friedelin notably decreased the formation of granuloma tissue. Friedelin also produced considerable analgesic activity in the acetic acid-induced abdominal constriction response and formalin-induced paw licking response. Treatment with friedelin showed a noteworthy dose-dependent reduction in pyrexia in rats[25]. The anti-inflammatory activity of *Salacia oblonga* root bark powder and *Azima tetracantha* leaf powder was assayed in male albino rats using carrageenan-induced rat paw oedema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methods by Syed Ismail *et al.,* in 1997. The study concluded that both the crude drugs were optimally active at a dose of 1000 mg/kg. In the cotton pellet granuloma assay, both the crude drugs were able to suppress the transudative, exudative and proliferative components of chronic inflammation[26].

Antinephrotoxic potential of *Azima tetracantha* Lam

The biochemical markers of nephrotoxicity are urea, creatinine and GGT. Their levels are significantly elevated in nephrotoxic situation due to metal induced damage to nephrons. In nephrotoxicity, the serum urea and creatinine accumulates because the rate of serum urea and creatinine production exceeds the rate of clearance due to defects in the glomerular filteration rate. The study designed by Konda et al., in 2015 evaluated the nephroprotective effect of root extract of *Azima tetracantha* in glycerol-induced acute renal failure in Wistar albino rats. The study report suggested that there was a considerable improvement in biochemical parameters and histopathological changes when compared with glycerol treated group. The antioxidant activity of the root extract of *A. tetracantha* was tested *invitro* and *invivo*. Both *invitro* and *invivo* assays showed significant antioxidant activity and due to this, the nephroprotective effect of *A. tetracantha* in glycerol-induced acute renal failure was estabilished[6]. The results of the study done by Manikandaselvi *et al.,* in 2012 indicated the significant elevation in the levels of urea, GGT and creatinine in ferrous sulphate induced group compared to control. After treatment with *Azima tetracantha* Lam there was a significant decrease in the levels near to normal compared to ferrous sulphate induced group. The study concluded that the nephroprotection could be attained due to its antioxidant and free radical scavenging activity[27].

Hepatoprotective activities of *Azima tetracantha* Lam

Hepatotoxicity induced in albino rats by ferrous sulphate and their hepatoprotective effect was studied by using aqueous extracts of *Azima tetracantha* by Soumya and Nagarajan in 2014. Aqueous extracts of *Azima tetracantha* showed a significant reduction in all the biochemical parameters of liver damage glucose, protein, bilirubin, cholesterol, ALP, SOD, CAT, Vitamin E, TBARS, Albumin and globulin elevated by ferrous sulphate[28]. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from *Azima tetracantha* Lam leaves were performed by Sunil *et al.,* in 2013. The report in the study indicated that friedelin restored the levels of SGOT, SGPT, LDH, SOD, catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) and showed liver protection, comparable to the standard silymarin[29]. A study report by Sambasivam et al., in 2013 on hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate-induced toxicity in rat revealed that the hydroalcoholic extract of leaf powder of *Azima tetracantha* and the fruit powder of *Tribulus terrestris* retrieved the liver parameters to usual level and possesses significant hepatoprotective activity[30]. A study done by Balakrishnan *et al*., in 2012 reported that the ethanol (50%) extract of *Azima tetracantha* Lam. (EEAT) root bark afforded significant protection against carbon tetrachloride (CCl4) induced hepatocellular injury. The report also revealed that the normal liver cellular architecture was retained when the liver sections of the rats treated with EEAT root bark extract for 7 days there by further confirming the intoxicating hepatoprotective effect of EEAT root bark[31]. Reports documented by Nargis *et al*., in 2011 revealed that the rats treated with ethanolic extract of *A. tetracantha* showed a significant reduction in all the five-biochemical parameters of liver damage (AST, ALT, ALP, ACP and total bilirubin) elevated by carbon tetrachloride[32].

Antiulcer Activity of *Azima tetracantha* Lam

Antonisamy *et al.,* in 2015 recorded the protective effects of friedelin isolated from *Azima tetracantha* Lam against ethanol-induced gastric ulcer in rats. The result from the study showed that the friedelin isolated from the hexane extract of leaves of *Azima tetracantha* protected from ethanol caused severe gastric damage and suggested that friedelin could be a new effective natural gastroprotective tool against gastric ulcer[33]. EEAT showed notable dose-dependent ulcer protective effect against cold restraint stress and aspirin plus pylorus ligation induced gastric ulcers on the study performed by Muthusamy et al., in 2009. The conclusion of the study stated that the gastro duodenal ulcer protecting effect of EEAT may be due to its predominant effect on the mucosal defensive factors rather than offensive factors[34].

PHYTOCHEMISTRY

The preliminary phytochemical screening carried out on various extracts of *A. tetracantha* revealed the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, steroids, carbohydrates, tannins, proteins and aminoacids. Other compounds such as friedelin, lupeol, glutinol and β- sitosterol have also been reported in *A. tetracantha.*

Physicochemical analysis[13]

The physicochemical characters such as organoleptic characters, fluorescence and the percentage of total ash, acid-insoluble ash, water-soluble ash and alkalinity of water soluble ash values of the powdered stem bark of *A.tetracantha* were evaluated.

Organoleptic characters of *A.tetracantha* stem bark powder

Colour: Pale brownish yellow

Appearance: Coarse powder

Odour: No characteristic odour

Taste: No characteristic taste

Table 4: Determination of consistency of *A. tetracantha* stem bark powder

Treatment	Observation
Powder treated with water	Non-sticky
Powder shaken with water	Honey comb like froth
Powder treated with 5% aqueous sodium	hydroxide Pale yellow
Powder treated with 60% aqueous sulphuric acid	Reddish brown
Powder pressed between filter paper for 24 hours	No oil stain

Table 5: Fluorescence characteristics of *A.tetracantha* stem bark powder

Treatment	Day light	UV light	
		254nm	365nm
Powder	Pale-brownish yellow	Pale green	Brown
Powder + 1N NaOH (aqueous)	Pale yellow	Pale yellow	Black
Powder + 1N NaOH (alcoholic)	Orange	Yellowish green	Black
Powder + 1N Hydrochloric acid	Pale yellow	Black	Black
Powder + 50% Sulphuric acid	Reddish brown	Dark brown	Black
Powder + 50% Nitric acid	Orange	Yellowish green	Black
Powder + Picric acid	Yellow	Green	Black
Powder + Acetic acid	Brown	No visible colour	Black
Powder + Ferric chloride	Orange	Green	Black
Powder +Con. Nitric acid	Brown	Green	Black
Powder + Nitric acid + Ammonia	Reddish orange with precipitate	Green	Black

Table 6: Ash values of *A.tetracantha* stem bark powder

Physicochemical Constants	Values
Total ash	21.625%
Water soluble ash	13.945%
Alkalinity of water soluble ash	1.73ml
Acid insoluble ash	0.665%

Phytochemical test[22]

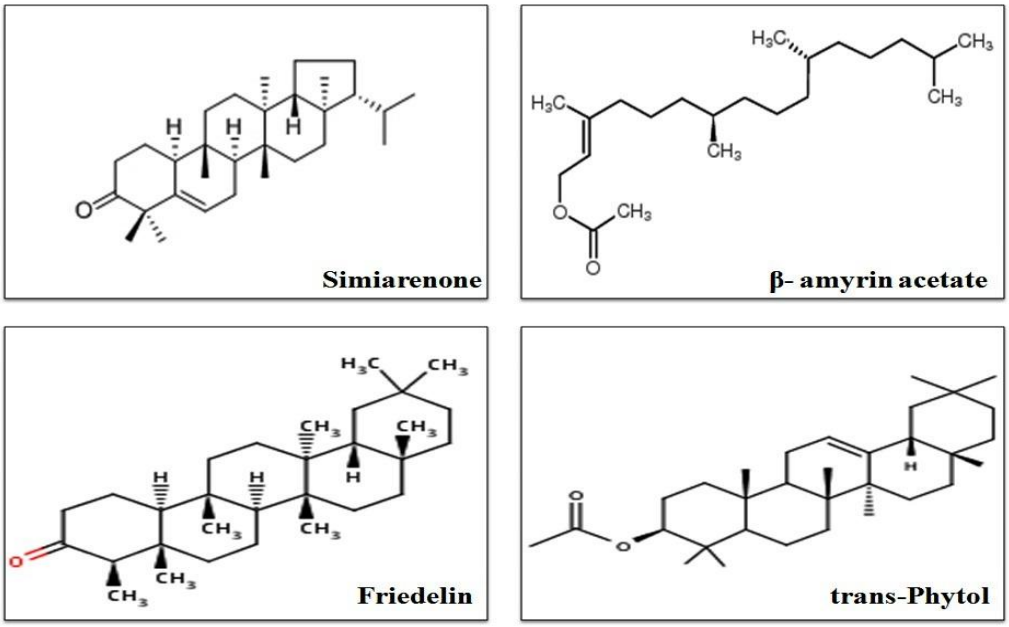
Table 7: Preliminary phytochemical test of various extracts of *A. tetracantha*.

Phytochemical Test	Pet. Ether extract	Ethylacetate Extract	Methanol Extract
Alkaloids	-	+	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Protein & Aminoacids	-	-	-
Flavonoids	+	+	+
Tannins	-	-	-
Steriods	+	+	+
Oil	+	+	+

Phytochemical structures[35]

The phytochemical constituents with their structures present in *A. tetracantha* are given in Fig. 1:

Fig. 1: The phytochemical constituents with their structures present in *A. tetracantha*[35]



CONCLUSION

The information summarized here is well-intentioned to serve as a reference tool to researchers in the field of ethanopharmacology of *Azima tetracantha*. Based upon the literature survey, it can be concluded that *Azima tetracantha* has been widely studied for its pharmacological activities. Science has always acknowledged the value of healing substances found in nature, such as digitalis, aspirin, penicillin, insulin, steroids etc. There is no doubt that valuable medicinal shrub, *Azima tetracantha* will be a treasure and will top the list of treasure hunters. Further research is needed to explore the unclaimed therapeutic effect of active compounds present in the shrub and the plausible molecular mechanisms of those active compounds.

REFERENCES

1. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharm Rev. 2010; 4(7): 58-61.
2. Prajapati ND, Purohit SS, Sharma AK, Kumar T. Handbook of medicinal plants. Jodhpur: Agarbios 2003.
3. Agarwal S S, Paridhavi M. Herbal drug technology. Hyderabad: Universities Press Private Limited 2007; 625.
4. Kokate CK, Purohit AP, Gokhle SB. Pharmacognosy. Delh: Vallabh Prakashan Publishers 2004; 597.
5. Khare CP. Indian medicinal plants, An Illustrated Dictionary 2007, 76.
6. Konda V R, Arunachalam R, Eerike M, Rao R, Radhakrishnan A K, Raghuraman L P, Meti V, Devi S. Nephroprotective effect of ethanolic extract of *Azima tetracantha* root in glycerol induced acute renal failure in Wistar albino rats. Journal of Traditional and Complementary Medicine. 2015; 1-8.
7. http://en.wikipedia.org/wiki/Azima_tetracantha. Retrieved on 25 August 2015.
8. Schmelzer GH, Gurib- Fakim A. Medicinal plants 1. Plant Resources of Tropical Africa 2008, 11 (1): 109-110.
9. The Wealth of India raw materials Vol - I: A Revised Edition, 512.
10. Bennett R N, Mellon F A, Rosa E A, Perkins L, Kroon P A. Profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of *Azima tetracantha* L. (Salvadoraceae). Journal of agricultural and food chemistry. 2004; 52(19): 5856-62.
11. Hebbar S S, Harsha V H, Shripathi V, Hegde G R. Ethnomedicine of Dharwad District in Karnataka, India: Plant used in oral health care. J. Ethnopharmacol. 2004; 94(2-3): 261-266.
12. Nadkarni K M. Indian Meteria Medica, Vol, 1, 3rd Edn, Popular Prakhasan, Bombay 1976; 45-52.
13. Balakrishnan M, Dhanapal R, Laksmi M M V, Chandra sekhar K B. Studies on pharmacognostical specifications of *Azima tetracantha* lam. International Journal of Phytopharmacology. 2010; 1 (1): 35-42.
14. Vinoth B, Manivasagaperumal R. Antimicrobial activity of different extracts of *Azima tetracantha* root. International Journal of Pharma and Bio Sciences. 2015; 6 (2): 613 - 620.
15. Vinoth B, Gomathinayagam M, Manivasagaperumal R. Screening of Phytochemical and Antimicrobial Activities of *Azima tetracantha* Lam. Leaf Extracts.

International Journal of Pharma Research & Review. 2014; 3 (10): 1-7.

16. Gowthami M, Tamil Selvi S, Senthil Kumar G, Panneerselvam A. Phytochemical analysis and antibacterial properties of leaf extract of *Azima tetracantha* (Lam.). Asian Journal of Plant Science and Research. 2012; 2 (2): 110-114.
17. Hema T A, Shiny M, Parvathy J. Antimicrobial activity of leaves of *Azima tetracantha* against clinical Pathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4 (4): 317- 319.
18. Duraipandiyan V, Gnanasekar M, Ignacimuthu S. Antifungal activity of triterpenoid isolated from *Azima tetracantha* leaves. Folia Histochemica Et Cytobiologica. 2010; 48 (2): 311-313.
19. Maruthi T E, Ramesh C K, Mahmood R. Evaluation of anthelmintic and antimicrobial activities of *Azima tetracantha* Lam. International Journal of Pharmaceutical Sciences. 2010; 2 (1): 375-381.
20. Yildrim A, Mavi A, Oktay M, Kara A A, Algur O F, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of Tila (*Tila argentea* Desf Ex DC), Sage (*Savia triloba* L.), and Black Tea (*Camelia sinensis*) extracts. J Agric Food Chem. 2000; 48 (10): 5030-5034.
21. Vinoth B, Manivasagaperumal R, Prakash P. Free radical scavenging potential of different extracts from *A. tetracantha* Lam. Int. J. Res. Ayurveda Pharm. 2015; 6 (1): 131-137.
22. Muthuswamy P, Elakkiya S, Manjupriya K, Deepa K, Ramachandran S, Shanumgapandiyan P. Preliminary phytochemical and in vitro antioxidant perspectives of the leaf extracts of *Azima Tetracantha* lam (Family: Salvadoraceae). Int J Pharm Biol Sci. 2012; 3 (1): 50- 58.
23. Gayathri G, Bindu R N, Babu V. *Invitro* antimicrobial and antioxidant studies on leaves of *Azima tetracantha* (Lam.). *International Journal of Current Research* 2011; 3 (12): 087-090.
24. Hepsibha B T, Sathiya S, Babu C S, Premalakshmi V, Sekar T. In vitro studies on antioxidant and free radical scavenging activities of *Azima tetracantha* Lam leaf extracts. Indian Journal of Science and Technology. 2010; 3 (5): 571-577.
25. Antonisamy P, Duraipandiyan V, Ignacimuthu S. Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. in mouse and rat models. Journal of Pharmacy and Pharmacology. 2011; 63, 1070–1077.
26. Ismail T S, Gopalakrishnan S, Begum V H, Elango V. Anti-inflammatory activity of *Salacia oblonga* Wall. and *Azima tetracantha* Lam. Journal of ethnopharmacology. 1997; *56* (2): 145-152.
27. Manikandaselvi S, Ramya D, Ravikumar R, Thinagarbabu R. Evaluation of antinephrotoxic potential of *Azima tetracantha* Lam. and *Tribulus terrestris* linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4, 566-568.
28. Soumya A, Nagarajan V. Hepatoprotective effect of *Azima tetracantha* Lam. on ferrous sulphate induced toxicity in albino rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014; 5 (1): 380-387.
29. Sunil C, Duraipandiyan V, Ignacimuthu S, Al-Dhabi N A. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from *Azima tetracantha* Lam. leaves. Food Chemistry. 2013; 139 (1–4): 860-865.
30. Sambasivam M, Ravikumar R, Thinagarbabu R, Davidraj C, Arvind S. Hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate-induced toxicity in rat. Bangladesh Journal of Pharmacology. 2013; 8 (3): 357-360.
31. Balakrishnan M, Dhanapal R, Vamsi M L M, Sekhar K C. Hepatoprotective activity of *Azima tetracantha* Lam. against Carbon tetra Chloride (CCl4)-induced hepatotoxicity in Wistar male albino rats. International Journal of Biological & Pharmaceutical Research. 2012; 3 (3): 366-372.
32. Nargis T B, Muhammad Ilyas M H, Vijaya Anand A. Hepatoprotective activity of *Azima tetracantha* Lam in experimental animals. Journal of Pharmacy Research. 2011; 4 (7): 2359-2360.
33. Antonisamy P, Duraipandiyan V, Aravinthan A, Al-Dhabi N A, Ignacimuthu S, Choi K C, Kim J H. Protective effects of friedelin isolated from *Azima tetracantha* Lam. against ethanol-induced gastric ulcer in rats and possible underlying mechanisms. European Journal of Pharmacology. 2015; 750: 167-175.
34. Muthusamy P, Suresh A J, Balamurugan G. Antiulcer activity of Azima tetracantha: a biochemical study. Research Journal of Pharmacy and Technology. 2009; 2 (2): 344-348.
35. Gayathri G, Bindu R N, Babu V. GC-MS Analysis of *Azima tetracantha* (LAM). Journal of Pharmacy Research. 2012;5 (7): 3746-3747.

Cite this article as:
Sundaresan Nandhini, Ramalingam Radha. Pharmacognosy of Azima Tetracantha: A Review. International Journal of Ayurveda and Pharma Research. 2015;3(12):13-19.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Nandhini Sundaresan
PG Scholar
Department of Pharmacognosy,
College of Pharmacy, Madras Medical College, Chennai-600003, Tamil Nadu, India.
Email: nandypharma1787@gmail.com
Mobile: +91 9566035357

WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.041

Volume 5, Issue 3, 1242-1246. Research Article ISSN 2278 – 4357

INVITRO ANTICANCER ACTIVITY OF LEAVES AND STEM OF *AZIMA TETRACANTHA* LAM.

Nandhini Sundaresan[1*], Ramalingam Radha[2] and Periyannan Muthusamy[3]

[*1]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

[2]Professor & Head of the Department, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

[3]Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

Article Received on
04 Jan 2016,

Revised on 24 Jan 2016,
Accepted on 14 Feb 2016

***Correspondence for Author**
Nandhini Sundaresan
PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

ABSTRACT

The present study deals with anticancer activity of *Azima tetracantha*. The leaves and stem of the plant material were extracted with hexane and ethanol using soxhlet extraction. Anticancer activities of various extracts were assayed with standard MTT colorimetric procedure against MCF-7 cell lines. From the analysis it was found that ethanolic leaf extract of *Azima tetracantha* showed MCF-7 cell line inhibition at ng range tested dose and other three extracts (hexane - leaf, stem and ethanolic stem) also displayed anticancer activities against MCF-7 cell line. Further work is in progress to evaluate the chemical constituents present in the extract.

KEYWORDS: *Azima tetracantha* Lam., Antioxidant, Anticancer, MTT Assay, MCF-7 cell lines.

INTRODUCTION

The word ‘cancer’ originated from *‘cancrum’* which is a Greek word for crab as it furnishes with claws on both sides of its body, so does the disease.[1] Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and in some cases, to metastasize, invade and spread to distant sites of the body.[2] Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism).[3,4] It

can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy.[5] Natural products have had a major impact on longevity and quality of life is in the chemotherapy of cancer. In fact, most major anticancer drugs are derived from plants or microorganisms. Examples include, Bleomycin, Doxorubicin, Daunorubicin, Vincristine, Mitomycin, Streptozocin, Paclitaxel (Taxol ^{TM}), Irinotecan (a camptothecin derivative), Etoposide and Tenoposide (podophyllotoxin derivatives).[6]

Azima tetracantha Lam., is a unique folk medicinal plant known as Mulsangu in Tamil, belonging to the family "Salvadoraceae", which has various medicinal properties like stimulant, antispasmodic, anti rheumatism, diuretic, anti inflammatory, anti microbial, hypoglycemic, antioxidant and hypolipidemic activity. It is also used in the treatment of cancer, dyspepsia and chronic diarrhoea.[7,8] Phytochemical composition of the plant like alkaloids[9], triterpenoids[10] and flavonoids[11] etc. were also reported. Clinical data showed that in the past there were herbs with anticancer property but the scientific validity of *Azima tetracantha* have not been established. Hence, the present study was designed to evaluate the anticancer activity of various extracts of leaves and stem of *Azima tetracantha.*

MATERIALS AND METHODS

Collection and identification of the Plant Material

The plant materials (leaves and stem) of *Azima tetracantha* Lam., were collected from Vellore district, Tamilnadu, India. The collected plant materials were botanically identified and authenticated by the Botanist Dr. Aravind, National Institute of Siddha, Tambaram, Chennai. The herbarium specimen was prepared and deposited at Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for future reference.

Extraction of plant material

Freshly harvested leaves and stem were air- dried at room temperature for a period for 2 weeks. Dried materials were coarsely grounded and stored in an air- tight container. About 200g of dried powdered leaves and stem were packed well in soxhlet apparatus and was subjected for hot extraction with hexane and ethanol (99.9%) at 18 hrs. The extract was filtered while hot and concentrated under Rotary vacuum evaporator in order to remove the solvent completely, dried and kept in a desiccator till further use.

IN VITRO ANTICANCER ACTIVITY

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture

MCF 7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37◦C in a humidified atmosphere of 5% CO2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay[12]

Cell viability was measured with the conventional MTT reduction assay. Briefly, MCF 7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37◦C for 4 h and then with DMSO at room temperature for 1h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments.

Cell viability (%) = Mean OD/Control OD x 100.

RESULTS

MCF-7 cell line inhibition of various extracts using MTT assay showed in Table 1 and Figure 1 and its graphical representation showed in Figure 2.

Table 1: In-vitro anticancer activity of the extracts of *Azima tetracantha*

Concentration (µg/ml)	MCF-7 Cell lines			
	Ethanolic Leaf Extract	Hexane Leaf Extract	Ethanolic Stem Extract	Hexane Stem Extract
Control	4.29	4.29	4.29	4.29
1ng	30.95	19.43	29.58	25.40
10ng	31.01	31.01	36.20	32.32
100ng	41.98	42.58	45.56	43.47
1µg	54.14	51.16	49.37	59.51
10µg	56.41	62.61	58.20	71.55
100µg	59.09	66.31	62.08	73.46

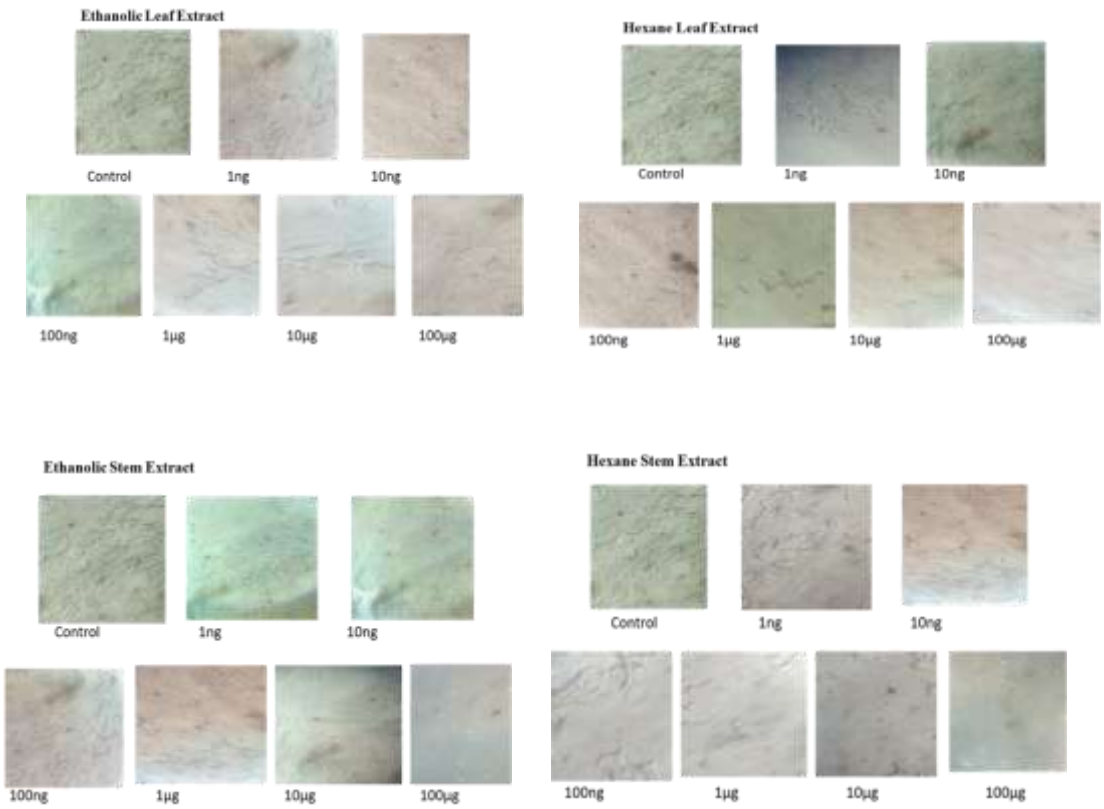


Fig 1: MCF-7 cell line inhibition of various extracts in different concentrations.

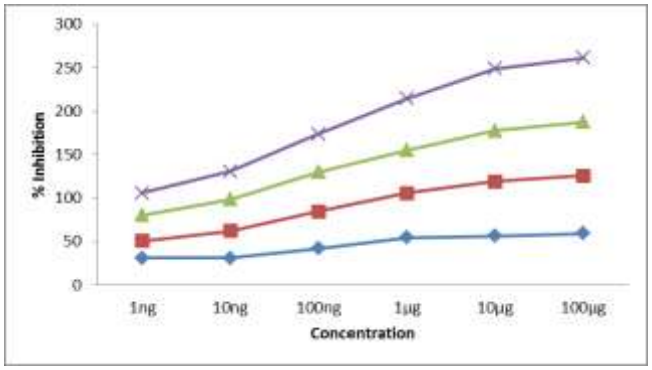


Fig 2: Graphical representation of MCF-7 cell line inhibition of various extracts.

DISCUSSION

Cancer is often associated with increased risk of death and the toxic side effects caused by the modern medicine, many cancer patients seek alternative and complementary methods of treatment such as usage of phyto medicine.

The present study shows a dramatic *in-vitro* anticancer activity of various extracts of *A. tetracantha* on human breast cancer cell line (MCF-7) at increasing concentrations. It showed that the ethanolic extract have more anticancer activity on MCF-7 cell line in the ng range.

CONCLUSION

The present study showed that *Azima tetracantha*, ethanolic leaf extract might be a potential alternative agent for human breast cancer therapy. Hence, it is anticipated that *A. tetracantha* would be a useful pharmaceutical material to treat breast cancer. Future research should focus on the molecular mechanism of *A. tetracantha* for anticancer action. There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s) to treat breast cancer.

REFERENCE

1. Kaushal SP. Fight cancer with herbs, 1st edn, Delhi. Vedic Cancer Research Centre., 1999; 1.
2. http://www.who.int/cancer/en/ accessed on 07/10/15.
3. Divisi D, Di TS, Salvemini S, et al., Diet and cancer. Acta Biomed, 2006; 77: 118-123.
4. Kuper, H; Boffetta, P; Adami, H.O. Tobacco used and cancer causation: association by tumor type. J. Intern., 2002; 252(3): 206-24.
5. https://en.wikipedia.org/wiki/Cancer accessed on 07/10/15.
6. Manuchair Ebadi. Pharmacodynamic basis of herbal medicine, 2nd ed. Taylor and Francis Group., 2009.
7. Khare CP. Indian Medicinal Plants. An illustrated dictionary. *Azima tetracantha* Lam. Ed, 2007; 76.
8. Raveesha HR, Sudhama VN. Ethnoveterinary Practices in Mallenahalli of Chikmagalur Taluk, Karnataka. Journal of Medicinal Plants studies., 2015; 3(1): 37-41.
9. Rall GJH, Smalberger TM, de Waal HL and Arndt RR. Dimeric piperdine alkaloids from *Azima tetracantha* Tetrahedron Letters., 1967; 36: 3465-6.
10. Venkatrao E and Prasad Rao PRS. Occrurrence of triterpenoids in *Azima tetracantha* Current Science., 1978; 47(22): 857.
11. Richard NB, Fred AM, Eduardo AS Rosa, Lionel P and Paul AK. Profiling Glucosinolates, Flavonoids, Alkaloids and Other Secondary Metabolites in Tissues of *Azima tetracantha* L. (Salvadoraceae). Journal of Agriculture and Food Chemistry., 2004; 52(19): 5856–5862.
12. Evelyn MLP, Fernando WCA, Ivone AS, Isla VGAB, et al. Pharmacological screening and acute toxicity of bark roots of *Guettarda platypoda*. Brazilian Journal of Pharmacognosy., 2012.

World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086

Available online at: http://www.wjpsonline.org/
Original Article

Pharmacognostical Studies of *Azima tetracantha* Lam. (Salvadoraceae)

Sundaresan Nandhini[1]*, Ramalingam Radha [2], Vijayabharathi Rajkishore[3]

*[1]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.
[2]Professor & Head of the Department, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.
[3]Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

Received: 22-02-2016 / Revised: 09-03-2016 / Accepted: xx-xx-2016 / Published:

ABSTRACT

The present study deals with evaluation of pharmacognostical characters of an important medicinal plant, *Azima tetracantha* Lam. The pharmacognostical studies were carried out such as organoleptic, macroscopic, microscopic and fluorescence analysis. Microscopic studies include transverse and longitudinal sections, powder microscopy and quantitative microscopy such as stomatal number, stomatal index, vein islet number and veinlet termination number. Various pharmacognostical characters observed in this study help in the identification and standardization of *Azima tetracantha.*

Keywords: *Azima tetracantha*, Microscopy, Organoleptic, Fluorescence analysis, Identification.

INTRODUCTION

Over the last few decades, there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment.[1] *Azima tetracantha* Lam., is a unique folk medicinal plant known as Mulsangu in Tamil, belonging to the family "Salvadoraceae", *Azima tetracantha* is a perennial shrub growing upto 3m height in hot, dry riverine scrub, particularly on alluvial or saline soil. It occurs naturally in central, eastern and southern Africa as well as in the Indian Ocean Islands and extends through Arabia to tropical Asia. It has various medicinal properties like stimulant, antispasmodic, anti-rheumatism, diuretic, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant and hypolipidemic activity. It is also used in the treatment of cancer, dyspepsia and chronic diarrhoea.[2,3] Microscopy is an important tool for authentication of crude drugs and study of powdered drugs.[4] It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance.[5] Establishment of the pharmacognostic, morphological and microscopical characters of leaves and stem of the plant will assist in standardization, which can guarantee quality, purity and identification of samples.

MATERIALS AND METHODS

Procurement of plant materials: Fresh leaves and stem of *Azima tetracantha* were collected from Vellore district, Tamilnadu, India. Identification of the plant was done by Dr. G.V.S Murthy, Scientist 'F', Botanical Survey of India, under reference number BSI/SRC/5/23/2016/Tech./178.

Organoleptic evaluation[6]: Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of particular drug. Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation.

Macroscopic evaluation[7]: Various macroscopic characters of fresh leaves and stem of *Azima*

***Corresponding Author Address: Sundaresan Nandhini,** PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India. Email: nandypharma1787@gmail.com*

tetracantha were recorded such as type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture. The stem is morphologically studied for its size, shape, surface, fracture and configuration.[6]

Microscopic evaluation: In microscopic evaluation, studies were conducted on both grounds qualitatively and quantitatively.

Qualitative microscopy: In this study, transverse and longitudinal sections of leaf and stem were studied under photomicrograph. Staining reagents (such as phloroglucinol-HCl and methyl orange) were used as per standard procedures.[8-11] The various identifying characters were studied with or without staining and recorded.

Powder microscopy: The dried leaves and stem were powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. A little quantity of leaves and stem powder were taken onto a microscopic slide, 1–2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain and calcium oxalate crystal was detected by the formation of blue colour on addition of 2–3 drops of 0.01 M iodine solution.[12] The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

***Quantitative microscopy*[9,13]**

Determination of stomatal number and stomatal index: Stomatal number is the average number of stomata per sq mm of epidermis of the leaf. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index. Stomatal index can be calculated by using following equation:

$$S.\ I = S / E + S \times 100$$

Where, I = stomatal index, S = number of stomata per mm^2 and E = number of ordinary epidermal cells per mm^2.

A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately by means of forceps. It was kept on slide and mounted in glycerin water. Camera lucida was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted. The numbers of stomata were counted as stomatal number and the stomatal index using the above formula was calculated separately for upper and lower surface.

Determination of vein-islet and vein termination number: Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per sq mm of leaf surface.

A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The average number of vein islet from the four adjoining square, to get the value for one square mm was calculated. The number of veinlet termination present within the square was counted and the average number of veinlet termination number from the four adjoining square to get the value for 1 sq mm was found known as vein termination number

Physicochemical analysis[14,15]: Physicochemical constants such as ash values, extractive values, loss on drying, volatile oil content, swelling index, foaming index and foreign organic matter were observed

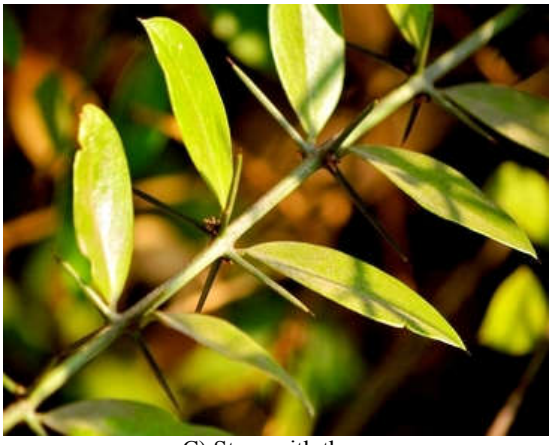
Fluorescence analysis[16]: Fluorescence analysis of powder of leaves was done by standard procedure. In this analysis the powder were treated with various acidic and basic solvents and were then observed in UV/ visible chamber under visible, short wave and long wave regions simultaneously.

RESULTS

Macroscopical characters

A) Leaf

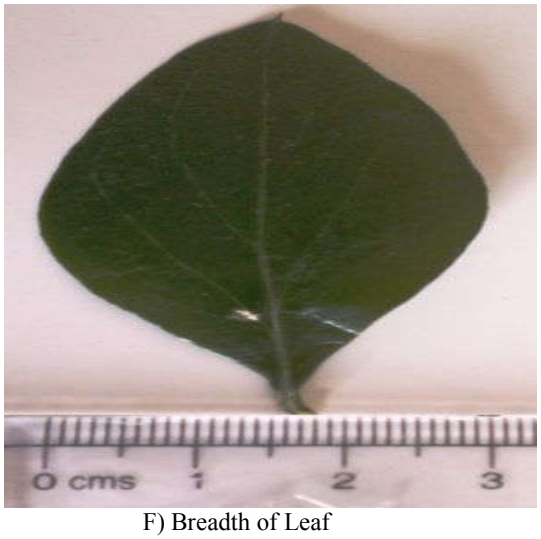
B) Flowers



C) Stem with thorns

D) Fruits

E) Length of leaf



F) Breadth of Leaf

Fig 1: Macroscopy of *Azima tetracantha* Lam.

Morphology of *Azima tetracantha* A) Leaf, B) Stem with thorns, C) Flower, D) Fruits, E) Length of Leaf and F) Breadth of Leaf

Organoleptic evaluation

Table 1: Organoleptic evaluation of *Azima tetracantha* Lam.

S. No	Particulars	Observations
1.	Colour	Dark green to pale green
2.	Odour	Characteristic odour
3.	Taste	Tasteless
5.	Margin	Simple and entire
6.	Apex	Mucronate, sharp-tipped
7.	Base	Acute base
9.	Shape	Aristate with a spine on tip
10.	Vein	Cross venulate
11.	Stipules	Absent or rudimentary
12.	Leaflets	Pinnate

Microscopic evaluation

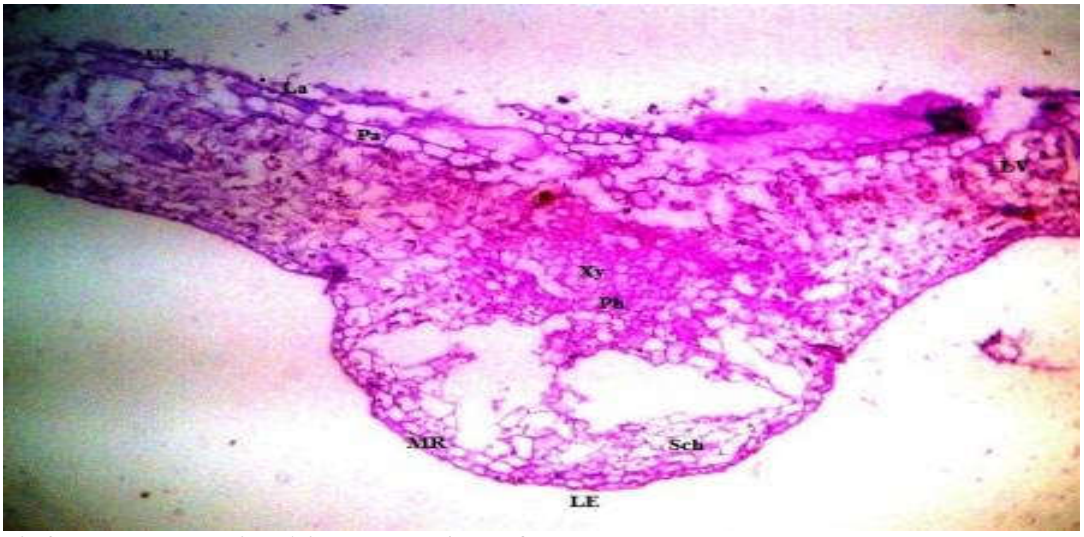
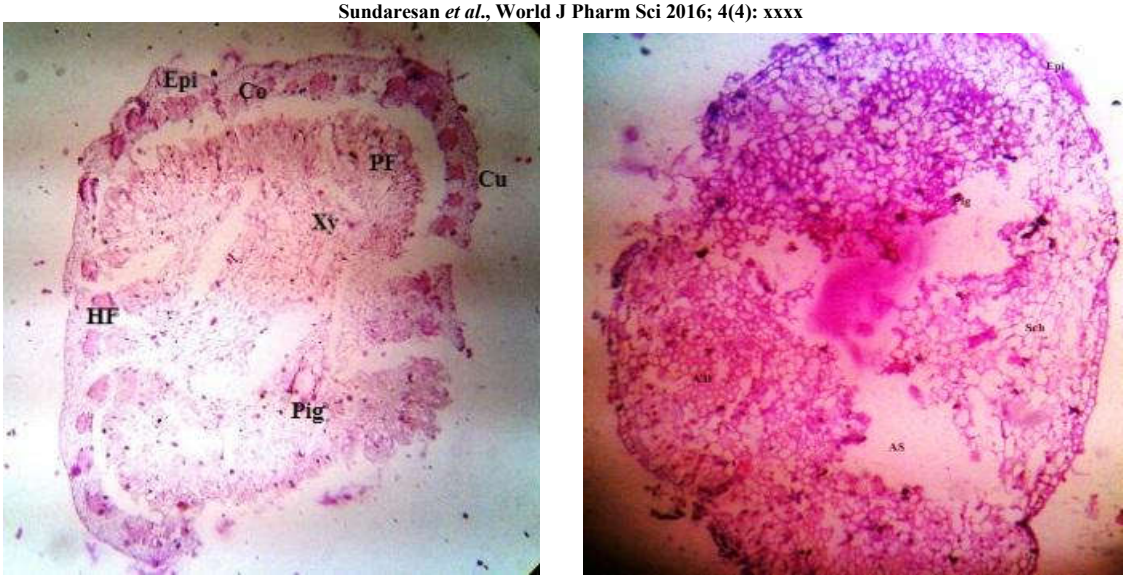


Fig 2: Transverse section *Azima tetracantha* Leaf



A B

Fig 3: Transverse section of *Azima tetracantha* A) Stem, B) Thorn

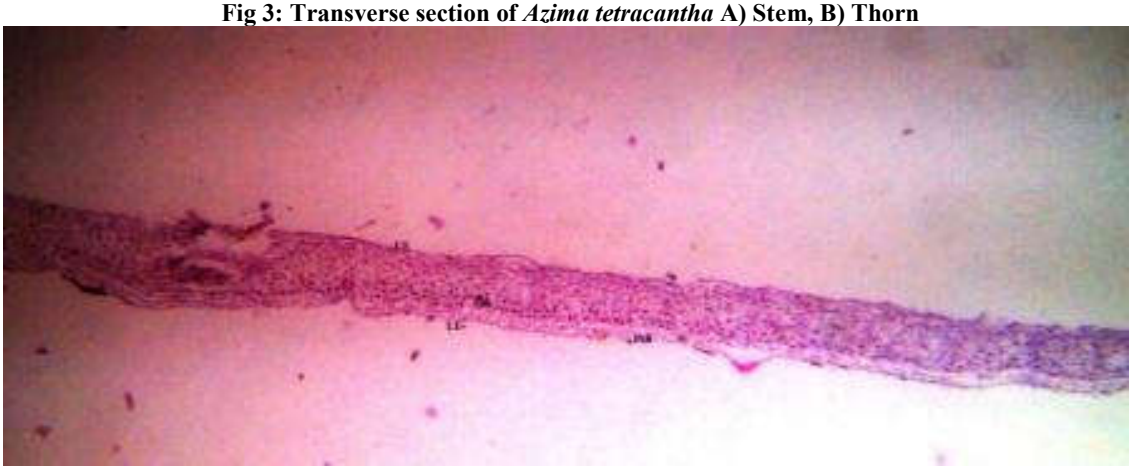
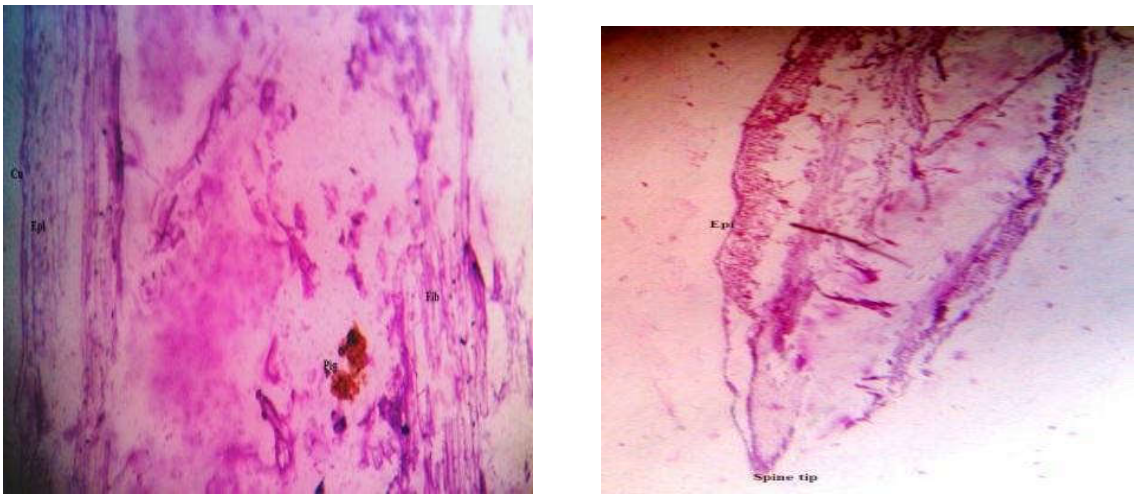


Fig 4: Longitudinal section of *Azima tetracantha* Leaf



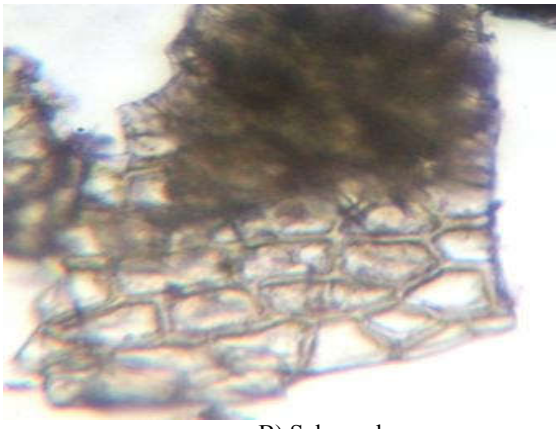
A B

Fig 5: Longitudinal section of *Azima tetracantha* A) Stem, B) Thorn

UE- Upper epidermis, La- Lamina, Pa- Palisade cells, Xy- Xylem, Ph- Phloem, MR- Midrib, LE- Lower epidermis, Sch- Sclerenchyma, LV- Lateral vein, AS- Airsac, VB- Vascular bundle, Pig- Pigments, HF- Hypodermal fibres, PF- Pericyclic fibres, Cu- Cuticle, Co- Cortex

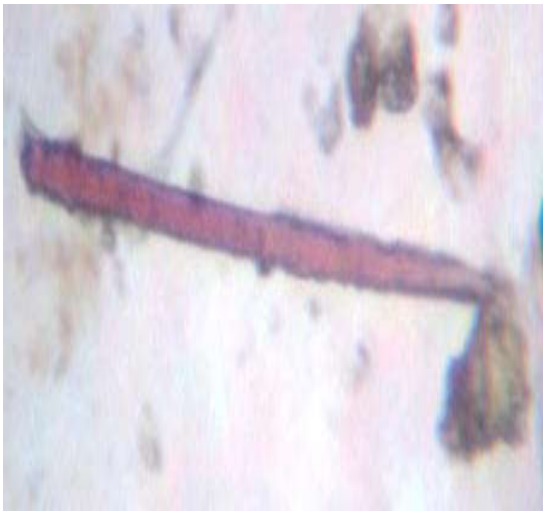
Powder microscopy

Parenchyma

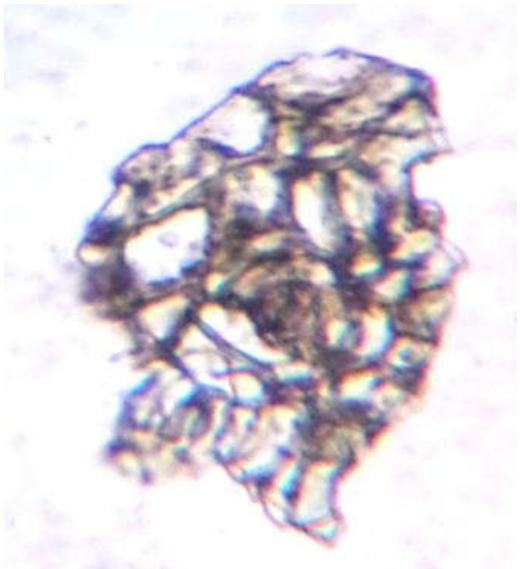


B) Sclerenchyma

Trichomes



D) Fibres



1. Stomata

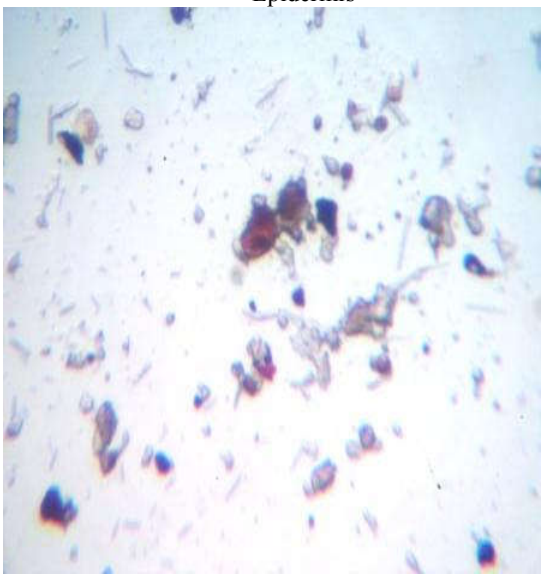
E) Crystal sheath

Xylem vessels

Fig 6: Powder microscopy of *Azima tetracantha* Leaf powder

Epidermis

B) Fibres



Pigments

D) Xylem vessels

Fig 7: Powder microscopy of *Azima tetracantha* Stem powder

Quantitative microscopy

Table 2: Quantitative microscopy of *Azima tetracantha* Leaf

S. No	Parameters	Values
1.	Stomatal number- Lower epidermis	23-27
2.	Stomatal index- Lowder epidermis	19-20
3.	Vein islet number- Lowder epidermis	9-15
4.	Veinlet termination number- Lowder epidermis	15-19

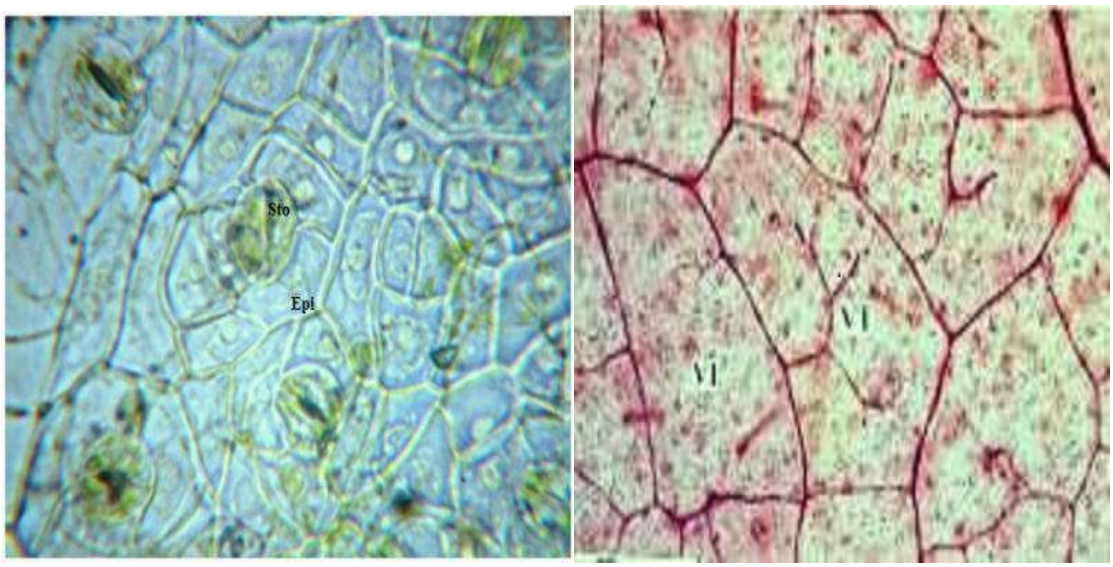


Fig 8: Quantitative microscopy of *Azima tetracantha* Leaf

Physicochemical analysis

Table 3: Physicochemical constants of *Azima tetracantha* Leaf and Stem

S. No	Parameters	Leaves	Stem
1.	Total ash	7.45%	21.039%
2.	Acid insoluble ash	1.06%	0.493%
3.	Water soluble ash	6.98%	13.985%
4.	Sulphated ash	15.35%	18.84%
5.	Loss on drying	15.27%	11.95%
6.	Water soluble extractive value	1.593%	1.394%
7.	Ethanol soluble extractive value	1.197%	0.399%
8.	Foreign organic matter	3.6%	No
9.	Volatile oil content	1.5%v/w	No
10.	Swelling Index	No	No
11.	Foaming Index	<100	<100

Flourescence analysis

Table 3: Flourescence analysis of *Azima tetracantha* Lam. leaves

Treatment	Day light	UV light	
		254nm	365nm
Powder	Pale-brownish green	Pale green	Brown
Powder + 1N NaOH (aqueous)	Yellowish green	Pale yellow	White
Powder + 1N NaOH (alcoholic)	Pale green	Brownish green	Brownish green
Powder + 1N Hydrochloric acid	Pale green	Brown	Black
Powder + 50% Sulphuric acid	Pale green	Bluish green	Pale green
Powder + 50% Nitric acid	Green	Yellowish green	Black
Powder + Picric acid	Dark yellow	Brown	Pale brown
Powder + Acetic acid	Pale green	Brownish green	Pale green
Powder + Ferric chloride	Orange	Brown	Pale brown
Powder +Con. Nitric acid	Brown	Brown	Black
Powder + Nitric acid + Ammonia	Green	Green	Black

DISCUSSION

Macroscopic study showed that leaf shape- blade elliptical-oblong to ovate-oblong or orbicular, base-pinnately veined with one pair of lateral veins and leaf margin was simple and entire with characteristic odour. Young stem measuring 1.5mm thick. Its outline is smooth and even. The characteristic microscopic features of leaves were observed the presence of epidermis, stomata, lamina and stem consists of a distinct continuous epidermis, cortex, vascular cylinder and pith. The characteristic microscopy of leaf powder showed the presence of parenchyma, sclerenchyma, trichomes, fibers, stomata, crystal sheath and xylem vessels. The characteristic microscopy of stem powder showed the presence of epidermis, fibres, pigments and xylem vessels. Quantitative microscopy showed the presence of stomata in the lower epidermis. There was no fluorescence compound in the plant drug.

CONCLUSION

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, microscopic, fluorescence analysis are done. Morphological and microscopical studies of the leaf will enable to identify the crude drug. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. Stomatal number, stomatal index, vein islet and vein termination value determination are equally important in the evaluation of crude drugs. These values help in the evaluation of purity of drugs. In conclusion, the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry/ trade and this can be included as microscopic standards in Indian Herbal Pharmacopeia.

REFERENCES

1. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal plants. Calcutta: Naya Prokash. 2001; 449–632.
2. Khare CP. Indian Medicinal Plants. An illustrated dictionary. 2007; 76.
3. Raveesha HR, Sudhama VN. Ethnoveterinary Practices in Mallenahalli of Chikmagalur Taluk, Karnataka. Journal of Medicinal Plants studies. 2015; 3(1): 37-41.
4. Rangari VD. Pharmacognosy and phytochemistry. 1st ed. New Delhi: Career Publications. 2006; 103-15.
5. Evans WC. Trease and evans pharmacognosy. 16th ed. Edingburgh: Saunders Elsevier. 2009; 541-70.
6. Kokate CK, Purohit AP, Gokhle SB. Pharmacognosy. Delh: Vallabh Prakashan, Publishers 2004.
7. Yunus MFLS, Yunus D. Systematic bark morphology of some tropical trees. Bot J Linn Soc. 1990; 103: 367-77.
8. Pandya DJ, Desai TR, Nadpara NP, Mehta HA, et al. Pharmacognostic study and establishment of quality parameters of leaves of *Bombax insigne* Linn. Int J Pharmacogn Phytochem Res. 2010; 2(3): 1-5.
9. Kokate CK. Practical pharmacognosy. New Delhi: Vallabh Prakashan. 2005; 107-8, 115-20, 122-3.
10. Wallis TE. Text book of pharmacognosy. 5th ed. New Delhi: CBS Publishers & Distributers. 1985; 68-101.
11. Ali M. Text book of pharmacognosy. New Delhi: CBS Publishers & Distributer. 2008; 181-211.
12. Thitikornpong W, Phadungcharoen T, Sukrong S. Pharmacognostic evaluations of *Lagerstroemia speciosa* leaves. J Med Plant Res. 2011; 5(8): 1330-37.
13. Srinivasa B, Kumar A, Prabhakarn V, Lakshman K, et al. Pharmacognostical studies of *Portulaca oleracea* Linn. Braz J Pharmacogn. 2008; 18(4): 527-31.
14. Indian pharmacopoeia, 4th edn., Vol. II, Government of India , Ministry of Health and Welfare, Controller of Publications, New Delhi. 1996: A53-4.
15. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright- Scientechnica, Bristol. 1975; 36- 45.
16. Chase CR, Pratt RJ. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Am. Pharmacol. Assoc. 1949; 38: 32.

World J Pharm Sci 7:49 AM (4 hours ago)

to me

Dear Author,

We have received revised galley proof.

Your article is scheduled to publish on 1st April 2016.

We are looking for your contribution in near future, and also Please encourage your colleagues and scholars to contribute their research work to World journal of pharmaceutical sciences (www.wjpsonline.org and editor@wjpsonline.org)

WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.041

Volume 5, Issue 4, XXX-XXX Research Article ISSN 2278 – 4357

BIOACTIVITY GUIDED ISOLATION OF CRUDE ETHANOL EXTRACT OF *AZIMA TETRACANTHA* LEAVES

Sundaresan Nandhini[1]*, Ramalingam Radha[2] and Narayanan Jayshree[3]

[*1]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

[2,3]Professor & Head of the Department, Department of Pharmacology, College of Pharmacy, Madras Medical College, Chennai, India.

Article Received on
27 Jan 2016,

Revised on 17 Feb 2016,
Accepted on 09 March 2016

DOI: 10.20959/wjpps20164-6378

***Correspondence for Author**

Sundaresan Nandhini

PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

nandypharma1787@gmail.com

ABSTRACT

Objective: To evaluate the antioxidant activity of the isolated fractions of crude ethanolic extract of *Azima tetracantha*. **Methods:** The crude ethanolic extract of *Azima tetracantha* leaves was subjected to chromatographic separation using column and thin layer chromatographic techniques. From the eluted fractions, F16, F27, F32, F36, F37, F38 and F45 fractions were screened for antioxidant activity using DPPH assay in *invitro*. **Results:** The extract yielded 7 component fractions. From which, fraction 32 (F32) exhibited the most profound anti-oxidant activity.

KEYWORDS: Azima tetracantha, Bioassay guided fractionation, Antioxidant, DPPH assay.

INTRODUCTION

Bioactivity guided isolation method is commonly employed in drug discovery research due to its effectiveness to directly link the analyzed extract and targeted compounds using fractionation procedure that followed with certain biological activity. In pharmacognosy, a typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity, followed by next round of separation and assaying.

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells.[1] As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease.[2]

MATERIALS AND METHODS

Preparation and extraction of the plant material

Fresh leaves of *Azima tetracantha* plant were collected and authenticated by a taxonomist from Botanical Survey India, Coimbatore. Freshly harvested leaves were air- dried at room temperature for a period for 2 weeks. Dried materials were coarsely grounded and stored in an air- tight container. About 200g of dried powdered leaves were packed well in soxhlet apparatus and was subjected for hot extraction with ethanol (99.9%) at 18 hrs. The extract was filtered while hot and concentrated under Rotary vacuum evaporator in order to remove the solvent completely, dried and kept in a desiccator till further use.

Isolation of the crude extract of *Azima tetracantha* leaves[3, 4]

Column chromatography

The crude extract of *Azima tetracantha* (8 g) was subjected to column chromatography to separate the extract into its component fractions. Silica gel 100-200 mesh was used as the stationary phase while varying solvent combinations of increasing polarity were used as the mobile phase. The column was prepared using silica gel (100- 200 mesh) with hexane. The crude extracts 8g was mixed with 16g of silica gel (1:2) separately (60-120 slurry) and loaded in the packed column. Elution of the extract was done with solvent systems of gradually increasing polarity using hexane, chloroform, and ethanol. The following ratios of solvent combinations were sequentially used in the elution process; Hexane: chloroform 100:0, 95:5, 90:10, 80:20, 50:50, 20:80, and 10:90; chloroform: Ethanol 100:0, 95:5, 90:10, 80:20, 50:50, 20:80, and 10:90; 0:100 The column flow rate was 1ml/min approximately 200 ml of 45 fractions was collected. TLC was carried out simultaneously for each fraction with the suitable mobile phase. The spots were visualized either by exposing to iodine vapors and/or

UV light. The fractions showing the same R_f was combined together. Based on TLC profile 15 major fractions was collected, saved and kept under air current to facilitate drying. The collected fractions were resulted in the **Table 1.**

***Thin layer chromatography*[5-7]**

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is a reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. TLC was carried out using 5 µl of 100 mg extract/ml solution on Merck TLC F254 by Hexane: Ethyl acetate (7:3) and Chloroform: Ethanol (5:5) mixture as eluents.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

***Invitro* Antioxidant activity**

***DPPH assay*[8]**

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). Two ml of 6 $\times 10^{-5}$ M methonolic solution of DPPH were added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound (Vitamin C) were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows.

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula.[9]

$$IP = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min.

$A_{A(t)}$ is the absorbance of the antioxidants at t = 16 min.

RESULTS AND DISCUSSION

Table 1: Isolation of the crude extract of *Azima tetracantha* leaves.

S. No	Eluent	Solvent ratio	Fractions
1.	Hexane	100	F1
2.	Hexane: Chloroform	95:5	F2-F6
3.	Hexane: Chloroform	90:10	F7-F15
4.	Hexane: Chloroform	80:20	**F16**
5.	Hexane: Chloroform	50:50	F17-F26
6.	Hexane: Chloroform	20:80	**F27**
7.	Hexane: Chloroform	10:90	F28-F31
8.	Chloroform	100	**F32**
9.	Chloroform: Methanol	95:5	F33-F35
10.	Chloroform: Methanol	90:10	**F36**
11.	Chloroform: Methanol	80:20	**F37**
12.	Chloroform: Methanol	50:50	**F38**
13.	Chloroform: Methanol	20:80	F39, F40
14.	Chloroform: Methanol	10:90	F41-F44
15.	Methanol	100	**F45**

Invitro Antioxidant activity

Invitro antioxidant activity of all the fractions were done using DPPH assay and resulted in **Table 2.**

Table 2: DPPH Scavenging Effect of various fractions.

Fractions	Control	Concentrations µg/ml									
		200		400		600		800		1000	
	OD	OD	%	OD	%	OD	%	OD	%	OD	%
Vit C	0.595	0.531	10.76	0.425	28.57	0.31	47.90	0.247	58.49	0.133	77.65
F1	0.595	0.578	2.86	0.544	8.57	0.512	13.95	0.489	17.82	0.477	19.83
F2	0.595	0.565	5.04	0.537	9.75	0.521	12.44	0.519	12.77	0.501	15.80
F3	0.595	0.58	2.52	0.576	3.19	0.558	6.22	0.527	11.43	0.508	14.62
F4	0.595	0.511	14.12	0.467	21.51	0.422	29.08	0.386	35.13	0.351	**41.01**
F5	0.595	0.593	0.34	0.581	2.35	0.578	2.86	0.556	6.55	0.532	10.59
F6	0.595	0.568	4.54	0.543	8.74	0.415	30.25	0.403	32.27	0.377	**36.64**
F7	0.595	0.581	2.35	0.572	3.87	0.481	19.16	0.422	29.08	0.407	31.60
F8	0.595	0.533	10.42	0.506	14.96	0.46	22.69	0.369	37.98	0.261	**56.13**
F9	0.595	0.591	0.67	0.574	3.53	0.512	13.95	0.493	17.14	0.469	21.18
F10	0.595	0.571	4.03	0.542	8.91	0.521	12.44	0.497	16.47	0.473	20.50
F11	0.595	0.588	1.18	0.571	4.03	0.535	10.08	0.503	15.46	0.477	19.83
F12	0.595	0.593	0.34	0.582	2.18	0.566	4.87	0.549	7.73	0.521	12.43
F13	0.595	0.559	6.05	0.532	10.59	0.511	14.12	0.496	16.64	0.47	21.01
F14	0.595	0.584	1.85	0.563	5.38	0.551	7.39	0.532	10.59	0.514	13.61
F15	0.595	0.552	7.23	0.516	13.28	0.451	24.20	0.382	35.80	0.317	**46.72**

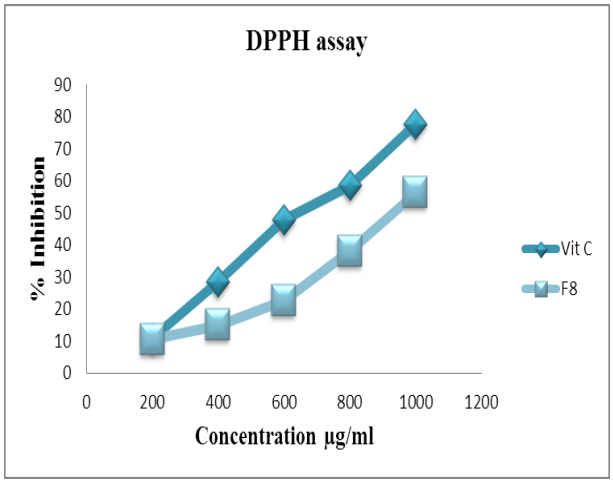


Fig 1: Percentage inhibition of Fraction 8 comparison with Standard.

CONCLUSION

Bioactivity guided isolation played an important role in the isolation of active compounds/ fractions from the natural products. In the present study, bioactivity guided isolation of various compounds/ fractions were isolated from the leaves of *Azima tetracantha*. From the entire isolated fraction, it is concluded that the fraction 8 showed 56.13% of antioxidant activity when compared to that of standard (Vitamin C) in DPPH assay. Based on the antioxidant activity, further studies are needed to identify the anticancer potential of the isolated fraction.

REFERENCES

1. Sies H. (ed.) Antioxidants in Disease, Mechanisms and Therapy, Academic Press, New York, 1996.
2. Bjelakovic G; Nikolova, D; Gluud, LL; Simonetti, RG; Gluud, C "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis". JAMA, 2007; 297(8): 842–57.
3. Ode OJ, Asuzu IU, Ajayi IE. Bioassay guided fractionation of the crude methanol extract of *Cassia singueana* leaves. Journal of Advanced Scientific Research., 2011; 2(4): 81-6.
4. Poornima SB, Gopalsamy S, Sarala S, Suma R, et al. Antiviral activity of bioassay guided fractionation of *Plumbago zeylanica* roots against herpes simplex virus type 2. World Journal of Pharmacy and Pharmaceutical sciences., 2014; 3(12): 1003-17.
5. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, 2nd edn. New Delhi: CBS Publishers., 2001; 115-26.

6. Stahl E. Thin layer chromatography, 2nd edn. New York (Heidenberg): Springer- Verlag., 1969; 30-160.
7. Wagner H. Plant drug analysis: A thin layer chromatography. Atlas, 2nd edn. Heidenberg: Springer- Verlag Belgium., 2002; 305: 227.
8. Von Gadow A, Joubert E, Hansmann CF. Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (Aspalathon linearis), α-tocopherol, BHT and BHA. Journal of Agricultural and Food Chemistry, 1997; 45: 632-8.
9. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. Journal of Agricultural and Food Chemistry, 1994; 42: 629-32.

WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

Impact Factor 6.041 ICV 6.21

Acceptance Letter

Manuscript No: WJPPS/6378/5/2016 **Date: 01/03/2016**

TITLE: BIOACTIVITY GUIDED ISOLATION OF CRUDE ETHANOL EXTRACT OF *AZIMA TETRACANTHA* LEAVES

Dear Sundaresan Nandhini

We are pleased to inform you that out of various research articles submitted, Experts/ Referees Panel of WJPPS has recommended your manuscript for publication, so World Journal of Pharmacy and Pharmaceutical Sciences has been accepted your manuscript for publication in Current (March) Issue of WJPPS.

World Journal of Pharmacy and Pharmaceutical Sciences publishes all its article in full open access format which are easily accessible for scientific community.

Kindly send the scanned copy of CTA form (Copyright Transfer Agreement). As early as possible. CTA form available at

www.wjpps.com

Thanking You

Editor in chief

Dr.Valentina Petkova

WJPPS

Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(1): 87-91
Received: 07-11-2015
Accepted: 09-12-2015

Nandhini S
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003.

Geethalakshmi S
Institute of Microbiology, Madras Medical College, Chennai-600003.

Selvam S
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003

Radha R
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003

Jerad Suresh A
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003

Muthusamy P
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003

Correspondence
Muthusamy Periyannan
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003, Tamil Nadu, India.
muthusamy_p99@Gmail.com

Preliminary phytochemical and anti-diabetic activity of *Cassia sophera* Linn

Nandhini S, Geethalakshmi S, Selvam S, Radha R, Jerad Suresh A, Muthusamy P

Abstract
The medicinal plant *Cassia sophera* was analysed for screening of phytochemical and anti-diabetic activity. The objective of the present study was to investigate anti-diabetic activity of *Cassia sophera* bark, using Streptozocin induced diabetic rats as model. The biochemical study was carried out and the results shown that ethanolic extract of dose 400 mg/Kg has more effective against Streptozocin induced diabetic rats. We conclude from the present study that the ethanolic extract of bark of *Cassia sophera* may be beneficial in the management of diabetes.

Keywords: *Cassia sophera* Linn. Poonaverie, Blood glucose, Anti-diabetic.

Introduction
Cassia sophera Linn. (Caesalpiniaceae) known as Poonaverie in Tamil, is an evergreen herb found in India and most tropical countries. It is common in waste lands, on roadsides and in the forests. The plant is used in the treatment of diabetes, hepatoprotective, asthma, psoriasis and in ringworm infections [1-4].
Diabetes is known as "diabetes mellitus" - where diabetes comes from the Greek word for siphon, which describes the excessive thirst and urination of this condition, and mellitus is the Latin word for honey, because diabetic urine is filled with sugar and is sweet. Diabetes is the common term for several metabolic disorders in which the body no longer produces insulin or uses the insulin it produces ineffectively. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. The cause of diabetes continues to be a mystery, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles [5-7].

Materials and Methods
The fresh bark of *Cassia sophera* was collected from Kerala state and the plant specimen was authenticated by Dr. V. Chelladurai, Research Officer Botany, Survey of Medicinal Plants Unit, Tirunelveli.

Preparation of Extracts
The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Cassia sophera* (500g) was first extracted with petroleum ether (60 – 80 °C) in Soxhlet apparatus and then with solvents of increasing polarity like chloroform, ethyl acetate and ethanol at 60 – 70 °C. Each extracts was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed phytochemical and pharmacological screening.

Phytochemical studies [8-10]
Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. Therefore a complete investigation is required to characterize the phyto constituents qualitatively and quantitatively.

Preliminary phytochemical screening
The chemical tests for various phyto constituents in the dried powder and exracts of bark of *Cassia sophera* Linn. were carried out as described below.

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

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

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

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

Modified Borntrager's test
The test material was boiled with 2ml of dil. Sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dil. Ammonia. A rose pink to red colour is produced in the ammoniacal layer.

Detection of Steroids and Triterpenoids
Libermann Burchards test
The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added from the sides of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

Salkowski test
The extracts was treated with few drops of Conc. Sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Detection of Flavonoids
Shinoda test
To the solution of extract, few piece of magnesium turnings and conc. HCl was added dropwise, pink to crimson red, occasionally green to blue colour appear after few minutes indicates the presence of flavonoids.

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

Detection of Carbohydrates
Molisch's test
To the test solution few drops of alcoholic α- naphthol solution and few drops of con. sulphuric acid were added through the sides of the test tube, purple to violet colour ring appears at junction.

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

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

Detection of Proteins
Biuret test
The sample was treated with 5-8 drops of 10%w/w copper sulphate solution, violet colour is formed.

Detection of Tannins
Lead acetate test
The test solution was mixed with basic lead acetate solution and examined for the formation of a white precipitate.

Ferric chloride test
A few drops of 5% aqueous ferric chloride solution was added to 2 ml of aqueous extract of the drug and examined for the appearance of bluish black colour.

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

Detection of Gum and Mucilage
Small quantities of test substances was dissolved in 5 to 10ml of acetic anhydride by means of heat, cooled and add 0.05ml of conc. Sulphuric acid; it is examined for the formation of bright purplish red colour.

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

Quantitative Estimation of Phytoconstituents
Glycosides Estimation [11]
Materials
Tincture
Baljet reagent (freshly prepared)

Preparation of tincture
A 10% extract in 70% alcohol is prepared by shaking for 2 hrs then filtered.

Spectrophotometric Determination
In the assay of tincture, 10ml of the 10% tincture (+0.8g powdered bark) are diluted during purification to 200ml, from which 10 ml (=0.04g powdered bark) are used in the assay, after being treated with 10 ml Baljet's reagent, and diluted with 20ml of water(to a total of 40ml). measure the absorbance of the tincture at 495 nm.

Fluorescence Analysis [12]

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

***In vitro* Glucose Diffusion Inhibition Study** [13]

A simple model system was used to evaluate the effects of plant extracts on glucose movements *in vitro*. The model was adapted from a method described by Edwards *et al*., which involved the use of a sealed dialysis tube into which 15ml of a solution of glucose and sodium chloride (0.15 M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube into which 1 ml of 50 g/l plant extract in 1% CMC and 2ml of 0.15 M sodium chloride containing 0.22 M D- glucose was added. The dialysis tube was sealed at each end and placed in a 50ml centrifuge tube containing 45 ml of 0.15 M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

***In vivo* Anti-Diabetic Activity** [14, 15]

Healthy Wistar albino rats weighing about 150-230g were kept fasting overnight but allowed for access to water. The rats were injected intra peritoneally with Streptozocin dissolved in citrate buffer of pH 4.5 at a dose of 555 mg/Kg body weight. After 48 hours, rats with blood glucose level 250 mg/dl were selected for the study.

Treatment

The animals were randomly divided into 5 groups of six animals each, after the induction of diabetes.

Group 1: Non diabetic control rats received 1ml of 1% CMC orally once daily for 4 weeks.

Group 2: Diabetic control rats received 1ml of 1% CMC orally once daily for 4 weeks.

Group 3: Diabetic control rats given Ethanolic bark extracts (200 mg/Kg) of *Cassia sophera* Linn. Made fine suspension with 1 ml of 1% CMC once daily for 4 weeks.

Group 4: Diabetic control rats given Ethanolic bark extracts (400 mg/Kg) of *Cassia sophera* Linn. Made fine suspension with 1 ml of 1% CMC once daily for 4 weeks.

Group 5: Diabetic control rats given Glibenclamide 3 mg/Kg body weight made fine suspension with 1ml of 1% CMC once daily for 4 weeks.

Biochemical analysis

Blood samples were collected from the retro orbital plexus of the rats at the end of 0 hr, 3 hr, 5 hr, 7 hr, 24 hr (Acute study) and 1st, 2nd, 3rd and 4th week (chronic study), samples were analysed for blood glucose content by glucometer.

Results

Table 1: Percentage yield of Successive solvent extracts of the bark of *Cassia sophera* Linn.

S. No	Extracts	Methods of Extraction	Physical Nature	Colour	Yield (%w/w)
1.	Petroleum ether	Soxhlet extraction	Semisolid	Green	4.5
2.	Chloroform		Semisolid	Green	3.8
3.	Ethyl acetate		Semisolid	Brownish Green	6.2
4.	Ethanol		Solid	Brownish Green	9.6

Table 2: Qualitative phytochemical analysis of bark of *Cassia sophera* Linn.

S. No	Chemical Constituents	Powder Drug	Petroleum Ether	Chloroform	Ethyl acetate	Ethanol
1.	Carbohydrate	-	-	-	-	-
2.	Alkaloids	-	-	-	-	-
3.	Steroids	+	+	-	-	-
4.	Glycosides	+	-	-	+	+
5.	Saponins	-	-	-	-	-
6.	Flavonoids	-	-	-	-	-
7.	Tannins	+	-	-	-	+
8.	Phenolic compounds	-	-	-	-	-
9.	Proteins	-	-	-	-	-
10.	Amino acids	-	-	-	-	-
11.	Gums and Mucilage	+	-	-	-	+
12.	Terpenoids	-	-	-	-	-
13.	Resins	-	-	-	-	-
14.	Chlorogenic acid	-	-	-	-	-
15.	Fats and Oils	+	+	-	-	-

Table 3: Fluorescence Analysis of powdered bark of *Cassia sophera* Linn.

S. No	Treatment	Day light	Short UV (254nm)	Long UV (366nm)
1.	Powder	Yellowish Green	Pale Green	Pale Green
2.	Powder + Water	Pale Green	Pale Green	Pale Green
3.	Powder+ 1N HCl	Pale Yellow	Pale Yellow	Pale Yellow
4.	Powder+ 1N H_2SO_4	Pale Yellow	Pale Yellow	Pale Yellow
5.	Powder+ HNO_3	Red	Red	Red
6.	Powder+ Acetic acid	Green	Pale Green	Pale Green
7.	Powder+ 1N NaOH	Pale Yellow	Pale Yellow	Pale Yellow
8.	Powder+ Picric acid	Yellow	Yellow	Yellow
9.	Powder+ 1N KOH	Pale Yellow	Pale Yellow	Pale Yellow

10.	Powder+ Acetone	Yellowish Green	Pale Green	Pale Green
11.	Powder+ Ammonia	Yellowish Brown	Brown	Brown
12.	Powder+ Iodine	Brown	Brown	Brown
13.	Powder+ $FeCl_3$	Green	Green	Green
14.	Powder+ Ethanol	Pale Yellow	Pale Yellow	Pale Yellow

Table 4: Fluorescence analysis of various extracts

S. No	Extracts	Day light	Short UV (254nm)	Long UV (366nm)
1.	Petroleum Ether	Dark Green	Green	Green
2.	Chloroform	Green	Green	Green
3.	Ethyl acetate	Brown	Brown	Brown
4.	Ethanol	Greenish Brown	Greenish Brown	Greenish Brown

Table 5: Quantitative estimation of phytoconstituents of *Cassia sophera* Linn.

S. No	Phytoconstituents	Quantity (%w/w)
1.	Glycosides	11.2

Table 6: *In vitro* anti-diabetic activity

Extract	1 hour	3 hours	5 hours	24 hours	27 hours
Control (Absence of extract)	172.3±0.3	204.73±0.8	268.93±0.2	291.03±2.5	319.43±1.9
Petroleum Ether (50g/l)	157.65±1.3	189.95±2.2	251.25±1.3	280.65±2.3	302.35±0.3
Chloroform (50g/l)	132.48±1.5	163.04±1.4	232.77±0.8	253.47±0.8	283.13±2.9
Ethyl acetate (50g/l)	105.76±3.2	134.19±2.9	204.49±3.3	218.99±2.4	248.55±2.0
Ethanol (50g/l)	90.45±2.2	99.23±2.5	177.12±1.9	201.77±3.4	214.22±0.6

Values are expressed as mean ±SD (n=3)

In vivo anti-diabetic activity

Table 7: Effects of ethanolic extracts of the bark of *Cassia sophera* Linn. on blood glucose level in streptozocin induced diabetic rats (mg/dl) – Acute study

Group	0 Hr	1 Hr	3Hr	5 Hr	7 Hr	24 Hr
Normal	83±1.3	93±1.6	102±1.2	112±1.8	99±1.1	101±1.9
Diabetic control	372±0.4	374±1.1	385±0.2	390±1.5	407±1.8	431±2.1
Diabetic+ Glibenclamide (3mg/Kg)	342±1.2	315±1.4	288±1.7	246±2.3	225±1.2	198±1.8
Diabetic+ Ethanolic Extract (200mg)	352±2.1	348±2.2	332±2.1	276±1.3	252±1.6	232±1.6
Diabetic+ Ethanolic Extract (400mg)	334±1.4	329±0.8	297±1.3	262±2.3	233±2.2	210±2.3

One way Anova values are expressed as mean ±SD n=6

*$p<0.05$ compared to diabetic control

Table 8: Chronic study

Group	Initial	1st week	2nd week	3 rd week	4th week
Normal	90±1.4	96±1.3	100±1.2	99±1.2	93±1.7
Diabetic control	264±1.4	278±1.6	282±0.8	290±1.1	307±1.9
Diabetic+ Glibenclamide (3mg/Kg)	250±0.9	248±0.5	200±1.3	182±1.3	148±1.2
Diabetic+ Ethanolic Extract (200mg)	278±1.3	274±2.7	216±1.9	209±2.3	197±1.5
Diabetic+ Ethanolic Extract (400mg)	270±1.2	261±2.3	206±1.5	191±2.5	173±1.6

Discussion

In the present study the preliminary phytochemical screening was carried out on *Cassia sophera* indicates the presence of steroids in petroleum ether and ethanol, glycosides in ethanol and tannins in ethanolic extract. The research has been carried out to evaluate the potential of various extracts to additionally retard the diffusion and movement of glucose in the intestinal tract. *In vivo* study showed that the administration of ethanolic bark extracts of *Cassia sophera* Linn. at the dose of 200mg/Kg and 400mg/Kg produced a significant reduction in blood glucose level. However, the ethanolic extract at the dose of 400mg/Kg was found to be more effective. It may be due to the presence of phytochemical constituents present in the plant. The results of the study strongly suggest that *Cassia sophera* Linn. Is useful in the treatment of diabetes.

Conclusion

From the overall study it is concluded that the ethanolic extract of *Cassia sophera* Linn. Is found to have anti-diabetic effect in Streptozocin induced diabetes in Wistar albino rats. Therefore, this treatment can safely be considered to be an alternative antihyperglycemic drug for diabetic patients. Further studies are warranted to isolate and characterise the anti-diabetic principles from the bark of *Cassia sophera*.

References

1. Nadkarni KM. Indian Materia Medica. 2009, 1:285-286.
2. Khare CP. Indian Medicinal Plants, 2007, 129-130.
3. Tagboto S, Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products. Adv Parasitol 2005; 50:199-295.
4. Gupta RK. Medicinal and Aromatic plants, 2010, 116-117.

5. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hypoglycemic crises in adult patients with diabetes. Diabetes care 2009, 32(7):1335.
6. Guyton and Hall. Text book of Medical Physiology. 11th ed. Pennysylvania: Elsevier, 2006, 848-851, 972-976.
7. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. 6thed. Elsevier, 403-408.
8. Harbone JB. Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall, 1973, 04-34.
9. Tyler VE, Brady Lr, Robber Je. Pharmacognosy. 9th ed. Philadelphia USA: Lea and Febriorer, 1998, 78.
10. Khandelwal KR. Practical Pharmacognosy. Pune: Nirali Prakashan, 1998, 20-42.
11. Bahoran T, Neergheen VS. Aruoma OI. Phytochemivcal constituents of *Cassia fistula*. African Journal of Biotechnology. 4, 1530-1540.
12. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drug under ultraviolet radiation. Journal of American Pharmaceutical system science. 1949, 28, 324-333.
13. Gallagher AM, Flatt PR, Duffy G, Abdul-Wahab YHA. The effects of traditional anti-diabetic plants on *in-vitro* glucose diffusion. Nutritional Research. 2003, 715-717.
14. Sakthi PS, Vadivu R, Jayshree N. *In vitro* and *In vivo* anti-diabetic activity of leaves of *Ravenala madagascariensis*. On alloxan induced diabetic rats. Journal of Pharmaceutical Science and Technology. 2010, 312-317.
15. Ramakrishna D, Vidyasagar G, Pavan Kumar K, Madhusudhana I, Gupta VSS. Evaluation of antidiabetic activity of *Triumfetta piosaroth* in streptozocin induced diabetic rats. International Journal of Pharma Sciences and Research. 2011, 2(3):107-109.

IJOPILS. FEB 2016; 4(3):1-05

International Journal of Pharmacy and Integrated Life Sciences
"Where improvisation meets innovation"
www.ijopils.com

RESEARCH ARTICLE ISSN: 2320 - 0782 V4-(I3) PG(1-05)

PRELIMINARY PHYTOCHEMICAL AND *IN-VITRO* ANTIBACTERIAL EVALUATION OF *ZIZIPHUS OENOPLIA* (L) MILL STEM BARK EXTRACTS

SUNDARESAN NANDHINI[1]*, RAMALINGAM RADHA[1]

1. Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, India.

ABSTRACT

Ziziphus oenoplia (L) Mill (Rhamnaceae) is a flowering plant with a broad distribution through tropical and subtropical Asia and Australia. It is a spreading, sometimes climbing, thorny shrub growing to 1.5m in height. The antibacterial activities of the hot and cold methanol extracts of the stem barks of *Ziziphus oenoplia* was evaluated on four bacterial strains like *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Proteus vulgaris.* The *in-vitro* antibacterial activity of hot and cold methanol extracts was performed by cup plate agar diffusion method. Ciprofloxacin (ciprozol-500) is used as a standard drug for the comparison of antibacterial activity. The hot methanol extract of *Ziziphus oenoplia* did produce considerable antibacterial activity than the cold maceration extract was observed. The maximum antibacterial activity of hot and cold methanol extracts was exhibited against *Staphylococcus aureus* when compared with standard drug. In addition the preliminary phytochemical screening of the hot and cold methanol extracts of *Ziziphus oenoplia* stem barks revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, and steroids. The results obtained in the present study suggest that *Ziziphus oenoplia* barks can be used in treating bacterial infections caused by the test organisms. Further studies on isolating chemical constituents of above extracts are necessary, in order to identify the active constituent responsible for the activity.

KEYWORDS: *Ziziphus oenoplia*, Phytochemicals, bacterial infections.

Article received on: 30/01/2016 Article accepted on: 20/02/2016

Corresponding Author: Nandhini Sundaresan
Address : Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003, Tamil Nadu, India.
Email : nandypharma1787@gmail.com.

INTRODUCTION

Medicinal plants are used in the treatment of infections caused by bacteria, fungi, viruses and parasites by the local population. *Ziziphus oenoplia (L) Mill* (Rhamnaceae) is a flowering plant with a wide distribution through tropical and subtropical Asia and Australia. It is a spreading, sometimes climbing, thorny shrub growing to 1.5m in height. *Ziziphus* species have been extensively used in indigenous system of medicine as folk remedy for various ailments like dysentery, inflammation, sore throats and digestive problems etc.[1]

EXPERIMENTAL METHODS

Plant Collection, Authentication and Extract Preparation

The stem barks of *Ziziphus oenoplia* were collected from Koovathur village, Ariyalur, Tamil Nadu. The plant was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The shade dried stem bark was powdered. The fine powder of the *Ziziphus oenoplia* stem bark was extracted with methanol at 48 h by hot continuous extraction and cold maceration method. The extracts were filtered and concentrated using a Rotary vacuum evaporator. The extracts were indicated as T_1 & T_2 respectively.

Phytochemical Analysis[2]

Both the hot and cold methanol extracts obtained from the powdered bark of the *Ziziphus oenoplia* were subjected to preliminary phytochemical investigation for alkaloids, carbohydrates, flavonoids, glycosides, lignin, saponins, terpenes, tannins, steroids and reducing sugar. The results are given in the **Table1**.

Antibacterial Screening[3]

The antimicrobial activity of the crude extracts were screened against gram positive bacteria and gram negative bacteria obtained from the laboratory stock of the Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu. The organisms that were tested are *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris.* 1ml of each bacterial strain was initially inoculated in 100 ml of sterile nutrient broth and incubated for 37°±1°C for 24 h (Working stock). The medium used was nutrient agar medium.

The Antimicrobial assay was performed by cup plate agar diffusion method. 0.2ml of working stock from each organism was seeded into 100ml sterile nutrient agar medium in a sterile petri dish. After the agar solidified, four holes of uniform diameter (7mm) were made in the medium

using a sterile borer. 0.2ml of varying concentrations (10-50 mg/ml) of T_1, T_2 and standard (Ciprofloxacin) and vehicle (DMSO) were placed in each hole for all the organisms under aseptic conditions. The plates were then maintained at room temperature for 2 h to allow the diffusion of the solution into the medium. All the bacterial plates were then incubated at 37°±1°C for 18 h and the zone of inhibition was measured. For each zone, an average of three independent measurements was determined. The values indicate the zone of inhibition in cm, including the diameter of the bore (7 mm). The results are given in **Table 2**.

RESULTS AND DISCUSSION

Table 1: Phytochemicals present in the hot and cold methanol extracts of *Ziziphus oenoplia* bark

Secondary metabolites	T1	T2
Carbohydrates	+	+
Flavonoids	+	+
Glycosides	+	+
Lignans	-	-
Saponins	+	+
Terpenes	-	-
Tannins	+	-
Steroids	+	+
Reducing sugars	+	-
Alkaloids	+	+

'+' Present, '-'Absent

The preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, steroids and reducing sugar.

Table 2: Zone of inhibition of the hot and cold methanol extracts of *Ziziphus oenoplia* bark

Extracts tested	Concentration (mg/ml)	Diameter of the zone of inhibition in (cm)			
		S. aureus	***B. subtilis***	***E. coli***	***P. vulgaris***
Hot methanol Extract (T_1)	10	2.0	1.7	1.7	1.3
	20	2.3	1.9	2.1	1.3
	30	3.0	2.1	2.2	1.6
	40	3.3	2.4	2.3	1.8
	50	3.7	2.5	2.6	2.1
Cold methanol Extract (T_2)	10	2.0	1.2	1.5	1.0
	20	2.2	1.4	1.8	1.2
	30	2.4	1.5	1.9	1.4
	40	2.5	1.8	2.2	1.5
	50	2.9	1.8	2.3	1.6
Ciprofloxacin	10	3.7	3.4	3.1	3.2
	20	4.0	3.5	3.2	3.3
	30	3.9	3.6	3.4	3.5
	40	4.1	3.7	3.5	3.6
	50	4.2	3.9	3.6	3.6

From the table, it is seen that both the methanol extracts T_1 & T_2 showed good antibacterial activity against both gram positive & gram negative bacteria. Their activity is comparable with that of the standard drug ciprofloxacin, though the activity is lesser. Among the two extracts, the methanol extract prepared by hot extraction method showed better activity against all the four organisms tested.

CONCLUSION

From this study, we conclude that the methanolic extract of the bark of *Ziziphus oenoplia* has good antibacterial activity. The method of extraction also plays an important role in extracting the phytoconstituents. The results open up the possibility of finding new clinically effective anti-bacterial compounds in an era where several microorganisms are to becoming resistant to conventional drugs.

References:

1. Indian medicinal plants - A Compendium of 500 species, Vol 1, Orient Longman Pvt Ltd, Arya vaidya sala, Kottakkal.
2. Harbone JB. Phytochemical methods - A guide to modern technique of plant analysis. 1998; 3rd Edn.
3. Gillespie SH. Medical bacteriology - A practical approach, Oxford university press, United Kingdom. 1994; 181-194.

ISSN: 2322-0902 (PRINT)
ISSN: 2322-0910 (ONLINE)

International Journal of Ayurveda and Pharma Research
http://ijapr.in

Certificate Of Publication

Is hereby awarding this certificate to

SUNDARESAN NANDHINI, RAMALINGAM RADHA

In recognition of the publication of the Review Article entitled

"PHARMACOGNOSY OF AZIMA TETRACANTHA LAM.: A REVIEW"

Published in International Journal of Ayurveda and Pharma Research

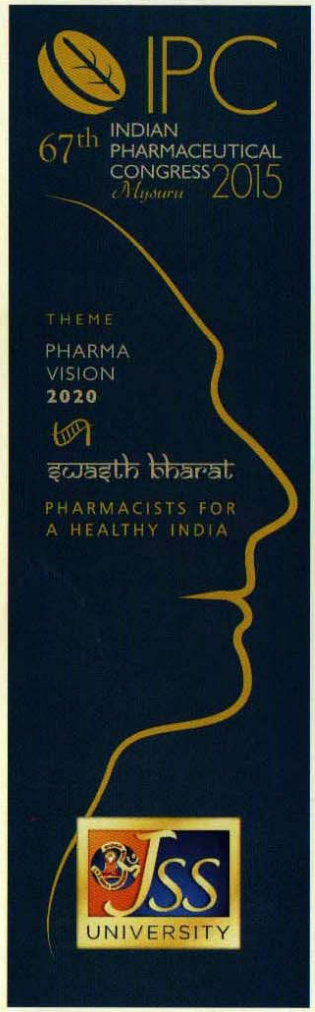
VOL 3, ISSUE 12: DECEMBER 2015

B. Srinu

EDITOR-IN-CHIEF
IJAPR

PUBLICATION MANAGER
MAHADEV PUBLICATIONS

ADDRESS: DR. B. SRINIVASULU, FLAT NO: 105, BLOCK - A, SRINIDHI APARTMENTS, BESIDES INDIAN OIL PETROL PUMP, PUPPALAGUDA MAIN ROAD, MANIKONDA POST OFFICE, RANGA REDDY DISTRICT, PIN: 500089, TELANGANA, INDIA. TELEPHONE: +919703393005, +918099660550



67th Indian Pharmaceutical Congress

19-21 December 2015

Mysuru, India

Certificate of Participation

This is to certify that

S. NANDHINI

has participated as a Delegate
in the 67th IPC held at JSS University, Mysuru
19 - 21 December, 2015.

Dr. B. Suresh	Dr. B. Manjunatha	Dr. (Lt Col) R. Vijaysimha
Chairman, LOC	Organising Secretary	Chairman, Registration
67th IPC, 2015	67th IPC, 2015	67th IPC, 2015

The Scientific Program is recognised as 'Continuing Pharmacy Education' under Regulation 4.2(IV) of the Pharmacy Practice Regulations 2015

Pharmacy Council of India
New Delhi

Organised by
Indian Pharmaceutical Congress Association

Hosted by
Indian Hospital Pharmacists Association

SRI VENKATESHWARAA MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

(A Unit of Ramachandra Educational Trust)
Ariyur, Puducherry-605 102

CREATING HEALTHIER SOCIETY

DEPARTMENT OF PHARMACOLOGY & MEDICAL EDUCATION

This is to certify that S. NANDHINI, M. PHARM II YEAR

MADRAS MEDICAL COLLEGE ✓ has participated as a Guest Speaker/Delegate/Poster Presentation in **"CME on Herbal Pharmacology"**, held on 7th July 2015 at Sri Venkateshwaraa Medical College Hospital & Research Centre, Puducherry.

Medical Education Unit | Organising Secretary | Dean

66th Indian Pharmaceutical Congress Hyderabad

66th INDIAN PHARMACEUTICAL CONGRESS

23rd TO 25th, JANUARY 2015, HITEX, HYDERABAD.

Pharma vision 2020

INDIA - PHARMACY OF THE WORLD

ROLE OF INDIAN REGULATORS AND PHARMA INDUSTRY

CERTIFICATE

THIS IS TO CERTIFY THAT

Dr./ Prof./ Mr./Ms. S. NANDHINI

HAS PARTICIPATED AS DELEGATE

IN 66th INDIAN PHARMACEUTICAL CONGRESS

HELD AT HITEX, HYDERABAD FROM 23rd TO 25th, JANUARY 2015.

RAVI UDAY BHASKAR	K. RAJA BHANU	P. VENKATESHWARLU
CHAIRMAN, 66th IPC	GENERAL SECRETARY	CHAIRMAN, REGISTRATION
SECRETARY GENERAL, AIDCOC.	66th IPC	66th IPC

HOST ALL INDIA DRUGS CONTROL OFFICERS' CONFEDERATION | www.aidcoc.in

66th INDIAN PHARMACEUTICAL CONGRESS

23rd TO 25th, JANUARY 2015, HITEX, HYDERABAD.

INDIA - PHARMACY OF THE WORLD

ROLE OF INDIAN REGULATORS AND PHARMA INDUSTRY

CERTIFICATE

THIS IS TO CERTIFY THAT

Dr./ Prof./ Mr./Ms. Nandhini S.

HAS PRESENTED A PAPER ENTITLED Preliminary phytochemicals and in-vitro Antibacterial Evaluation of Ziziphus Oenoplia(L) Mill stem Bark Extracts.

IN THE SCIENTIFIC POSTER SESSION OF

66th INDIAN PHARMACEUTICAL CONGRESS

HELD AT HITEX, HYDERABAD FROM 23rd TO 25th, JANUARY 2015.

VENKAT JASTI	RAVI UDAY BHASKAR	DR. T.K. RAVI	DR. PRABHA SHANKAR
PRESIDENT, IPCA 66th IPC	CHAIRMAN, 66th IPC SECRETARY GENERAL, AIDCOC.	CONVENOR SCIENTIFIC SERVICES, IPCA	CHAIRMAN, SCIENTIFIC SERVICES COMMITTEE, 66th IPC

HOST ALL INDIA DRUGS CONTROL OFFICERS' CONFEDERATION | www.aidcoc.in

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE
CHENNAI-3.

CERTIFICATE

This is to certify that Dr/Mr/Mrs/Miss Nandhini.S *of*

Department of Pharmacognosy, College of Pharmacy, MMC *has participated in the*

The Tamil Nadu Dr. M.G.R. Medical University Sponsored "Guest Lecture Series - 2014" conducted on

26th & 27th September, 2014 at the College of Pharmacy, Madras Medical College, Chennai - 600003.

Dr. N. Jayshree
Co-Ordinator,
College of Pharmacy, MMC.

Dr. A. Jerad Suresh
Chair Person
Principal, College of Pharmacy, MMC.

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE, CHENNAI-3.

Certificate

This is to certify that NANDHINI. S (I-M.PHARM) PHARMACOGNOSY *College of Pharmacy, Madras Medical College has attended the guest lecture "Recent Trends in Herbal Research" on 13th June 2014 at the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003.*

Dr. N. Jayshree
Professor & Head,
Department of Pharmacognosy,
College of Pharmacy

Dr. A. Jerad Suresh
Principal
College of Pharmacy

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE, CHENNAI - 3

AND

BIODISCOVERY - SOLUTIONS FOR FUTURE

AGRA, U.P, INDIA - 282002

WORKSHOP ON DRUG DESIGN

CERTIFICATE

This is to certify that ~~Mr.~~ /Ms/~~Mrs~~ S. Nandhini *of* College Of pharmacy Madras Medical College. *has attended the WORKSHOP ON DRUG DESIGN AND HANDS ON TRAINING between January 30th and 3rd February 2012 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.*

Mr. Syed Asif Husain Naqvi
Bio Discovery-Solutions for future

Dr. A. Jerad Suresh
Principal
College of Pharmacy,MMC

Dr. V. Kanagasabai
Dean
Madras Medical College