CHEMO PREVENTIVE ACTIVITY OF SOME MEDICINAL PLANT ON 7, 12-DIMETHYLBENZ (a) ANTHRACENE INDUCED BREAST CANCER AND THEIR ANTIOXIDANT ACTIVITY ON RAT MODEL

THESIS SUBMITTED TO

The Tamilnadu Dr. M.G.R. Medical University

Chennai – 32

for the award of the Degree of

DOCTOR OF PHILOSOPHY

By

Mr.R.SURESH M.Pharm

Under the Guidance of

Dr. D.BENITO JOHNSON M.Pharm., Ph.d

Professor and Head

Department of Pharmacology

R.V.S. College of Pharmaceutical Sciences,

242-B, Trichy Road, Sulur, Coimbatore – 641 402

JUNE – 2017

CERTIFICATE

This is to certify that the work embodied in this thesis entitled **CHEMO PREVENTIVE ACTIVITY OF SOME MEDICINAL PLANT ON 7, 12-DIMETHYLBENZ (a) ANTHRACENE INDUCED BREAST CANCER AND THEIR ANTIOXIDANT ACTIVITY ON RAT MODEL** is the bonafied research work carried out by **Mr R.Suresh., M.Pharm**., under my guidance and supervision in the department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore. This work is original and has not been submitted in part or whole to this or any other university.

SUPERVISOR AND GUIDE

Place: Sulur

Date: **Dr D BENITOJOHNSON M.Pharm.,Ph.D**

DECLARATION

I hereby declare that the research work embodied in this thesis entitled **CHEMO PREVENTIVE ACTIVITY OF SOME MEDICINAL PLANT ON 7, 12-DIMETHYLBENZ (a) ANTHRACENE INDUCED BREAST CANCER AND THEIR ANTIOXIDANT ACTIVITY ON RAT MODEL** submitted by me, for the degree of Ph.D in Pharmacology. This record of research was done by me and carried out under the supervision of **Dr D.BENITO JOHNSON M.Pharm.,Ph.d**, **Professor and Head, Department of Pharmacology**, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore. This work is an original and has not formed the basis of the award of any degree, Diploma, Associateship, Fellowship or any similar title by me in part or whole to this or any other university.

Place: Sulur **Mr R.SURESH**

Date:

ACKNOWLEDGEMENT

First I express my gratefulness to ***God*****,** who has given me the wisdom and capability to complete this work successfully.

I would like to thank my guide **Dr D.Benito Johnson** Professor and Head, Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore who guiding me in the right direction.

I would like to give my sincere thanks to **Dr.R.Venkatnarayanan**, Principal R.V.S. College of Pharmaceutical Sciences, Sulur, Coimbatore, who helped me in my research. Without his help I could not have finished my dissertation successfully.

My most sincere gratitude to **Dr.E.P.Kumar ,** M.Pharm, Ph.D., Professor, Nehru College of Pharmaceutical Sciences and Dr Alwin Jose, Drug Inspector, Thirunelveli for their personal guidance which have provided a good basis for the present thesis.

I convey my special thanks to my department staff members **Dr C.Maheswari**, **Dr C. Senthilkumar,** Asst Professor, Department of Pharmacology, and **Mr R.Sivasakthi,** Asst Professor, Department of Pharmacy Practice for their support and timely help to complete the work successfully.

I wish to acknowledge the kind assistance of **Mr.P.Sakthivel,** Lab-Assistant, for his kind cooperation and assistance during my research work.

It is a pleasure to express my gratitude whole heartedly to **Mr .K. Senthil Ganesh** , Managing trustee, R.V.S. Group of Institutions, Sulur, Coimbatore for permitting me to do the research programme.

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

(Mr R.SURESH)

INDEX

LIST OF TABLE

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

DNA	- Deoxyribonucleic acid
MYC	- Myelocytomatosis Oncogene
RAS	- Intracellular GTPase switch protein
RB	- Retinoblastoma
FPC	- Familial adenomatous polyposis of the colon
BRCA2	- Breast cancer gene 2
BRCA1	- Breast cancer gene 1
UV	- Ultra violet
XP	- Xeroderma Pigmentosum
BLM	- Bloom syndrome
BCL_2	- B Cell lymphoma 2
SERMs	- Selective Estrogen Receptor Modulators
SARMs	- Selective Androgen Receptor Modulators
MDR	- Multiple Drug Resistance
HER 2	- Human Epidermal Growth factor Receptor 2
DCIS	- Intra Ductal Carcinoma or Ductal Carcinoma in situ
IDC	- Invasive Ductal Carcinoma
ILC	- Invasive Lobular Carcinoma
IBC	- Inflammatory Breast Cancer
DMBA	– 7,12, Dimethyl benz Anthracene
4 NP	- 4 Nonyl Phenol
HAA	- Heterocyclic Aromatic Amines
NAT 2	- N Acetyl transferase 2
PFOA	- Perfluoro octanoic acid
CT	- Computer Tomography
PCBs	- Polychlorinated Biphenyls
DDT	- Dichlorodiphenyl trichlororthane
T4	- Thyroxine
DEHP	- Di (2-ethylhexyl) phthalate
PUFA	- Polyunsaturated fatty acids
LDL	- Low Density Lipoprotein
LOOH	- Lipid hydroperoxides

ROS	- Reactive Oxygen Species
Hep G2	- Liver hepatocellular cells
CYP3A	- Cytochrome P450 3A
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
NADH	- Nicotinamide Adenine Dinucletide
PMS	- Phenazine methosulphate
NBT	- Nitroblue Tetrazolium chloride
NO	- Nitric oxide
OECD	- Organisation for Economic Co-operation and Development
CPCSEA	- Committe for the purpose of control and supervision in experimental Animals
LD50	- Lethal dose to kill 50% of animal
DMSO	- Dimethyl suphoxide
ML	-Milli litre
TRM	- Triumfetta rhomboidea methanol leaf extract
Mcg	- Micrograms
Mg Kg^{-1} b.wt	- Milligram/ Kilogram body weight
TBARS	-Thiobarbituric Acid reactive substances
SOD	- Superoxide dismutase
CAT	- Catalase
GPx	- Glutathione Peroxidae
GSH	- Reduced glutathione
CLIA	- Chemiluminescent immunoassay
E2	- 17 β estradiol
DLA	- Daltons Ascites Lymphoma cells
EAC	- Ehrlich ascites carcinoma cells
PBS	- Phosphate Buffered saline
% ILS	- Percentage increase life span
ANOVA	- One way Analysis of Variance
GLIDE	- Grid-based Ligand Docking with Energetics
PDB	- Protein Data Bank
S.C	- Subcutaneus
U/L	-Unit/Litre
LDH	- Lactate dehydrogenase

CPK	- Creatine phosphokinase
IU/L	- International unit/Litre
NaCl	- Sodium Chloride
Mcg	- Microgram
I.P	-Intraperitoneal
NSAIDs	- Non Steroidal Anti-inflammatory Drugs
SOB	- Simple ointment base

1.INTRODUCTION

Cancer results from different molecular events that changes the normal fundamental properties of cells. In cancer cells the normal systems prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells grow and divide in the presence of signals that n inhibit cell growth normally . As these cells grow they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These changes allow the cell and its progeny to grow and divide, even in the presence of normal cells that inhibit the growth of nearby cells. This changes allow the cancer cells to spread and invade other tissue cells. Abnormalities in cancer cells result from mutations in protein-encoding genes that regulate cell division. This process is often because the genes that make the proteins that normally repair DNA damage are themselves not functioning normally and they are also mutated.Mutations begin to increase in the cell, causing further abnormal changes in that cell and the daughter cells. Some of these mutated cells die, other alterations may give the abnormal cell a selective advantage that allows it to multiply more rapidly than the normal cells. This type of enhanced growth describes most cancer cells, which have improved functions present in the normal, healthy cells. As long as these cells remain in their original place, they are known as benign; if they become invasive, they are known as malignant.

Genetics of Cancer

Thirty five thousands genes in the human genome had been associated with cancer. Alterations in the same gene often are associated with different types of cancer.

This malfunctioning genes can be classified into three groups. The first group, called proto-oncogenes, produces protein products that normally enhance cell division or inhibit normal cell death and the mutated form is known as oncogenes. The second group is known as tumor suppressors,which makes proteins that normally prevent cell division or cause cell death. The third group is DNA repair genes, which help prevent mutations that lead to cancer. Cell growth control is maintained by regulation of proto-oncogenes, which accelerate growth, and tumor suppressor genes and slow cell growth. Mutations produce oncogenes enhance growth while those that affect tumor suppressor genes prevent the normal inhibition of growth. In both case, uncontrolled cell growth occurs. [1,2]

Oncogenes

In normal cells, proto-oncogenes code for the proteins that send a signal to the nucleus to stimulate cell division. These signaling proteins act in a series of steps called signal transduction cascade or pathway. This cascade includes a membrane receptor for the signal molecule, intermediary proteins that carry the signal through the cytoplasm. The transcription factors in the nucleus that activate the genes for cell division. Oncogenes are altered versions of the proto-oncogenes that code for these signaling molecules. The oncogenes activate the signaling cascade continuously, resulting in an increased production of factors that stimulate growth.MYC is a proto-oncogene that codes for a transcription factor. Mutations in MYC convert it into an oncogene associated with seventy percent of cancers. RAS is another type of oncogene that normally functions as an “on-off” switch in the signal cascade. Mutations in RAS cause

the signaling pathway to remain "on," leading to uncontrolled cell growth. About thirty percent of tumors such as lung, breast, colon, thyroid, and pancreatic carcinomas have a mutation in RAS.[3,4]

The conversion of a proto-oncogene to an oncogene occur by mutation of the proto-oncogene, by rearrangement of genes in the chromosome which moves the proto-oncogene to a new location, or by an increase in the number of copies of the normal proto-oncogene. A virus inserts its DNA in or near the proto-oncogene, causing it to become an oncogene. The result of any of these events is an altered form of the gene, which leads to cancer. Most of the oncogenes are dominant mutations; a single copy of this gene is sufficient for expression of the growth trait. This is also a "gain of function" mutation because the cells with the mutant form of the protein have gained a new function not present in cells with the normal gene [5,6]

Biology of Tumour

Cancer cells behave as independent cells, growing without control to form tumors. Tumors grow in a series of steps. The first step is hyperplasia, defining that there are more no of cells resulting from uncontrolled cell division. These cells appear normal, but changes have occurred that result in loss of control of growth. The second one is dysplasia, resulting from further growth, accompanied by abnormal changes to the cells. The third one requires additional changes, which result in cells that are more abnormal and now spread over a wider area of tissue. These cells begin to lose their original function; such cells are known as anaplastic. At this stage, because the tumor is

still present within its original location (called in situ) and is not invasive, it is not considered malignant — it is potentially malignant. The last step occurs when the cells in the tumor metastasize, which means that they can invade surrounding tissue, including the bloodstream, and spread to other locations. This is the most serious type of tumor, but not all tumors progress to this point. Non-invasive tumors are said to be benign.

The type of tumor that produces depends on the type of cell that was initially altered. There are five types of tumors [7,8]

• Carcinomas produced from altered epithelial cells, which cover the surface of the skin and internal organs. Most cancers are carcinomas.

• Sarcomas produced from changes in muscle, bone, fat, or connective tissue.

• Leukemia rproduced from malignant white blood cells.

• Lymphoma is a cancer of the lymphatic system cells that occour from bone marrow.

• Myelomas are cancers of specialized white blood cells that produce antibodies.

Although tumor cells are no longer dependent on the control mechanisms that govern normal cells, they still require nutrients and oxygen in order to grow. All living tissues are supplied with capillary vessels, which bring nutrients and oxygen to all cell. As tumors enlarge, the cells in the center and cannot receive nutrients from the normal blood vessels. To give a blood supply for all the cells in the tumor, it should form new

blood vessels to supply the cells in the center with nutrients and oxygen. In the pocess of angiogenesis, tumor cells produce growth factors and induce formation of new capillary blood vessels. The blood vessels cells that divide to produce new capillary vessels are inactive in normal tissue and tumors make angiogenic factors, which activate the blood vessel cells to divide. Without a blood supply, tumor cells also cannot spread and metastasize, to new tissues. The tumor cells can cross through the walls of the capillary of blood vessel at a rate of about one million cells per day. Not all cells in a tumor are angiogenic. Both angiogenic and nonangiogenic cells in a tumor cross into blood vessels and spread and non-angiogenic cells give rise to dormant tumors when they grow in other areas [9,10]. Oncogene called BCL2 has been shown to greatly increase the production of a potent stimulator of angiogenesis. It appears, then, that oncogenes in tumor cells may cause an increased expression of genes that make angiogenic factors. There are at least fifteen angiogenic factors and production of many of these is increased by a variety of oncogenes. Therefore, oncogenes in some tumor cells allow those cells to produce angiogenic factors. The progeny of these tumor cells will also produce angiogenic factors, so the population of angiogenic cells will increase as the size of the tumor increases. Dormant tumors are those that do not have blood vessels; they are generally less than half a millimeter in diameter. Several autopsy studies in which trauma victims were examined for such very small tumors revealed that thirtynine percent of women aged above 40 have very small breast tumors, while 60 percent of men aged above 60 have very small prostate tumors. 90 percent of people aged above 50 have very small thyroid tumors. However, for those age groups in the general

population, the incidence of these particular cancers is only one-tenth of a percent (thyroid) or one percent (breast or prostate cancer). The conclusion is that the incidence of dormant tumors is high compared to the incidence of cancer. So that angiogenesis is critical for the progression of dormant tumors into cancer.[11]

Traditional Treatment [12]

Cancer comprises many diseases, doctors use many different treatments. The course of treatment depends on the type of cancer, its location, and its state of advancement. Surgery is the first treatment option, which is used to remove solid tumors and only treatment necessary for early stage cancers and benign tumors. Radiation kills cancer cells targeted directly to the tumor. And acts primarily by damaging DNA and inhibiting its replication; so that , it kills cancer cells, which rapidly divide. It also kills some normal cells, particularly those that are dividing. Surgery and radiation treatment are often used together. Chemotherapy drugs are toxic compounds that kills rapidly growing cells. Many of these drugs are designed to interfere with the synthesis of precursor molecules needed for DNA replication mechanis and interfere with the activity of the cell to complete the S phase of the cell cycle.

Modern Treatments [13]

Many of the factors that affect normal cell growth are hormones. Although cancer cells have lost some of the normal responses to growth factors, some cancer cells still require hormones for growth. Hormone therapy for cancer attempts to starve the cancer cells of these hormones. This is usually done with drugs that block the activity of the hormone, although some drugs can block synthesis of the hormone. For example,

some breast cancer cells require estrogen for growth. Drugs that block the binding site for estrogen can slow the growth of these cancers. These drugs are called selective estrogen receptor modulators (SERMs) or anti-estrogens. Tamoxifen and Raloxifene are examples of this type of drug. A ten-year clinical trial of these two drugs with 20,000 women began in 1999 to determine their effectiveness in preventing breast cancer. Similarly, testosterone (an androgen hormone) stimulates some prostate cancer cells. Selective androgen receptor modulators (SARMs) are drugs that block the binding of testosterone to these cancer cells, inhibiting their growth and possibly preventing prostate cancer. Newer chemotherapeutic drugs target specific, active proteins or processes in cancer cell signal transduction pathways, such as receptors, growth factors, or kinases . Because the targets are cancer-specific proteins, the hope is that these drugs will be much less toxic to normal cells than conventional cancer drugs. The oncogene RAS is mutated in many types of cancer, particularly pancreatic cancer, which has a poor rate of survival for those afflicted. The RAS protein is only active after it is modified by the addition of a specific chemical group. Scientists are developing drugs to inhibit the action of the enzyme that adds the chemical group to the RAS protein, resulting in an inactive form of RAS. Early tests indicate that these drugs show promise for reducing tumors in cancer patients. Chemotherapy may fail because the cancer cells become resistant to the therapeutic drugs. One of the characteristics of cancer cells is a high frequency of mutation. In the presence of toxic drugs, cancer cells that mutate and become resistant to the drug will survive and multiply in the presence of the drug, producing a tumor that is also resistant to the drug. To overcome this problem,

combinations of chemotherapy drugs are given at the same time.[14] This decreases the probability that a cell will develop resistance to several drugs at once; however, such multiple resistances do occur. Some drug-resistant cancer cells express a gene called MDR1 (multiple drug resistance). This gene encodes a membrane protein that can not only prevent some drugs from entering the cell, but can also expel drugs already in the cell. Some cancer cells make large amounts of this protein, allowing them to keep chemotherapy drugs outside the cell. Another promising target for cancer therapy is angiogenesis. Several drugs, including some naturally occurring compounds, have the ability to inhibit angiogenesis. Two compounds in this class are angiostatin and endostatin; both are derived from naturally occurring proteins. These drugs prevent angiogenesis by tumor cells, restricting tumor growth and preventing metastasis. One important advantage of angiogenesis inhibitors is that, because they do not target the cancer cells directly, there is less chance that the cancer cells will develop resistance to the drug. One contributing factor in cancer is the failure of the immune system to destroy cancer cells. Immunotherapy encompasses several techniques that use the immune system to attack cancer cells or treat the side effects of some types of cancer treatment. The least specific of these are the immunostimulants, such as interleukin 2 and alpha interferon, which enhance the normal immune response. A technique called chemoimmunotherapy attaches chemotherapy drugs to antibodies that are specific for cancer cells. The antibody then delivers the drug directly to cancer cells without harming normal cells, reducing the toxic side effects of chemotherapy. These molecules contain two parts: the cancer-cell-specific antibody and a drug that is toxic once it is

taken into the cancer cell. A similar strategy, radioimmunotherapy, couples specific antibodies to radioactive atoms, thereby targeting the deadly radiation specifically to cancer cells. Another immunological approach uses antibodies that inactivate cancer-specific proteins, such as growth factors or tumor cell receptors, which are required by tumor cells. For example, many breast and ovarian cancer cells over-express a receptor protein called HER2. An antibody called Herceptin, which binds HER2, inhibits tumor growth by preventing the binding of growth factors to these cells. Some cancers, particularly leukemia, are treated with very high doses of chemotherapy drugs and radiation intended to kill all the cancer cells.[15] The side effect of this harsh treatment is destruction of the bone marrow, which contains stem cells. Stem cells, immature cells that develop into blood cells, are essential. After treatment, the patient's bone marrow must be restored, either from bone marrow removed from the patient before drug therapy or from a compatible donor. Although the patient's own bone marrow is best, it can contain cancer cells that must be destroyed before it is returned to the patient

Preventing Cancer

Cancer appears to result from a combination of genetic changes and environmental factors. A change in lifestyle that minimizes exposure to environmental carcinogens is one effective means of preventing cancer. Individuals who restrict their exposure to tobacco products, sunlight, and pollution can greatly decrease their risk of developing cancer. Many foods contain antioxidants and other nutrients that may help to prevent cancer. The National Cancer Institute recommends a diet with large amounts of colorful fruits and vegetables. These foods supply ample amounts of vitamin A, C, and

E, as well as phytochemicals and other antioxidants that help to prevent cancer. There is strong evidence that a diet rich in vegetables and fruits will not only reduce the risk of cardiovascular disease, obesity, and diabetes, but will also protect against cancer.[16] Vaccines also offer some promise for prevention of cancer. The first vaccine to prevent cancer was for hepatitis B, which is associated with liver cancer. An effective hepatitis B vaccine is available that can prevent both hepatitis and the cancer that may follow this infection. In 2002, test results of a papillomavirus vaccine were reported. Human papillomavirus type 16 infects about twenty percent of adults. Although most papillomavirus infections do not cause cancer, some are associated with cervical cancer. A vaccine against this virus was administered to 1,200 young women in the United States. Within eighteen months, the vaccine produced high levels of antibodies to the virus, and prevented both papillomavirus infection and precancerous lesions in all the women. In the control group of about 1,200 women who did not receive the vaccine, forty-one infections and nine precancerous lesions were found. The vaccine can also prevent genita warts caused by this virus strain. It appears that vaccines such as these may help in the fight to prevent cancers associated with viruses[17]

BREAST CANCER

Breast cancer is a type of malignant tumour that derived from the cells of the breast. Malignant tumour is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too. The female breast is made up mainly of lobules (milk-producing glands), ducts (tiny tubes that carry the milk from the

lobules to the nipple), and stroma (fatty tissue and connective tissue surrounding the ducts and lobules, blood vessels, and lymphatic vessels). Most breast cancers begin in the cells that line the ducts (ductal cancers). Some begin in the cells that line the lobules (lobular cancers), while a small number start in other tissues. The lymph system is important to understand because it is one way breast cancers can spread. This system has several parts. Lymph nodes are small, bean-shaped collections of immune system cells (cells that are important in fighting infections) that are connected by lymphatic vessels. Lymphatic vessels are like small veins, except that they carry a clear fluid called lymph (instead of blood) away from the breast [18] Lymph contains tissue fluid and waste products, as well as immune system cells. Breast cancer cells can enter lymphatic vessels and begin to grow in lymph nodes. Most lymphatic vessels in the breast connect to lymph nodes under the arm (axillary nodes). Some lymphatic vessels connect to lymph nodes inside the chest (internal mammary nodes) and either above or below the collarbone (supraclavicular or infra clavicular nodes). If the cancer cells have spread to lymph nodes, there is a higher chance that the cells could have also gotten into the bloodstream and spread (metastasized) to other sites in the body. The more lymph nodes with breast cancer cells, the more likely it is that the cancer may be found in other organs as well. Because of this, finding cancer in one or more lymph nodes often affects the treatment plan. Still, not all women with cancer cells in their lymph nodes develop metastases, and some women can have no cancer cells in their lymph nodes and later develop metastases[19,20]

TYPES OF BREAST CANCERS

Breast cancer can be separated into different types based on the way the cancer cells look under the microscope. Most breast cancers are carcinomas, a type of cancer that starts in the cells (epithelial cells) that line organs and tissues like the breast. In fact, breast cancers are often a type of carcinoma called adenocarcinoma, which is carcinoma that starts in glandular tissue. Other types of cancers can occur in the breast, too, such as sarcomas, which start in the cells of muscle, fat, or connective tissue. In some cases a single breast tumor can be a combination of different types or be a mixture of invasive and in situ cancer. And in some rarer types of breast cancer, the cancer cells may not form a tumor at all. Breast cancer can also be classified based on proteins on or in the cancer cells, into groups like hormone receptor-positive or triple-negative[21]

Ductal carcinoma in situ

(DCIS; known as intra ductal carcinoma) is considered non invasive or pre-invasive breast cancer DCIS cells that lined the ducts have changed to look like cancer cells. The difference between Intra ductal carcinoma and invasive cancer is that the cells have not spread through the walls of the ducts into the surrounding breast tissue. Because it hasn't invaded and can't spread outside the breast. Intra ductal carcinoma is considered a pre-cancer because some cases can go on to become invasive cancers.

Invasive ductal carcinoma

It starts in a milk ducts of the breast and grows into the fatty tissue of the breast. In this stage, it may be able to spread to other parts of the body through the lymphatic

system and bloodstream. About 2 in 10 invasive breast cancers are infiltrating ductal carcinomas.

Invasive lobular carcinoma

Invasive lobular carcinoma (ILC) starts in the milk-producing glands (lobules) and it can spread (metastasize) to other parts of the body. About 1 in 10 invasive breast cancers is an Invasive lobular carcinoma and may be harder to detect by a mammogram than invasive ductal carcinoma.

Less common types of breast cancer

1. **Inflammatory breast cancer**

   The uncommon type of invasive breast cancer accounts for about 2% to 3% of all breast cancers. There is no single lump or tumor and they are inflammatory breast cancer makes the skin on the breast look red and feel warmth. It also give the breast skin a thick, pitted appearance that looks a lot like an orange peel. The affected breast may become larger or firmer, tender, or itchy.

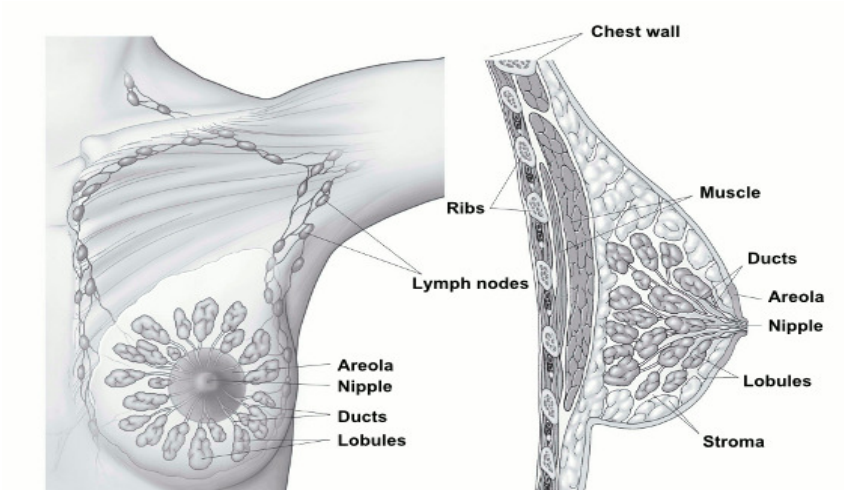


Figure 1: Normal Breast Tissue

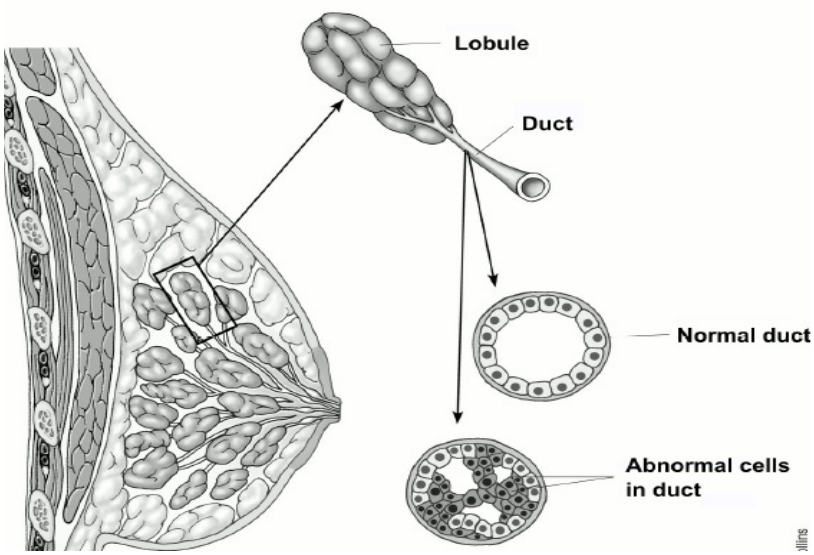


Figure 2: Ductal carcinoma in situ

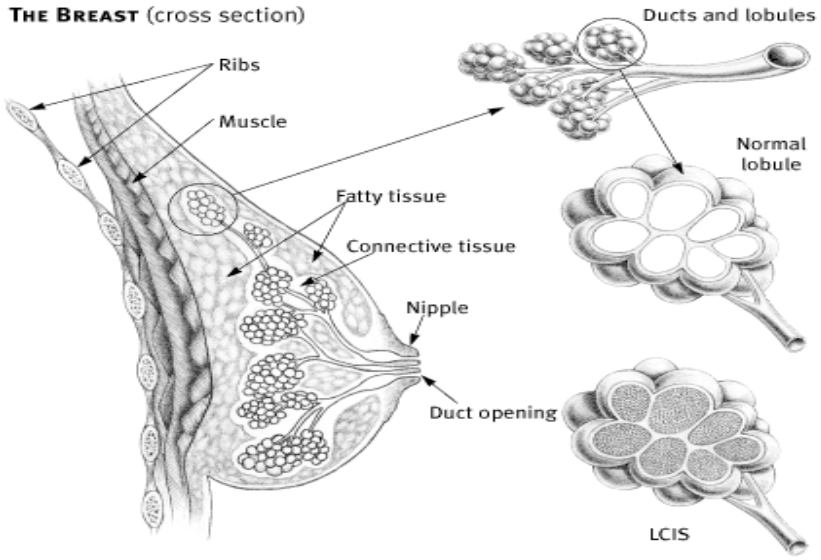


Figure 3: Inflammatory breast disease

2. Paget disease of the nipple

This type of breast cancer starts in the breast ducts and spreads to the skin of the nipple and then to the areola, the dark circle around the nipple. It is rare, accounting for only about 1% of all cases of breast cancer. The skin of the nipple and areola often appears crusted, scaly, and red, with areas of bleeding or oozing.

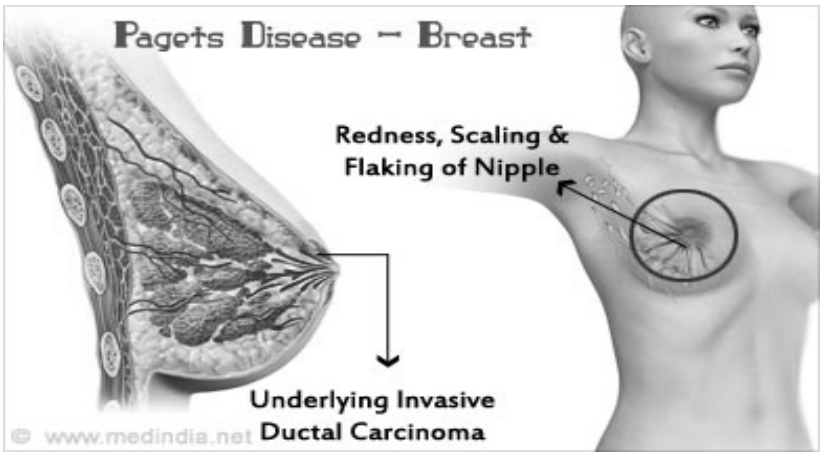


Figure 4: Paget disease of the nipple

Paget disease is almost always associated with either ductal carcinoma in situ (DCIS) or infiltrating ductal carcinoma. Mastectomy is the treatment . If no lump can be felt in the breast tissue, and the biopsy shows DCIS but no invasive cancer, the outlook (prognosis) is excellent. If invasive cancer is present, the prognosis is not as good, and the cancer will need to be staged and treated like any other invasive cancer.

3. Phyllodes tumor

The rare breast tumor develops in the stroma (connective tissue) of the breast, in contrast to carcinomas, which develop in the ducts or lobules and other names of these tumors are phylloides tumor and cystosarcoma phyllodes. This type tumors are usually benign and on rare occasions may be malignant. The Benign Phyllodes tumors are

treated by removing the tumor along with a margin of normal breast tissue. The malignant phyllodes tumor is treated by mastectomy.

Angiosarcoma

This type of cancer starts in cells that line blood vessels or lymph vessels. It occurs in the breasts. This is an extremely rare complication of breast radiation therapy that can develop about 5 to 9 years after radiation. Angiosarcoma can occur in the arms of women who develop lymphedema as a result of lymph node surgery or radiation therapy to treat breast cancer.

DRUGS AND CHEMICALS CAUSES BREAST CANCER

7,12-Dimethylbenz[a]anthracene (DMBA) [22]

DMBA is an immunosuppressant agent and a powerful organ-specific laboratory carcinogen. DMBA is widely used in many research laboratories for studying cancer. It is highly lipophilic and requires metabolic activation for its carcinogenicity. Several tissues are capable of activating DMBA, and these include the mammary gland. In the breast, DMBA is converted to epoxides, active metabolites with a capacity for damaging the DNA molecule, the main event in carcinogenesis initiation. With the higher cellular proliferative index of types 1 and 2 lobules, there is higher metabolic activity and more epoxide formation

1,3-butadiene

1,3-butadiene is an air pollutant created by internal combustion engines and petroleum refineries. It is also used in the manufacture and processing of synthetic rubber products and some fungicides and also found in tobacco smoke. 1,3-butadiene is

carcinogenic to humans, with the main route of exposure being through inhalation. The National Toxicology Program classifies 1,3-butadiene as a known human carcinogen .Data from research on animals indicate that females may be more vulnerable to the carcinogenic effects of 1,3-butadiene which is known to cause mammary and ovary tumors in female mice and rats.

Pesticides

Chlordane, Malathion and 2,4-D

A case-control study of 128 Latina agricultural workers diagnosed with breast cancer in California with three pesticides namely chlordane, malathion and 2,4-D—associated with an increased risk of the disease. Scientists found that use of these chemicals were higher in young women and in those with early-onset breast cancer than in unexposed women. In the animal especially in rat mammary tissue development, malathion treatment led to increased proliferation of mammary cells

Alkyl Phenols

Alkylphenols are industrial chemicals used in the production of detergents,cleaning products, and as antioxidants in products made from plastics and rubber. They found in hair products. As such, people are exposed to these compounds in a variety of ways, including through the skin and from contamination of both air and water. . The alkylphenols, including 4-NP, shown to mimic the actions of the natural estrogenestradiol, with their biological effects being regulated by interactions with the cellular estrogen receptor and bound to the cell membrane estrogen receptor and mimic cellular signaling responses usually controlled by estradiol .

Ionizing Radiation

Ionizing radiation is any form of radiation can break the chemical bonds in molecules, including DNA molecules, thereby disturbing their normal functioning. X-rays, gamma rays are the only common forms of radiation with sufficient energy to penetrate and damage body tissue.. In 2005, the National Toxicology Program classified X-radiation and gamma radiation as human carcinogens. Repeated low-dose exposures over time may have the same harmful effects as a single high-dose exposure.

. Ionizing radiation increase the risk for breast cancer through a number of different mechanisms, including direct mutagenesis, genomic instability, therefore increasing the likelihood of future mutations, and changes in breast cell microenvironments that can lead to damaged regulation of cell-to-cell communication within the breast.

Phytoestrogens (Plant Estrogens)[23,24]

Foods such as whole grains, dried beans, peas, fruits, broccoli, cauliflower and especially soy products are rich in phytoestrogens. Several epidemiological studies have shown that regular consumption of soy-based products, or other vegetables high in phytoestrogens, as part of a normal balanced diet can exert a protective influence against later development of breast cancer.. Other studies have found protective effects of soy intake for both pre- and post-menopausal cancer, independent of the tumors' receptor profile (estrogen-receptor and progesterone-receptor positive or negative). A prospective study of women aged 44 to 55 years who had never been diagnosed with breast cancer

but were considered to be at high risk, six months of dietary isoflavone intake was associated with increased proliferation of breast cells.

KEY STATISTICS OF BREAST CANCER [25]

Breast cancer cases in urban Indian women is 30-35 and the age adjusted rate is 35-40 new cases per lakh women per year. Breast cancer is increasing - the average increase over a 35 year period in Mumbai was 12 per cent per decade. Breast cancer is increasing both in young (11 % per decade) and old women (16 % per decade) There are an estimated 1 lakh - 1.25 lakh new breast cancer cases in India every year. The number of breast cancer cases in India is estimated to double by 2025. In 2014, an estimated 232,670 new cases of invasive breast cancer will be diagnosed in U.S. women. In addition to invasive breast cancer, an estimated 62,570 cases of carcinoma in situ will be diagnosed.

MEDICINAL PLANTS IN BREAST CANCER THERAPY

Different active constituents derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of breast cancer. Active oxygen may cause cancer through two possible mechanisms: one is gene mutations and the effects on signal transduction and transcription factors. Another one is oxidative stress causes damages to DNA, phospholipids, proteins and carbohydrates on the cell membrane. Oxidation and injury to DNA induce genetic mutation. Free radicals presence may enhance the mutation of some genes. An antioxidant is “any substance that delays, prevents or removes oxidative damage to a target molecule” Antioxidants act by diverse mechanisms in the oxidative sequence. Antioxidants can be classified into a number of

different groups as enzymatic and non enzymatic strategies. The enzymatic antioxidant involve superoxide dismutase, glutathione peroxidise,catalase and glutathione reductase, while non enzymatic antioxidants are the vitamins A, C, and E, glutathione, and lipoic acid, several bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), etc [26] Antioxidants may work either alone, or in association with each other against different types of free radicals. Vitamin E inhibits the propagation of lipid peroxidation; the combination of vitamin C and vitamin E suppress the formation of hydroperoxide; metal complexing antioxidant such as penicillamine inhibit free redical formation in lipid peroxidation. Human body is continuously generating free radicals, which produces oxidative stress. Factors such as drugs, pollution, immune responses to viruses, deficiency of natural antioxidants, uv rays and tobacco destroy the body potential of stabilizing free radicals. Endogenous sources of oxidative stress include mitochondria, or microsomes,. The body has the power to neutralize the free radicals , but if there is an imbalance between the free radicals and the ability of the body to neutralize it, it produces oxidative stress. Oxidative stress may cause different problems and various diseases such as diabetes, Parkinson's disease, aging and cancer. Oxidative stress causes Lipid peroxidation in cell membranes reacting with polyunsaturated fatty acids. Cell membranes contain polyunsaturated fatty acids and low density lipoproteins They are sensitive to free radicals [27,28] The molecular oxygen reacts with carbon-centered free radicals, and thus, lipid hydroperoxides are formed. Lipid peroxidies alter the membrane structure and its functions. Free redicals cause proteins oxidation and are different markers of protein oxidation, which including protein carbonyl derivates,

oxidized amino acid side chains.In the oxidation process, Protein carbonyl derivates are formed early, and are generated as the peptide main chain as some amino acid side chains that are cleaved (arginine, lysine, or threonine), are also oxidized.[29,30] The carbonyl groups are relatively stable and may result in loss of protein function, as well as increased degradation of soluble proteins . Protein oxidation has also been shown to be a chain reaction and may be inhibited by chain-breaking antioxidants . Oxidation of proteins may develop some problem, but protein damage can be repaired, and is a nonlethal event for the cell . DNA is especially sensitive to damage due to its potential to create cumulative mutations, which may disrupt cellular homeostasis [31,32] DNA may be damaged by ROS and cause permanent structural changes in, as base-pair mutations, deletions, insertions, rearrangements and sequence amplification. Continuous oxidative damage to DNA may lead to alterations in signaling cascades or gene expression, and may cause replication errors and genomic instability. Cancer development is a multistage process which involves mutations in critical genes required for maintenance of the cellular homeostasis . 25% of all cancers in the world is due to chronic inflammation due to infection or injury. Various chemicals such as chlorinated compounds, metal ions, aromatic hydrocarbons and some peroxisome proliferators have been shown to induce oxidative stress, which damages the DNA. They may, therefore, partly account for the development, especially of workrelated cancers. Many cancers are associated with increased production of ROS. Natural products, especially plants, have been used for the treatment of various diseases from ancient times. People of Egypt, China, India and Greece have been using terrestrial plants as medicines, and a large

number of modern drugs have been developed from them. Medicinal plants are used in the treatment of different diseases, such as liver and kidney failure , obesity,diabetes and cancer, etc. Many evidences showed that the production of free radicals inside the body cause damages to DNA and leads cancer, etc., and these free redicals are neutralized by the antioxidants from different medicinal plants, then it prevents cancer. Different studies have shown that plant derived antioxidant scavenge free radicals and modulate oxidative stress.[33,34]. The National Cancer Institute reported about 30,000 plant samples from 25 different countries, and screened around 115,000 extracts for anticancer activity. 60 percentage of the preparations are commercialy available anticancer drugs are derived from natural plant sources. Treatment by herbal medicines may have some advantages over treatment by single purified chemicals as hebal medicine are the mixtures of more therapeutic or preventive components, and so might have more activity than single products seperately . The antioxidant and anti-tumor effects of extracts from various herbs and medicinal plants have been proved clinically. Different in vitro or in vivo studies have proved the anticancer potential of the extracts from several medicinal plants. [35,36].

Among Complementary and alternative medicines, herbal medicine is the most commonly used group of treatment of various diseases. Herbal treatment is the oldest method of system of medicine in the world with more than 2000 years history. Other names used for herbal therapy are phytomedicine, phyto-therapy and botanical medicine and made exclusively from plants such as roots, bark, flowers, seeds, fruits, leaves, or branches and is used in all societies and common to numerous cultures

including Asia, Africa, and America..Phytoconstituents resulting from the herbs such as Vinca rosea, Taxus species, Allium sativum, Aloe vera, Angelica sinensis, Astragals, Glycyrrhiza, Hordeum vulgare, Hydrocotyle asiatica, Morinda citrifolia, Panax pseudoginseng, Saussurea lappa, Taxus, Tinospora cordifolia, Viscum album, Withania somnifera, Zingiber officinale etc. have been used in numerous preparations to improve function of the body's immune cells that stimulates production of cytokines including interleukin, interferon, tumor necrosis factor as well as colony stimulating factor. These herbal preparations assist the body to fight cancer more efficiently and also decrease the harmful side effects of chemotherapy and radiotherapy. Most cancer patients combine herbal remedies with conventional therapy in the hope of boosting the effect of conventional medicine .[37,38] A study of women being treated for early stage breast cancer showed that 10.7% had been using one or more herbal remedies at the time of diagnosis, while an additional 28.5%began using herbal remedies after surgery [39,40] . Similarly a multinational survey found that 35 % of cancer patients were either past or present users of complementary and alternative medicine. Herbal medicines were by far the most commonly used group of treatments, escalating in use from 5 % before the diagnosis of cancer to 13% after the diagnosis of cancer Generally, herbal products are utilized for two reasons, first, to lessen symptoms of disease and second to prevent sickness. Examples include palliative use of St. John's Wort (Hypericum perforatum) for relief of acute depression, the use of Ginkgo biloba for enhancement in perception/understanding and the use of Echinacea for improving cold symptom. In the second circumstance, herbal supplements are taken especially in the anticipation of

averting disease or modifying the effects of threat for certain illnesses. Such as intake of green tea and other flavonoid rich botanicals to yield benefit of the natural antioxidants in them and the consumption of garlic due to high organo sulfur compounds that have been experimentally proven to prevent cancer in animals. Initiation of phase I and phase II metabolic enzymes by herbal supplements is quite typical and maybe liable for some of this action**.** These phase I and II enzymes provide major protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by carcinogens through initiation of their metabolism, particularly phase 2 enzymes such as glutathione S-transferases (GSTs), UDP-glucuronosyl transferases, and quinone reductases . Taking example of garlic, its intake and supplement use is prevalent in both, Eastern and Western cultures . Garlic along with numerous other organosulfur compounds derived from garlic demonstrate robust chemo-preventive action against experimentally induced cancers of the mammary gland as well as esophagus, stomach, colon, liver and lungs. Initiation of phase I and phase II enzymes, nonetheless, result in a significant side effect of herbal products. [41]

Free radicals are the cause of oxidative stress, which may causes injury to cells, gene mutation, and may lead to cancer. Oxidative stress causes cancer, by the interaction with intracellular signal transduction and transcription factors, directly or indirectly. Medicinal plants are main sources in healing of the cancer around the world. The property of the plants is because of the presence of potent anti cancer substances present in that. Several medicinal plants have been known to cure and control cancer. Most of the medications used world wise contains herbal product, with less and no side effects.

Medicinal Plants in Clinical Use

The anticancer agents such as vinblastine and vincristine from the Apocynaceae family , introduced a new era of the use of plant material as a medication for cancer treatment. They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are used in combination with other cancer drugs, for the treatment of different types of cancers, including lymphomas, advanced testicular cancer, breast and lung cancers.[49-50] The isolation of paclitaxel from the bark of the Pacific Yew, Taxus brevifolia Nutt. (Taxaceae), is in the discovery of natural product drug. Various parts of Taxus brevifolia and other Taxus species (e.g., Taxus Canadensis Marshall, Taxus baccata L.) have used by several native American tribes for the treatment of different types of diseases, while Taxus baccata in India as a medicine for the treatment of cancer.[51,52] Paclitaxel is active against a number of cancer types, for example: ovariancancer, advanced breast cancer and non-small cell lungcancer. Camptothecin was isolated from the Chinese ornamental tree, Camptotheca acuminate Decne (Nyssaceae), the clinical trials in the 1970s and dropped it because of severe bladder toxicity. Camptothecin derivatives, Topotecan and irinotecan, are used for the treatment of ovarian and small cell lung cancers, and colon cancers.[53]

2.AIM AND OBECTIVES OF THE STUDY

Treatment for Breast cancer is problematic. Because Allopathic drugs are effective against this type of breast cancer but exhibit severe toxicity which leads to death . Physicians and patients are in need of maximum therapeutic value with no or less side effects to improve the quality of the life . Several herbal plants potentially constitute such a group. In recent years, researchers have examined the activity of plants used traditionally by indigenous healers and herbalists to support to treat cancer. Several hundred plants have been reported for use in different types of cancer treatment . Only a handful have been fairly well researched. After the extensive search of medicinal plant for the treatment of cancer, the plant selected for the study is *Triumfetta rhomboidea* Jacq, which belongs to the family Tiliaceae.[42] The genus Triumfetta consists of about 150 species out of which 8 are found in India. It is known as Burweed in English.It is a herb or under shrub with stellate pubescence. Roots of *Triumfetta rhomboidea* have been traditionally used in intestinal ulcer,dysentry and as diuretic. Leaves and stem are used in treatment of tumors,leprosy and Gonorrhoea. However, no reports are available to show the antioxidant property of the plant and the effectiveness in breast cancer..

Based on the literature review there is no evidenced work done in the Chemo preventive activity of *Triumfetta rhomboidea* in 7, 12-Dimethylbenz (A) Anthracene induced Breast cancer in Sprague –Dawley Rat model. The present study is planned to evaluate the Chemo preventive effect of methanol extract of leaves of *Triumfetta rhomboidea* in 7, 12-Dimethylbenz (A) Anthracene induced Breast cancer in Sprague – dawley rat model.

3.REVIEW OF LITERATURE

1. **Devamurari et al** [54] investigated the ethanolic extract of *Triumfetta rhomboidea* Jacq was subjected to various phytochemical tests. Preparative Thin layer Chromatography study of the extract was performed and active constituents were isolated. Spectral analysis of the isolated constituent indicates that *Triumfetta rhomboidea (Tiliaceae)* contains carbohydrate glycosides, phytosterol, steroids, flavonoids, tannin & phenolic compounds and triterpenoids. Antibacterial activity of ether and alcoholic extract of the plant was performed. Results exhibited that *Triumfetta rhomboidea* Jacq contain good antibacterial action.
2. **CD Odimegwu et al** [55] investigated the antimicrobial activity of methanol extract of *Triumfetta rhomboide*a leaves was evaluated against *Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumoniae*. Agar diffusion and agar dilution techniques were employed for the antimicrobial sensitivity and interaction studies. Phytochemical analysis of the crude extract was also carried out using standard procedures. The results obtained showed that the crude extract exhibits a substantial antimicrobial activity against *Klebsiella pneumoniae* (which recorded the greatest sensitivity), *Salmonella typhi* but not against *Staphylococcus aureus*. Thus, this study shows that *Triumfetta rhomboidea* possesses promising antimicrobial activity especially against *Klebsiella pneumoniae*.

3. **Uche et al** [56] studied the analgesic and anti-inflammatory effects of the methanolic extract of the leaves of *Triumfetta rhomboidea* on mice and rats respectively. And to screen the phytochemical constituent of the extract. The analgesic effect was determined by acetic acid-induced writhing test in mice. While the anti-inflammatory activity was determined by egg albumin-induced oedema of the rat paw. *Triumfetta rhomboidea* extract caused a statistically significant reduction in the number of acetic acid-induced writhing in mice, with$P<0.001$ (ANOVA).
4. **Prasad et al**[57]determined experimentally the possible Antibacterial Activity of Water Extract and Active constituents were Isolated of Butanol from a *Triumfetta rhomboidea* and Analytical Study by HPLC Method. The Antibacterial activity of the Water Extract of *Triumfetta rhomboidea* was evaluated at two different concentrations by the diffusion method. The Bacteria B. cereus was found to be more active and E. coli, S. aureus was found to be less active in inhibition zone. .Antibacterial activity of water extract of the plant was performed. Results exhibited that *Triumfetta rhomboidea* contain good Antibacterial action and Active constituents were Isolated of Butanol.
5. **Rozina Parul et al**[58] studied the methanolic extracts of three medicinal plants named *Phyllunthus freternus*, *Triumfetta rhomboidae* and *Casuarina littorea* were examined for their possible regulatory effect on nitric oxide (NO) levels using sodium nitroprusside as a NO donor in vitro. Most of the extracts tested demonstrated direct scavenging of NO and exihibited significant activity and the

potency of scavenging activity was in the following order: *Phyllunthus freternus* > Leaves of *Triumfetta rhomboidae* > *Casuarina littorea* > barks of *Triumfetta rhomboidae* > roots of *Triumfetta rhomboidae*. All the evaluated extracts exhibited a dose dependent NO scavenging activity.

6. **P Sivakumar et al** [59] proved experimentally the possible antitumor effect and antioxidant role of methanol extract of *Triumfetta rhomboidea* (METR) leaves against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The METR administered at the doses of 100, 200 mg/kg, in mice for 14 days after 24 hours of tumor inoculation. The effects of METR on the growth of murine tumor, life span of EAC bearing mice were studied. Hematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were also estimated.
7. **P. Lissy et al** [60] studied methanolic extract of *Sida retusa* Linn. (Malvaceae),*Urena lobata* Linn.(Malvaceae)and *Triumfetta rhomboidea* Jacq.(Teliaceae) roots were found to inhibit lipid peroxidation, scavenge hydroxyl and superoxide radicals in vitro.. IC 50 of root extract of U.lobata was 470. 60 ug/ml, 1627.35ug/ml and 1109.24 ug/ml for superoxide radical scavenging, hydroxyl radical scavenging and lipid peroxidation respectively. T.rhomboidea root extract required for IC 50 was 336.65 ug/ml, 1346.03 ug/ml and 1004.22 ug/ml for superoxide scavenging, hydroxyl radical scavenging and lipid peroxidation respectively.

8. **N Suganthi et al**[61] investigated the antidiabetic effect of ethanolic extract of *Triumfetta rhomboidea* was investigated in alloxan induced diabetes rats. Oral administration of ethanolic extract of *Triumfetta rhomboidea* at the doses of 100, 200, and 400 mg / kg body weight was studied in alloxan induced diabetic rats. The activity of the plant extracts was comparable with glibenclamide, a well known antidiabetic drug. The result clearly suggests that ethanolic extract of *Triumfetta rhomboidea* posses' significant antidiabetic activity.
9. **J P Mevy et al** [62] proved the essential oil of the aerial parts of *Triumfetta rhomboidea* was analysed by GC and GC-MS and assayed for its antibacterial and antifungal activities. The main constituents identified were *trans-β*-caryophyllene (22.4%), kessane (14%) and caryophyllene oxide (13%). The antimicrobial tests showed a mild activity against *Escherichia coli* and *Enterococcus hirae*.
10. **Natla Sashidhar Reddy et al** [63] studied that Quercetin supplementation at the dose of 100 mg kg-1 body weight was most effective in alleviating cancer symptoms and was comparable to vincristine. The plasma TBARS were reduced and breast tissue TBARS were elevated. The antioxidant enzymes were rejuvenated by quercetin supplementation at all three dose levels. Quercetin is found to be an effective chemotherapeutic agent in the treatment of breast cancer on par with vincristine. Being a plant product, quercetin can also be used in chemoprophylaxis in high risk individuals with genetic predisposition towards breast cancer. Besides, it can be given orally and has a wide margin of safety.

11. **T.Rengarajan et al** [64] reported 7, 12-dimethylbenz (a)anthracene(DMBA) acts as a potent site and organ specific carcinogen by generating various reactive metabolic intermediates leading to oxidative stress. Female Sprague Dawley rats were divided into four groups and each group consisting of six animals. Group I and group IV were vector and drug control. The group II and group III animals were treated with DMBA 20 mg/kg bodyweight to induce mammary carcinoma. Rats received cancer bearing Group III animals were treated with D-Pinitol at the concentration of 30 mg/kg bodyweight for 45 days orally. At the end of the experimental period all the rats were sacrificed. The breast and liver tissues levels of the enzymic and non-enzymic antioxidants were significantly decreased in cancer bearing animals when compared to the control animals. Phase I and II xenobiotic metabolizing enzymes and Lipid peroxide levels (LPO) were estimated. Western blotting analysis showed over expression of Bcl-2 in the mammary tissue of DMBA induced group II rats. From our results, we conclude that D-Pinitol is a potent antioxidant and play a protective role against DMBA induced breast cancer.
12. **Margarida F et al** [65] studied that Combined chemopreventive effects of alpha tocopherol and selenium in rodent tumours seem to be more promising than that of selenium or alpha tocopherol alone.On the otherhand the effect of ascorbic acid alone in some tumour model system is not consistent or appears to be weak. The combined effects vitamin C and selenium depends on the chemical form of the selenium compound. This experimental study was designed to evaluate

mixture of three micro nutrients. Results point to an effective chemopreventive action of this mixture in reducing the incidence and preventing the development of malignant tumours in this animal model.

13. **Haiming Ding et al[66] reported** Avocado extract Inhibited (DMBA)- Induced Carcinogenesis in Hamster Cheek Pouches and shown that an avocado extract selectively inhibits proliferation in premalignant and malignant and not normal primary human oral epithelial cell lines via an ROS-mediated mechanism. Herein, the in vivo anticancer effect of D003 extract and freeze-dried avocado (FA) was determined in the DMBAinitiated hamster cheek pouch (HCP) model.
14. **Veena Sharma et al [67]** studied Present investigation shows that hydroethanolic extract of Moringa oleifera (MOHE) and its isolated saponin (SM) attenuates DMBA induced renal carcino-genesis in mice. Isolation of SM was achieved by TLC and HPLC and characterization was done using IR and 1 H NMR. Animals were pre-treated with MOHE (200 and 400 mg/kg body weight; p.o), BHA as a standard (0.5 and 1 %) and SM (50 mg/kg body weight) for 21 days prior to the administration of single dose of DMBA (15 mg/kg body weight). Administration of DMBA significantly (p \ 0.001) enhanced level of xenobiotic enzymes. It enhanced renal malondialdehyde, with reduction in renal glutathione content, antioxidant enzymes and glutathione-S-transferase. The status of renal aspartate transaminase, alanine transaminase, alkaline phosphatase and total pro-tein content were also found to be decreased along with increase in total cholesterol in DMBA administered mice. Pretreatment with MOHE and SM significantly

reversed the DMBA induced alterations in the tissue and effectively suppressed renal oxidative stress and toxicity

15. **Jyoti Parma et al** [68] reported that Investigation of cancer chemopreventive and anti-oxidative property of S.cumini seed extract (SCE) on 7,12-dimethyl benz(a)anthracene (DMBA) induced skin carcinogenesis in mice. Animals were divided into four groups on the basis of their respective treatments wherein mice of Group I & II served as vehicle treated and SCE treated controls respectively. For induction of skin tumors, mice of Group III and IV were applied topically with 7,12-dimethylbenz(a)anthracene (DMBA) followed 2 weeks later by repeated application of croton oil (1% in acetone three times a week) and continued till the end of the experiment (i.e. 16 weeks). Mice of Group IV were administered S.cumini seed extract (SCE) at peri- & post-initiational stage. The results of the study revealed a significant decrease in incidence, cumulative number of papillomas, tumor yield and tumor burden in mice of Group IV as compared with DMBA alone at the end of experiment. A significant reduction in tumor weight and tumor volume was also observed. Reduction in the incidence and number of papilloma, the preneoplastic lesions, was considered to be the mean of assessment.

4.MATERIALS AND METHODS

4.1 PLANT PROFILE

4.1.1.Botanical Information

TRIUMFETTA RHOMBOIDEA N. Jacq

Classification	***Triumfetta Rhomboidea***
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	*Triumfetta*
Species	*Triumfetta Rhomboidea*

Vernacular Names

Hindi	: Burr Bush, Chinese Burr,
Tamil	: Ottu Pulli
Telugu	: Bankathuthara
Bengali	: Bon okhra
Kannada	: Kadu bende
Assamese	: Agra

4.1.2. Description of the Plant [43-45]

An annual undershrub, 0.6-1.5 m high; branches slender, more or less pubescent. Leaves variable, lower leaves 5-7.5 cm diam, usually 3-lobed, irregularly serrate, clothed with stellate hairs on both surfaces; upper leaves usually simple, with very short petioles. Flowers 6 mm across, yellow in dense terminal and leaf-opposed cymes. Capsule subglobose, 4 mm diam., echinate with hooked bristles.

ChemicalConstituents:[46]

A flavone glycoside, triumboidin, Friedelin, Friedelinol has been isolated from this plant. It also contains small amounts of other flavones and flavonoids. Seeds contain a fixed oil and proteins

Ethnomedicinal uses:[47,48]

Fruits, flowers and leaves are used as astringent and demulcent . Bark and fresh leaves are traditionally used in diarrhoea ,dysentery,Inflammation, Diabetes Mellitus, Peptic ulcer and cancer of Breast. Flowers rubbed with sugar and water is given in gonorrhoea to stop burning sensation . Fruits are used to promote parturition. Roots are

used as tonic, diuretic and cooling; used in dysentery; hot infusion is taken by the women to facilitate childbirth.

4.2 PHYTOCHEMICAL EVALUATION

4.2.1.Collection and Authentication of plant

The fresh healthy plant leaves of *Triumfetta rhomboidea* was collected from Irular society, Thandarai, TamilNadu, India during the month of December 2013. The plant was identified and authenticated by Botanical Survey of India, Coimbatore and a voucher specimen has been preserved in the Department of Pharmacognosy, R.V.S. College of Pharmaceutical sciences, sulur, Coimbatore. After authentification, the fresh, healthy plant leaves of *Triumfetta rhomboidea* was properly dried in shade for 2-3weeks. It was pulverized in a blender, sieved and used for further studies.

4.2.2.Preparation of the Extracts

About 2 kg of shade dried plant leaves of *Triumfetta rhomboidea* extracted in soxhlet successively extracted with n-hexane, chloroform, ethyl acetate and methanol. Each extract was evaporated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant leaves. The Consistency and Colour of the extract were noted. All the solvents used for this work were of analytical grade.

4.2.3.Qualitative chemical tests [69]

The n-hexane, chloroform, ethyl acetate, methanol extracts of the leaf powder of *Triumfetta rhomboidea* was subjected to qualitative chemical analysis.

1. **Test for alkaloids**

   The extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with different alkaloidal reagents

a) Mayer's reagent

Treated with Mayer's reagent (mixture of potassium and mercuric chloride). Appearance of cream colour precipitate indicates the presence of alkaloids

b) Dragondroff's reagent

Extract is treated with Dragondroff's reagent (Sodium nitoprusside and iodine). Appearance of orange colour precipitate indicates the presence of alkaloids.

c) Hager's reagent

Extract is treated with Hager's reagent (saturated aqueous solution of picric acid). Appearance of yellow precipitate indicates the presence of alkaloids.

d) Wagner's reagent

Extract is treated with Wagner's reagent (Iodine in Potassium iodide). Appearance of reddish brown precipitate indicates the presence of alkaloids.

2. Test for carbohydrates

The minimum amount of the extracts were dissolved in 5ml of distilled water and filtered. The filtrate was used to test for carbohydrates.

a) Molisch's test

It was treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of violet colour ring at the junction of two layers confirms the presence of carbohydrates.

b) Fehling's test

The filtrate was treated with 1 ml of Fehling's A and B and heated in a boiling water bath for 5-10min. Appearance of reddish orange precipitate confirms the presence of carbohydrates .

3. Test for glycosides

a) Cardiac glycoside Keller-Killani test- To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid were added. Formation of reddish brown colour at the junction of the two liquid layers indicates the presence of cardiac glycosides.

b) Saponin glycosides Foam test – The extract and powder was mixed vigorously with water. Appearance of foam shows the presence of Saponins

c) Anthraquinone glycosides Borntrager's Test – To extract dilute sulphuric acid was added, boiled and filtered. The cold filtrate equal volume benzene or chloroform was added. The organic layer was separated and ammonia was added. Ammonical layer changed to pink or red.

d) Coumarin glycosides -Methanol extract when made alkaline, shows blue or green fluorescence.

4. Test for phytosterol

1gm of the methanol extract was dissolved in few drops of dry acetic acid; acetic anhydride (3ml) was added and few drops of concentrated sulphuric acid. Bluish green colour confirms the presence of phytosterol.

5. Test for fixed oils and fats

(a) Filter paper Test:- Various extracts was separately pressed between two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil.

(b) Saponification Test:- Few drops of 0.5N alcoholic KOH is added to a small quantity of extracts along with a drop of phenolphthalein. The mixture was heated for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

6. **Test for tannins and phenolic compounds**

Small quantity of various extracts were taken separately in water tested for the presence of phenolic compounds and tannins with

(a) Dilute ferric chloride solution (5%) formation of violet colour indicates the presence of phenolic compounds.

(b) 1% solution of gelatin with 10%NaCl formation of white precipitate indicates the presence of tannins.

(c) 10% lead acetate solution formation of white precipitate indicates the presence of tannins.

7. Test for proteins

Various extracts were dissolved in few ml of water and treated with

(a) Millon's reagent gives the appearance of red colour shows the presence of proteins and free amino acids.

(b) Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

8. Test for gums and mucilage

About 10ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

9. Test for flavonoids

(a) With aqueous solution of sodium hydroxide blue to violet colour (Anthrocyanins), yellow colour (Flavones), yellow to orange (Flavonones).

(b) With concentrated sulphuric acid yellowish orange colour (Anthrocyanins), orange to crimson colour (Flavonones).

(c) Shinoda's test – the extracts were dissolved in alcohol, to that a piece of magnesium and followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

10. Test for terpenoids Noller's test

The substance was warmed with thionyl chloride and Tin. Pink color indicates the presence of triterpenoids.

11. Test for steroids Libermann – Burchard Reaction

2ml extract was mixed with chloroform. To this 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. First red, then blue and finally green colour appears.

4.2.4. Thin Layer Chromatography of methanol extracts of *Triumfetta rhomboidea*[70,71]

Of the various methods of separating and isolating plant constituents, thin layer chromatography (TLC) is one of the most powerful techniques used for the separation, identification and estimation of single or mixture of components present in various extracts. Mechanism employed in this reliable technique is adsorption in which solute adsorbs on the stationary phase according to its affinity. Substances are separated by differential migration that occurs when a solvent flows along the thin layer of stationary phase. The substance which is having more affinity towards mobile phase moves faster when compared to the substance which has less affinity leading to the separation of the compounds.

TLC Plates

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

Sample application

The extracts to be analyzed were diluted with respective solvents and then spotted with help of capillary tube just 2 cm above its bottom.

Selection of mobile phase

Solvent mixture was selected on the basis of the phytoconstituents present in each extract. Solvents were analyzed as its order of increasing polarity. Several mobile phases were tried for the separation of maximum components. After several trials, the best solvent system was selected which showed good separation with maximum number of components.

Solvent system

Methanol: Ethyl acetate: Water (6:3:1)

Rf values were noted down for each selected extracts after elution by using different detecting agents such as Dragondroff's, Ninhydrin, Libermann Burchard, concentrated sulphuric acid and ferric chloride.

4.2.5. High Performance Thin Layer Chromatography of methanol extract of *Triumfetta rhomboidea*

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantization of nanograms level of samples. Thus this method can be conveniently adopted for routine

quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.

Basic steps involved in HPTLC

Extracts used : Methanol

Application mode : CAMAG Linomet IV.

Development mode : CAMAG Twin Trough chamber.

Sample application

The samples were dissolved in same solvent and 10µl quantity of sample was applied on the HPTLC silica merk 60F 254 graded plate sized 6cm x 10 cm as narrow bands using CAMAG Linomat 5 injector.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 254nm. The data's obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

Mobile Phase

n-Butanol(4): Water(5): Acetic acid(1).

4.3. INVITRO FREE RADICAL SCAVENGING ACTIVITY

4.3.1.DPPH Radical Scavenging Activity [72-75]

Free radical scavenging effect was determined according to the method of Blois as modified by zang et al . Briefly, a 1mM solution of DPPH radical solution in methanol was prepared, and then 1mL of this solution was mixed with different concentrations of methanol extract; the mixture was then vortexed vigorously and left for 30 min at room temperature in the dark and the absorbance was measured at 517 nm with a spectrophotometer and was calculated. DPPH Scavenging activity % = [(Control Absorbance – Extract Absorbance)/Control Absorbance] x 100. For control 1.0 mL of methanol was added to 1mL of 1mM solution of DPPH radical solution and the rest of the procedures remain the same.

4.3.2.Superoxide Radical Scavenging Assay [76-79]

The superoxide radical scavenging activity of the extracts was determined according to the literature method and containing PMS (0.1 mmol/L), NADH (1 mmol/L), NBT (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4) with different concentrations of the extract was incubated at room temperature for 5 min and the color was read at 560 nm against a blank. The scavenging effect was calculated against the control

4.3.3.Nitric Oxide Radical Inhibition Assay [80]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by

Griess reaction. The reaction mixture (3ml) containing sodium nitropruside (10mM) in phosphate buffered saline and the varying concentrations of extract and control were incubated in water both at 25oC for 30 minutes. After incubation, 1.5ml mixture were removed and 1.5 of Griess reagent was then added. The absorbance of the chromophore formed was evaluated using spectrophotometer at 546nm. NO Scavenging activity % = [(Control Absorbance – Extract Absorbance)/Control Absorbance] x 100. For control 1.0 mL of buffer was added to 3mL of 10mM sodium nitropruside and the rest of the procedures remain the same.

4.4. ACUTE TOXICITY STUDY

4.4.1.Plant extract

The therapeutically active extract of *Triumfetta rhomboidea* Methanol extract (TRM) to carry out the pharmacological studies in animals was selected on the basis of in- vitro studies like in-vitro free radical scavenging activity and phytochemical analysis.

4.4.2.Experimental animals

Adult male Wister rats weighing 100-150g were obtained from R.V.S. College of Pharmaceutical Sciences, Sulur, Coimbatore. They were maintained at standard housing conditions and fed with commercial diet and provided with water and libitum during the experiment. The institutional animal ethics committee (Reg.no 1012/C/06/CPCSEA) permitted the study. The animals were kept in clean and dry polycarbonate cages and maintained in a well ventilated animal house with 12hrs light – 12 hrs dark cycle.

4.4.3.Acute toxic class method [81,82]

Acute toxicity study was done according to OECD guidelines 423 (Organization of Economic Co-Operation and Development). It is a procedure with three animals of single sex per step. Based on the mortality and morbidity status of the animal, average of 2-4 steps necessary to allow decision on the test substance. The procedure is to fix a minimal number of animals, which allows acceptable database scientific conclusion. The method uses different doses (5, 50, 500, 2000mg/kg body weight) Procedure Three healthy, Wistar Albino rats weighing 150-200gms were selected for the study. The rats were fasted over-night and provided with water ad libitum. Following the period of

fasting, the animals were treated with the methanolic extract at the dose of 2000mg/kg body weight, orally. As most of the crude extracts possess LD50 value more than 2000mg/kg body weight and this was used as starting dose. After oral administration, the rats were observed for 24hrs to access mortality and to observe any changes in the autonomic or behavioral responses viz. aggressiveness, alertness, irritability, spontaneous activity, corneal reflex, tremor, salvation, urination, convulsion and respiration etc. The rats were observed regularly for 14 days to note the mortality or toxic symptoms. Since there was no death as per the guidelines, the study was repeated with the same dose to confirm the results.

4.5 PHARMACOLOGICAL SCREENING

4.5.1. SCREENING OF 7,12 DIMETHYL BENZ ANTHRACENE INDUCED BREAST CANCER [83-86]

Experimental design

Forty rats were divided into four groups and each group contained ten rats.

Group I rats received the excipient (single dose of 1 mL of emulsion of sunflower oil and physiological saline, s.c.) and 1 mL of 2% DMSO (p.o.) throughout the experimental period served as vehicle treated control.

Groups II , III and IV rats were induced mammary carcinogenesis by providing single subcutaneous injection of 25 mg of DMBA. Group 2 rats received no other treatment.

Group III, IV and V rats were orally administered with TRM 100 , 200 mg kg^{-1} b.wt., and 5mg/kg of Tamoxifen as a standard dissolved in 2% DMSO starting one week before the exposure of the carcinogen respectively and continued till the experimental period.

The experiment was terminated at 16th week to evaluate the chemopreventive effect of TRM during DMBA-induced mammary carcinogenesis. All rats were sacrificed by cervical dislocation at the end of experimental period to evaluate the tumor incidence and tumor volume in control and experimental rats in each group.

Biochemical Estimation

Blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 1000x g for 15 min. Tissue samples from rats were washed with ice cold saline and dried between folds of filter paper, weighed and homogenized using appropriate buffer in an All-glass homogenizer with teflon pestle and used for biochemical estimations Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma were assayed by the method of Yagi [87] (1987). Tissue lipid peroxidation was done by the method of Ohkawa *et al*. [88](1979). The activities of superoxide dismutase(SOD), catalase (CAT) and Glutathione Peroxidase (GPx) were assayed by the method of Kakkar *et al*. [89] (1984), Sinha [90] (1972) and Rotruck *et al*. [91](1973) respectively. The GSH level in plasma and mammary tissues was determined by the method of Beutler and Kelley (1963)[92]. Chemiluminescent immunoassay (CLIA) was used for the estimation of serum 17 β-estradiol (E2)

Histopathology

For histopathological studies, tumor tissues and normal mammary gland tissues were fixed in 10% formalin and were routinely processed and paraffin embedded, 2-3 μm sections were cut in a rotary microtome and were stained with hematoxylin and eosin.

4.5.2. IN VIVO ANTI-TUMOR ACTIVITY

Cell Lines

Dalton's lymphoma ascites tumor cells (DLA) and Ehrlich ascites carcinoma cells (EAC) maintained in Amala Cancer Research Centre, Thrissur, Kerala, were used for the assay.

Maintaining of tumor cell lines

Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells were maintained in the ascites fluid of the peritoneal cavity of mouse. The aspirated tumor cells were washed 3 times with PBS (pH-7.4) and adjusted the cell count to 1×10^6 cells/ml using haemocytometer. Approximately, 1×10^6 cells were injected intraperitoneally to develop ascites tumor in mice.

In vivo Anti-tumor activity

For assessing the antitumor activity, Dalton's Lymphoma Ascites (DLA) cell induced solid tumor model and Ehrlich Ascites Carcinoma (EAC) cell induced ascites tumor model were employed.

EAC induced ascites tumor model system [93-95]

Male Swiss Albino mice were grouped into four groups (6 animals per each group).

Group I: Control – Untreated;

Group II: Standard – Cyclophosphamide 10mg/kg;

Group III: TRM (100 mg/kg b.wt)

Group IV: TRM (200 mg/kg b.wt)

EAC cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1ml containing $1x10^6$cells/ml was injected intraperitoneally into all the animals. The drug administration has started next day after the induction of tumor and continued for 10 consecutive days. The animals were observed for the development of ascites tumor and death due to tumor burden was recorded for 30 consecutive days. The life span of animals was calculated using the formula,

%ILS = (T-C)/C × 100, where T and C are mean survival of treated and control mice respectively.

DLA induced solid tumor model system [96-97]

Assessment of tumor volume

Male Swiss Albino mice were grouped into four groups (6 animals per each group).

Group I: Control – Untreated;

Group II: Standard – Cyclophosphamide 10mg/kg;

Group III: TRM (100 mg/kg b.wt)

Group IV: TRM (200 mg/kg b.wt). DLA cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1ml containing $1x10^6$cells/ml was injected intramuscularly into the right hind limb of all the animals. The drug administration was started next day after the induction of tumor and continued for 10 consecutive days. The tumor development on animal in each group was determined by measuring the diameter of the tumor growth in two perpendicular planes using a vernier caliper at fixed intervals (on each 3rd day) and the volume was calculated using the formula, **Tumor volume =** $4/3\ \pi$

r_1^2 X r_2 where $r_1$is the minor radius and r_2 is the major radius.And the percentage of inhibition of tumor volume in animals was calculated by, **% of inhibition** = [(tumor volume of control on 30^{th} day – Tumor volume of treated on30^{th}) / (Tumor volume of control on 30^{th} day)] × 100

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM of six animals from each group. The statistical analysis were carried out by one way analysis of variance (ANOVA) P values < 0.05 were considered significant**.**

4.5.3.DOCKING ANALYSIS

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational drug design helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor and docking is the process by which two molecules fit together in 3D space. A bioactive compounds has been already reported from the leaves of *Triumfetta rhomboidea* .The aim of the present study is to investigate the inhibitory activity of the compounds on breast cancer by molecular docking studies and to analyze the ADME/T properties of the compounds

Compound 1- Friedelin

Compound 2- Friedelinol

Compounds 1 and 2 were used for docking on BRCA1(Breast Cancer Gene 1). Glide docking uses the assumption of a rigid receptor, although scaling of vander Waals radii of nonpolar atoms, which decreases penalties for close contacts, can be used to model a slight “give” in the receptor and/or ligand. Docking studies of designed compounds were carried out using GLIDE (Grid-based Ligand Docking with Energetics) module version 5.9. Schrödinger, LLC, New York, NY, 2013. The software package running on multi-processor Linux PC. GLIDE has previously been validated & applied successfully to predict the binding orientation of many ligands.

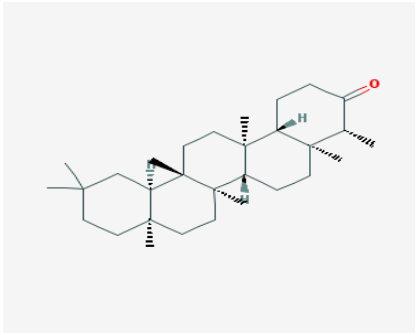
Software's handled [98-101]

- GLIDE module version 5.9., mastero 9.4
- Quik prop -3.6 -Schrödinger, LLC, New York, NY, 2013- docking
- Swiss PDB viewer-4.04 – protein viewer
- Pymol viewer 1.3 – image viewer
- Marveen sketch 5.5 – drawing ligand structure

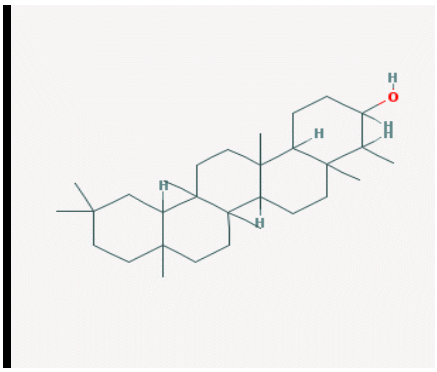
Ligand structure

The chemical structure of each ligand was drawn using build module**.**

1. Structure of Structure of Friedelin



2. Structure of Friedelinol



Ligand preparation

In order to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with the 3D structures in SD Maestro format, Lig Prep was used. Lig Prep produced a single, low-energy, 3D structure with corrected chiralities for each successfully processed input structure.

Preparation of protein

The typical structure file from the PDB is not suitable for immediate use in molecular modelling calculations. A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, and cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the placement of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges. This was done using the Protein Preparation Wizard.

Receptor Grid Generation

Receptor grid generation requires a "prepared" structure: an all atom structure with appropriate bond orders and formal charges. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The options in each tab of the Receptor Grid Generation panel

allow defining the receptor structure by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids, and set up Glide constraints. A grid area was generated around the binding site of the receptor.

Ligand Docking:

This is carried out using GLIDE DOCK. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand acts as single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide was run in rigid or flexible docking modes; the latter automatically generated conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarily of ligand-receptor interactions using a grid-based method patterned after the empirical Chem-Score function. Poses that passed these initial screens entered the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non bonded ligand-receptor interaction energy. Final scoring is then carried out on the energy-minimized poses.

Glide Extra-Precision Mode (XP)

The extra-precision (XP) mode of Glide combines a powerful sampling protocol with the use of a custom scoring function designed to identify ligand poses that would be expected to have unfavourable energies, based on well-known principles of physical chemistry. The presumption is that only active compounds will have available poses that avoid these penalties and also receive favourable scores for appropriate hydrophobic contact between the protein and the ligand, hydrogen-bonding interactions, and so on. The chief purposes of the XP method are to weed out false positives and to provide a better correlation between good poses and good scores. Extra-precision mode is a refinement tool designed for use only on good ligand poses. Finally, the minimized poses are re-scored using Schrödinger's proprietary *Glide-Score* scoring function. Glide-Score is based on Chem-Score, but includes a steric-clash term and adds buried polar terms devised by Schrodinger to penalize electrostatic mismatches:

Glide Score = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

Docking Procedure

Docking studies of 2 compounds were performed using 1protein obtained from the RCSB Protein Data Bank, http://www.rcsb.org/pdb.

Experiments were performed using the program GLIDE (Grid-based Ligand Docking with Energetic) module version 5.9, Schrödinger, LLC, New York, NY, 2013 (Schrodinger Inc.). Coordinates of the full-length substrate-complexed dimmer were

prepared for Glide 5.9 calculations by running the protein preparation wizard. The p-prep script produces a new receptor file in which all residues are neutralized except those that are relatively close to the ligand (if the protein is complexed with a ligand) or form salt bridges. The impref script runs a series of restrained impact energy minimizations using the Impact utility. Minimizations were run until the average root mean square deviation (rmsd) of the non-hydrogen atoms reached 0.3Å. Glide uses two boxes that share a common centre to organize its calculations: a larger enclosing box and a smaller binding box. The grids themselves are calculated within the space defined by the enclosing box. The binding box defines the space through which the centre of the defined ligand will be allowed to move during docking calculations. It provides a measure of the effective size of the search space. The only requirement on the enclosing box is that it be large enough to contain all ligand atoms, even when the ligand centre is placed at an edge or vertex of the binding box. Grid files were generated using the co-crystallized ligand at the centre of the two boxes.

Components of Glide Score of Docking (Extra-Precision Mode)

Component	Description
VdW	Van der Waals energy is calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums.
Coul	Coulomb energy is calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums.

Lipo	Lipophilic contact term. Rewards favourable hydrophobic interactions.
HBond	Hydrogen-bonding term is separated into differently weighted components that depend on whether the donor and acceptor are neutral, one is neutral and the other is charged, or both are charged.
Metal	Metal-binding term used only for the interactions with anionic acceptor atoms is included. If the net metal charge in the apo protein is positive, the preference for anionic ligands is included; if the net charge is zero, the preference is suppressed.
BuryP	Penalty for buried polar groups.
RotB	Penalty for freezing rotatable bonds.
Site	Polar interactions in the active site. Polar but non-hydrogen-bonding atoms in a hydrophobic region are rewarded.

4.5.4. SCREENING OF CARDIO PROTECTIVE ACTIVITY [102]

Rats were treated with different doses of Methanol extract of *Triumfetta Rhomboidea* (TRM) orally using an intra-gastric tube daily for 14 days. On 14th day, myocardial injury was induced in experimental rats by injection of ISO (200 mg kg-1, s.c.) twice at an interval of 24 h (i.e., on 14th and 15th day of extract treatment) while normal control and Isoproterenol (ISO) treated rats were given an equivalent volume of the vehicle.

Treatment protocol

The experimental rats were divided into four groups of 6 animals each and treated as follows:

Group 1: Normal Control Rats received single daily dose of 5 % Tween 80 (5 ml/Kg; po)

Group 2: Rats treated with 5 % tween 80 (5 ml/Kg; po) and ISO (200 mg kg/day for 2 days, s.c.)

Group 3: Rats pretreated with TRM (100 mg/ kg/ day, p.o.) and then ISO (200 mg kg-1day-1; for 2 days, s.c.)

Group 4: Rats pretreated with TRM (200 mg/ kg/ day, p.o.) and then ISO (200 mg kg-1day-1; for 2 days, s.c.)

Biochemical analysis:

At the end of experimental period (after 24hr of second ISO injection or 16th day of extract/vehicle treatment) the rats were anaesthetized with light anaesthetic ether. Blood was collected from retro-orbital puncture, serum was separated and used for

estimation of marker enzymes. The activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)6 in serum were determined spectrophotometrically and the absorbance was measured at 520 nm and enzyme activity was expressed as U L−1.The Lactate Dehydrogenase5 (LDH) activity in serum was assayed and the absorbance was measured 520 nm and the enzyme activity was expressed as U L−1.The Creatine Phosphokinase (CPK)4 activity in serum was determined and the absorbance was measured at 640 nm and the enzyme activity was expressed as IU L−1.

4.5.5. IN VIVO ANTI-SNAKE VENOM ACTIVITIY [103-105]

Snake venom

Lyophilized snake venom of Naja Naja was obtained from Haffkine Institute, Parel, Mumbai, and were preserved at 2 to 8°C. The *Naja naja* snake venom was dissolved in normal saline (0.9% w/v NaCl), centrifuged and the supernatant was used whenever required. The venom concentration was expressed in terms of dry weight (mg/ml) of the stock venom.

In vivo anti- snake venom activity

Oral administration of extract 5 min prior to the injection of venom .Twenty four adult Swiss albino mice of both sexes were divided into four groups of six mice each. The control group was injected with only venom (lethal dose 61mcg/20 g of the mice , i.p), while the other groups were treated separately with venom, after 5 min of oral administration of anti snake venom serum (10mg/kg) and methanolic extract of *Triumfetta rhomboidae* (100 and 200mg/kg) respectively. The mice were observed for 24 hours for the number of mice which were survived.

4.5.6.ANALGESIC ACTIVITY BY ACETIC ACID INDUCED WRITHING IN MICE [106-107]

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid is administered intraperitoneally to the experimental animals to induce pain sensation. As a positive control, any NSAIDS drug can be used. In the present study Diclofenac sodium was used to serve the purpose. Methanol extracts of *Triumfetta rhomboidae* were administered orally in two different doses (100 and 200 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (5 mg/kg) was used as a reference standard (positive control).

4.5.7.CARRAGEENAN INDUCED PAW EDEMA METHOD [108-110]

Acute anti-inflammatory activity was evaluated by carrageenan–induced rat paw edemas as described by Turner et al. Wistar albino rats were divided into 4 groups (n=6). Acute inflammation was produced by injecting 0.1 mL of 1% carrageenan into sub- plantar surface of rat hind paw. The control group I received Tween 80 (0.5%) 0.1mL.The test groups 200 mg/kg methanol extracts of *Triumfetta rhomboidae* by oral route. The standard group received the drug diclofenac 40 mg/kg by oral route. All the suspensions were administered 30 minutes before carrageenan injection (0.1mL of 1%). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer at 0, 1, 2, 3 and 6 hrs.

4.5.8.WOUND HEALING ACTIVITY [111-112]

Excision wound model

The groups were:

Group 1: Control (treated with Simple ointment base (SOB))

Group 2: Standard group treated with povidone iodine ointment

Group 3: Treated with 100mg/kg of TRM

Group 4: Treated with 200mg/kg of TRM

Group 5: Treated with 10mg/kg of TRF

Group 6: Treated with 20mg/kg of TRF

The animals in each group were anaesthetized by the open mask method with anesthetic ether. Depilations of the rats were done on the dorsal side. The excision wound was inflicted by cutting away a 100 mm^2 full thickness of skin from

predetermined shaved area Excision wound was left undressed. Topical application of the drugs to the divided group's viz. SOB, Reference Standard (Povidone Iodine ointment), respectively, were done until the wound heals completely. Wound contraction was monitored.

5. RESULTS AND ANALYSIS

5.1. EXTRACTION

The percentage yield of successive extractive values for leaves of ***Triumfetta Rhomboidea*** is tabulated in **Table 1**

Table 1

The percentage yield of successive extracts of the leaves of *Triumfetta Rhomboidea*

S.No	Name of the Extract	Colour of the extract	Physical nature	Percentage yield (w/w)
1.	**n-Hexane**	Green/Sticky mass	Waxy Semisolid	1.9
2.	**Chloroform**	Green/Sticky mass	Semisolid	2.1
3.	**Ethyl Acetate**	Brownish green solid	Solid	3.3
4.	**Methanol**	Brownish green solid	Solid	7.7

In the phytochemical analysis different polarity of phytoconstituents were sorted out from the powdered leaves of ***Triumfetta rhomboidea*** by using solvents like n-

hexane, chloroform, ethyl acetate and methanol by successive extraction using soxhlet apparatus. Successive extractive values revealed the solubility and polarity particulars of the metabolites in the plant. Methanolic extract showed high extractive yield of 7.7% w/w when compared to other extract of ***Triumfetta rhomboidea***

5.2. PHYTOCHEMICAL EVALUATION

Qualitative chemical analysis of phytoconstituents of the leaf extracts of

Triumfetta rhomboidea tabulated in **Table 2**

Table 2

S.NO	TEST	n-Hexane	Chloroform	Ethyl acetate	Methanol
1.	Alkaloids	**-**	-	**-**	**+**
2.	Carbohydrate	**-**	-	**+**	**+**
3.	Glycosides	**-**	-	**+**	**+**
4.	Phytosterol	**-**	-	**-**	**-**
5.	Fixed oils and Fats	**-**	-	-	**-**
6.	Tannins	**-**	-	**-**	**-**
7.	Phenols	**-**	-	**-**	**+**
8.	Proteins	**-**	-	**+**	**+**
9.	Gums and Mucilages	**-**	-	-	**-**
10.	Flavonoids	**-**	-	**-**	**+**
11.	Terpenoids	**-**	-	**+**	**+**
12.	Steroids	**+**	**+**	-	**-**
13.	Saponins	**-**	-	**+**	**+**

Phytochemical screening of *Triumfetta rhomboidea*

Note: + ve indicates positive result, whereas – ve indicates negative result

Qualitative preliminary phytochemical analysis of ***Triumfetta rhomboidea*** was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract. n-Hexane and chloroform extracts showed the presence of steroids. Ethyl acetate extract was found to contain terpenoids, glycosides, carbohydrates, proteins and saponins. Methanolic extract showed the presence of Alkaloids, terpenoids, carbohydrates, flavonoids, phenols, saponins, proteins and glycosides.

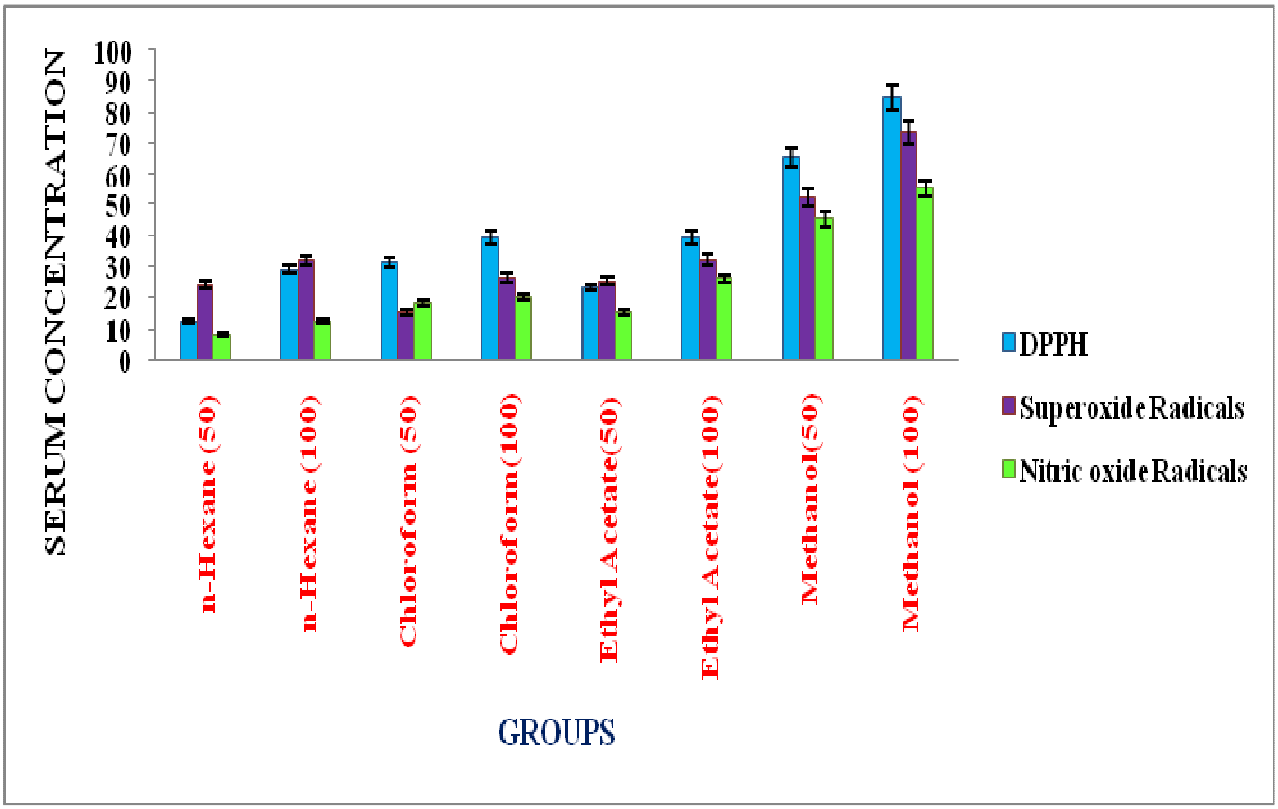
5.3. INVITRO FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of various extracts of ***Triumfetta Rhomboidea*** were detected. The methanol extract of ***Triumfetta Rhomboidea*** showed the dose dependent free radical scavenging activity in all in vitro assay models. Free radical scavenging activity of various extract of ***Triumfetta Rhomboidea*** was tabulated in **table 3 and Figure 5**

Table 3
Free radical scavenging activity of various extracts of *Triumfetta Rhomboidea*

S. No	Name of the Extract	Concentration Mcg/ml	Scavenging activity (%)		
			DPPH	Superoxide Radicals	Nitric oxide Radicals
1	n-Hexane	50	13.14±0.12	25.18±0.15	09.14±0.21
2	n-Hexane	100	28.11±0.31	33.01±0.23	14.12±0.13
3	Chloroform	50	31.14±0.14	19.12±0.24	19.23±0.26
4	Chloroform	100	38.32±0.28	28.21±0.14	21.12±0.09
5	Ethyl Acetate	50	26.04±0.22	28.12±0.14	16.12±0.15
6	Ethyl Acetate	100	41.31±0.22	33.13±0.18	27.12±0.08
7	Methanol	50	64.43±0.24	51.18±0.27	48.12±0.19
8	Methanol	100	85.69±0.21	71.33±0.21	55.09±0.12

Figure 5: Free radical scavenging activity of various extracts of *Triumfetta Rhomboidea*



5.4. ACUTE TOXICITY OF TRIUMFETTA RHOMBOIDEA

Table 4

Acute toxicity of methanol extract of *Triumfetta Rhomboidea*

S.No	Dose (mg/kg/b.wt)	Sex	No of rats per dose	Weight of rat (gm)	Symptoms of toxicity
1	0	Male/ Female	6	150	Nil
2	5	Male/ Female	6	150	Nil
3	50	Male/ Female	6	150	Nil
4	300	Male/ Female	6	150	Nil
5	2000	Male/ Female	6	150	Nil

Table 5

Behavioural Responses of Acute toxicity of *Triumfetta Rhomboidea*

S.No	PARAMETERS	OBSERVATION
1.	Locomotor activity	Normal
2.	Gait	Normal
3.	Eyelid closure	Normal
4.	Effect of Auditory Stimulus	Normal
5.	Effect of tactile stimulus	Normal
6.	Abnormal secretion	Normal
7.	Alertness	Normal
8.	Body position	Normal
9.	Lighting reflex	Normal
10.	Lacrimation	Normal
11.	Urination/Defecation	Normal
12.	Piloerection	Normal

5.5 PHARMACOLOGICAL SCREENING OF 7,12 DIMETHYL BENZ (a) ANTHRACENE INDUCED BREAST CANCER

Table 6

Incidence of mammary tumor in control and experimental rats in each group

Groups	Treatment	Tumour incidence	Total no of Tumours	Tumour Volume
I	Control	0	0	0
II	DMBA	100%	10/10	15.68±1.2
III	DMBA + TRM (100mg/kg)	60%	06/10	2.14±0.12
IV	DMBA+TRM (200mg/kg)	30%	03/10	1.09±0.07
V	STD Tamoxifen 5mg/kg	30%	03/10	1.09±0.06

Figure 6

Tumour Incidence

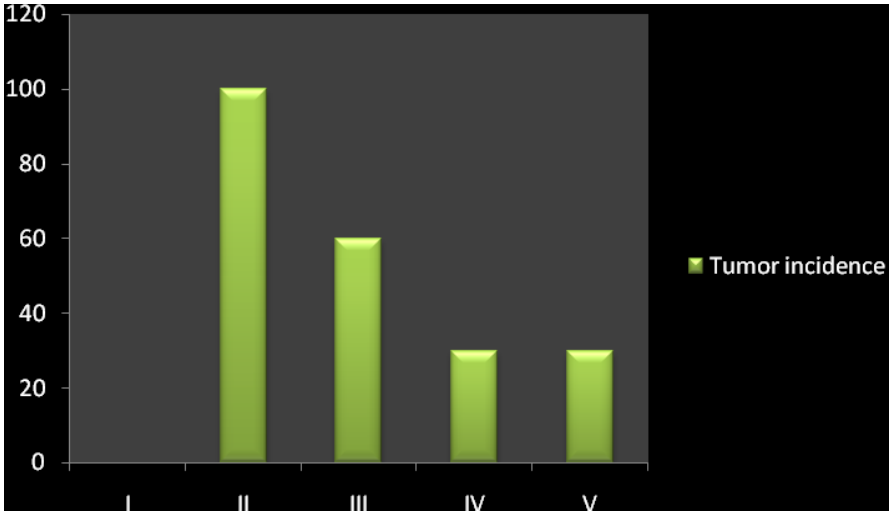


Figure 7

Total Number of Tumours

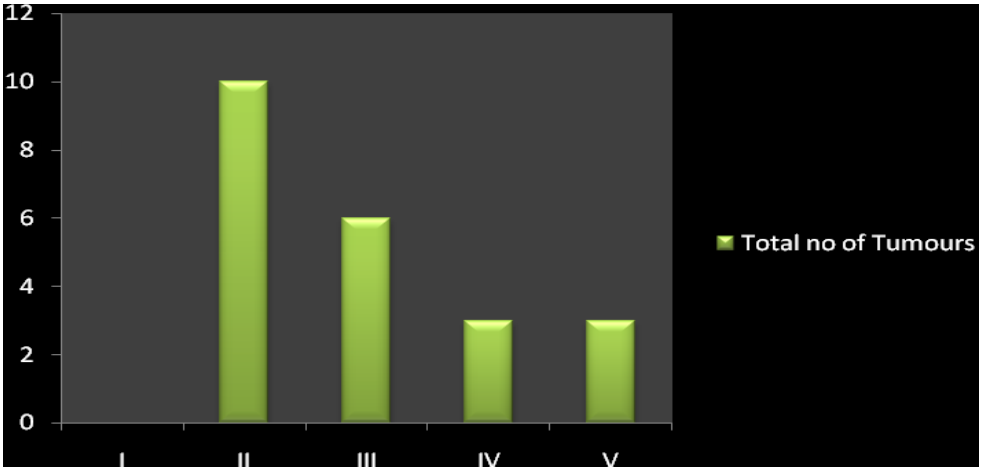


Figure 8

Tumour Volume

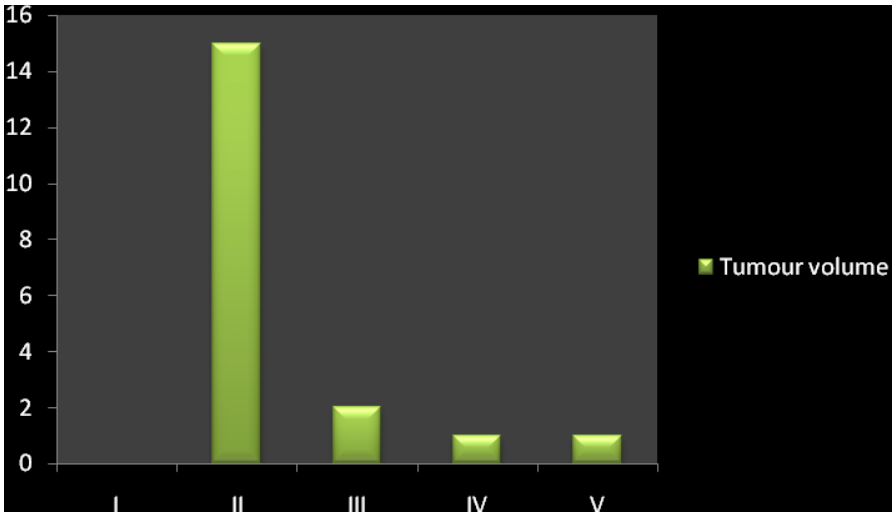


Table 7

Effect of plasma 17-β estradiol (E2) in control and experimental rats in each group

Groups	17-β estradiol (pg/ml)
Control	45.1±0.12
DMBA	58.3±0.34
DMBA+TRM(100mg/kg)	51.1±0.16
DMBA+TRM(200mg/kg)	48.2±0.21
STD Tamoxifen (5mg/kg)	47.1±0.25

Figure 9

Effect of plasma 17-β estradiol (E2) in control and experimental rats in each group

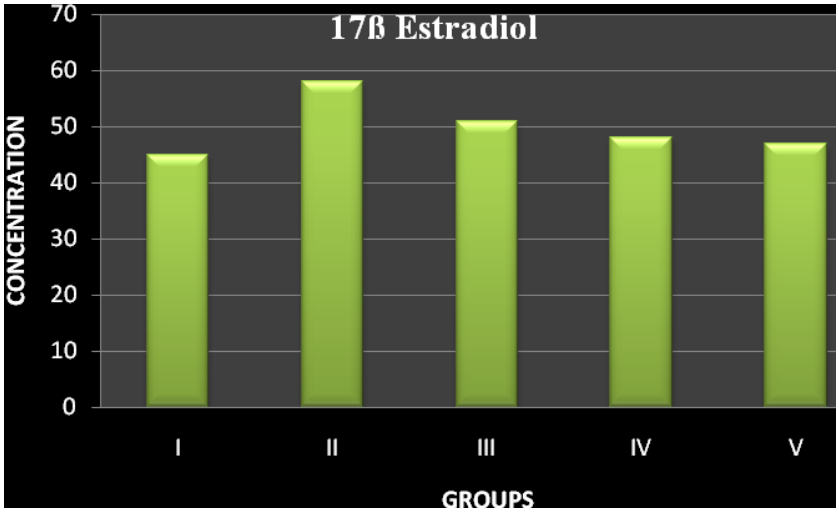


Table 8

Effect of plasma TBARS and antioxidants in control and experimental rats in each groups

Groups	TBARS (nmols/ml)	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)	GSH (mg/dl)
Control	1.84±0.12	2.89±0.01	1.59±0.11	154±0.09	31±0.12
DMBA	3.45±0.21	1.73±0.05	1.09±0.24	109±0.04	19±0.16
DMBA+TRM(100mg/kg)	2.59±0.09	2.31±0.09	1.21±0.32	139±0.07	24±0.09
DMBA+TRM(200mg/kg)	1.34±0.11	2.74±0.12	1.45±0.19	146±0.03	29±0.20
STD Tamoxifen (5mg/kg)	1.64±0.12	2.84±0.10	1.55±0.25	148±0.02	29±0.11

Figure 10

Effect of plasma TBARS,SOD and CAT in control and experimental rats in each groups

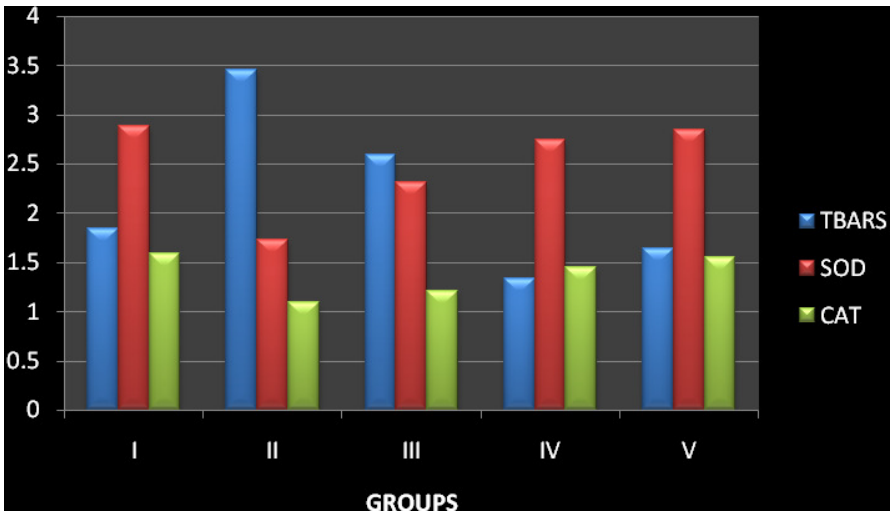


Figure 11

Effect of plasma GPx and GSH in control and experimental rats in each groups

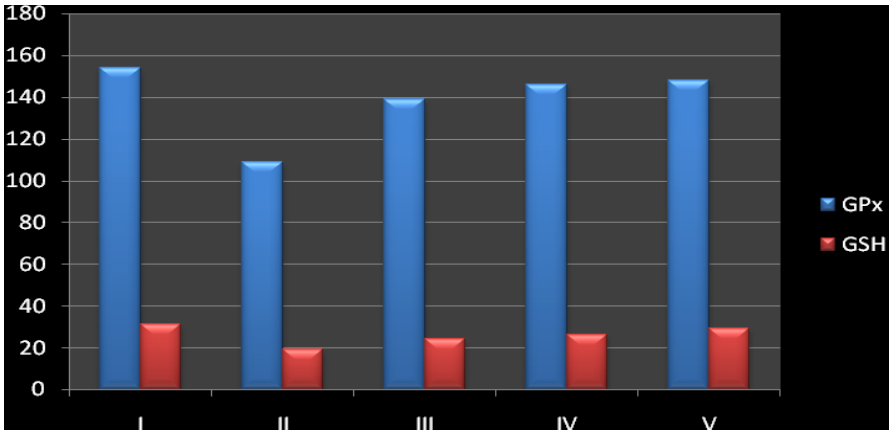


Table 9

Effect of mammary tissues TBARS and antioxidants in control and experimental rats in each group

Groups	TBARS (nmols/100g of tissue)	SOD (U/mg of protein)	CAT (U/mg of protein)	GPx (U/mg of protein)	GSH (mg/100mg of tissue)
Control	0.74±0.11	12.69±0.21	41.59±0.10	18±0.19	08±0.32
DMBA	1.15±0.31	07.30±0.05	28.09±0.24	29±0.08	13±0.46
DMBA+TRM(100mg/kg)	0.99±0.19	10.31±0.19	35.01±0.12	21±0.17	11±0.69
DMBA+TRM(200mg/kg)	0.78±0.12	12.74±0.20	39.25±0.14	19±0.13	09±0.42
STD Tamoxifen(5mg/kg)	0.77±0.14	12.64±0.10	40.15±0.12	18±0.17	08±0.52

Figure 12

Effect of mammary tissues SOD,CAT,GPx and GSH in control and experimental rats

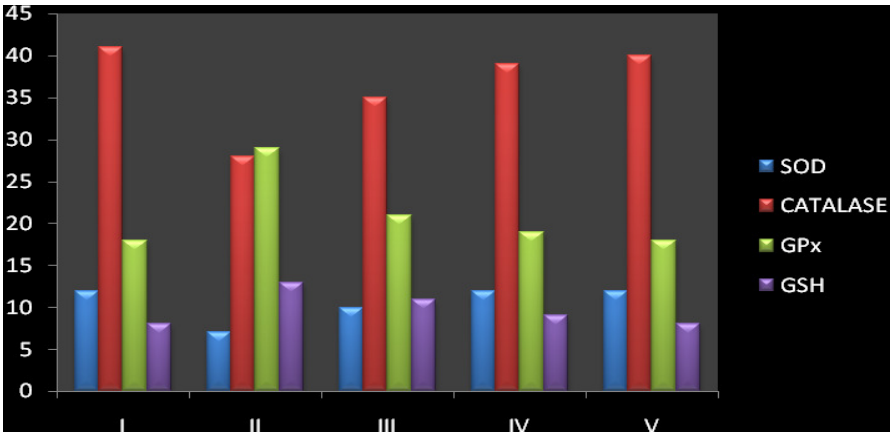
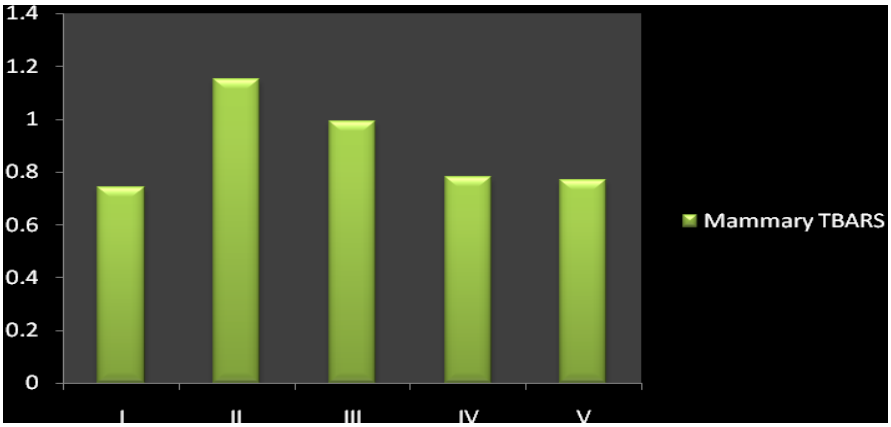


Figure 13

Effect of mammary tissues TBARS in control and experimental rats



HISTOPATHOLOGY

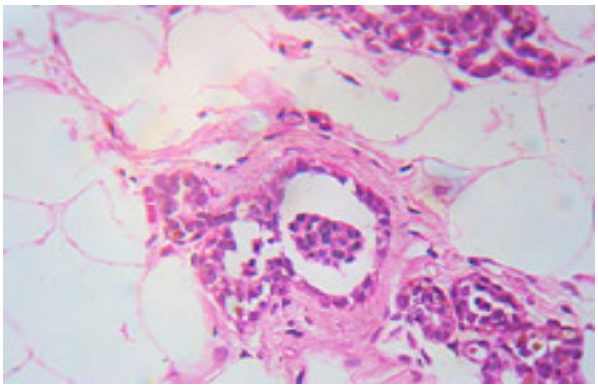


Fig 14 : Control Rat shows normal ductal epithelium

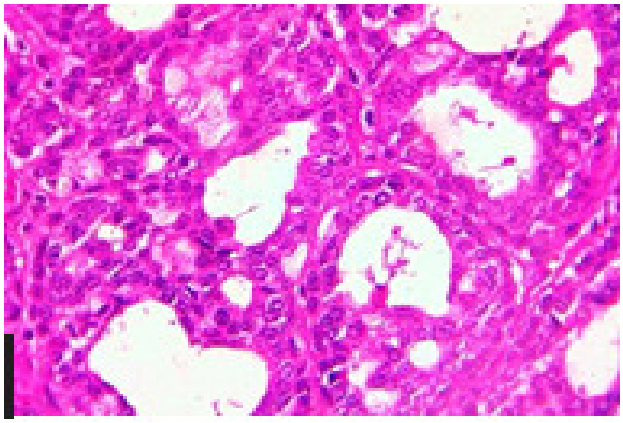


Fig 15: DMBA induced rats showing ductal carcinoma and abnormal cellular proliferation

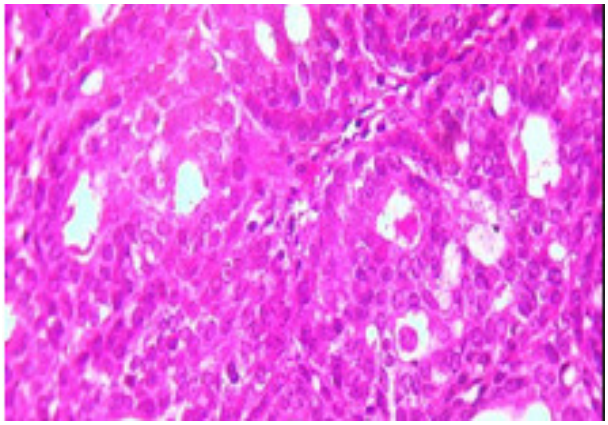


Fig:16 DMBA+TRM(200mg/kg) rats showing ductal dysplasia

FIGURE 17 & 18

The gross appearance of the mammary adenocarcinoma in DMBA(Figure 17) and DMBA+ TRM (200mg/kg) (Figure 18) treated female Sprague-Dawley rats.

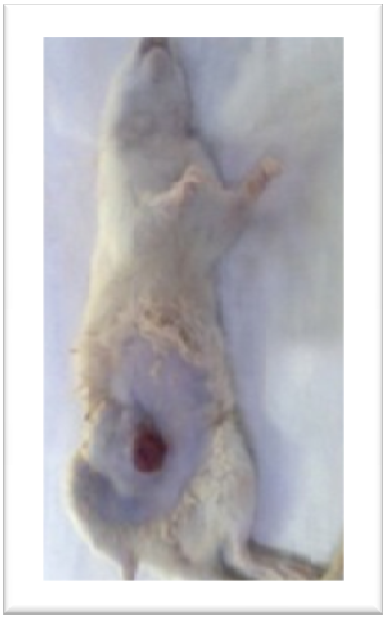


FIGURE 17 **FIGURE 18**

Table 6 shows the incidence of mammary tumors in DMBA, DMBA+TRM 100mg/kg DMBA+TRM 200mg/kg and Standard Tamoxifen treated rats. The present study observed 100% tumor incidence in rats treated with DMBA alone. The tumors were histopathologically confirmed as moderately and poorly differentiated adenocarcinoma. Oral administration of TRM 100mg/kg, 200mg/kg and Tamoxifen to DMBA-treated rats reduced the tumor incidence (40% , 70% and 70%, respectively) and tumors in this group (60% , 30% and 30%) (**Figure 6**) were histopathologically confirmed as well-

differentiated adenocarcinoma. The size of the tumor and tumor volume observed in DMBA+ 200mg/kg and Tamoxifen treated rats were very small as compared to rats treated with DMBA alone (**Figures 7&8**) . **Figure 17 and 18** show the gross appearance of mammary tumors in DMBA treated and DMBA +TRM 200mg/kg treated rats and **(Figures 14-16**) show histological features in Control ,DMBA, and DMBA+200mg/kg treated rats.

Control Rat shows normal ductal epithelium(**Figure 14**) DMBA alone treated rats showing ductal carcinoma and abnormal cellular proliferation(**Figure 15**) TRM 200mg/kg treated rats showed ductal dysplasia(**Figure 16**)

The level of plasma E2 was significantly increased in DMBA treated rats as compared to control rats (**Table 7 & Figure 9**). Oral administration of TRM 200mg/kg to DMBA-treated rats as well as control rats significantly ($p<0.05$) decreased the level of E2 which is comparable with standard.

The levels of TBARS were significantly increased whereas GSH content and activities of SOD, CAT and Gpx in plasma were decreased in DMBA treated rats as compared to control rats (Table 8). Oral administration of 200mg/kg of TRM to DMBA-treated rats significantly ($p<0.05$) decreased the levels of TBARS and improved the levels of GSH and activities of SOD, CAT and GPx. The activity of 200mg/kg is equal to stanadard drug (**Table 8 and Figures 10 & 11**)

The levels of TBARS, GSH and activity of GPx were increased whereas the activities of SOD and CAT were decreased in the tumor tissues as compared to normal tissues of control rats (Table 9). Oral administration of 200mg/kg of TRM to DMBA-treated rats significantly ($p<0.05$) restored the status to near normal which is comparable with stamdard..(**Table 9 and Figures 12 & 13**)

5.6. EAC INDUCED ASCITES TUMOR MODEL SYSTEM

Table 10

Effect of TRM treatment on average life span of ascites tumour bearing mice

Groups	Treatment	Mean survival days	% ILS
I	Control	19.28±1.79	-
II	Standard(Cyclophosphamide)	27.42±0.97	42.22
III	TRM (100mg/kg)	22.14±1.06	14.83
IV	TRM (200mg/kg)	24.5±1.64	27.04

Figure 19: Mean Survival Days

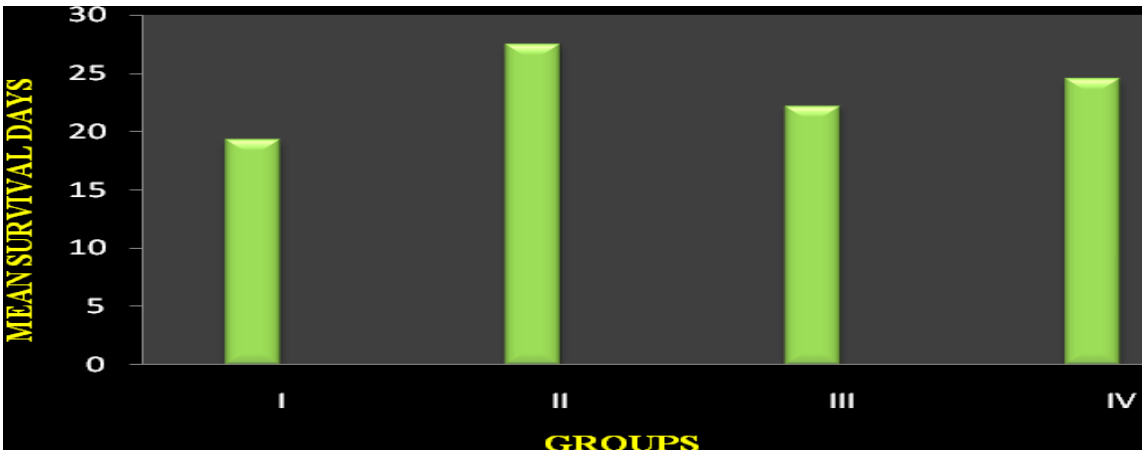
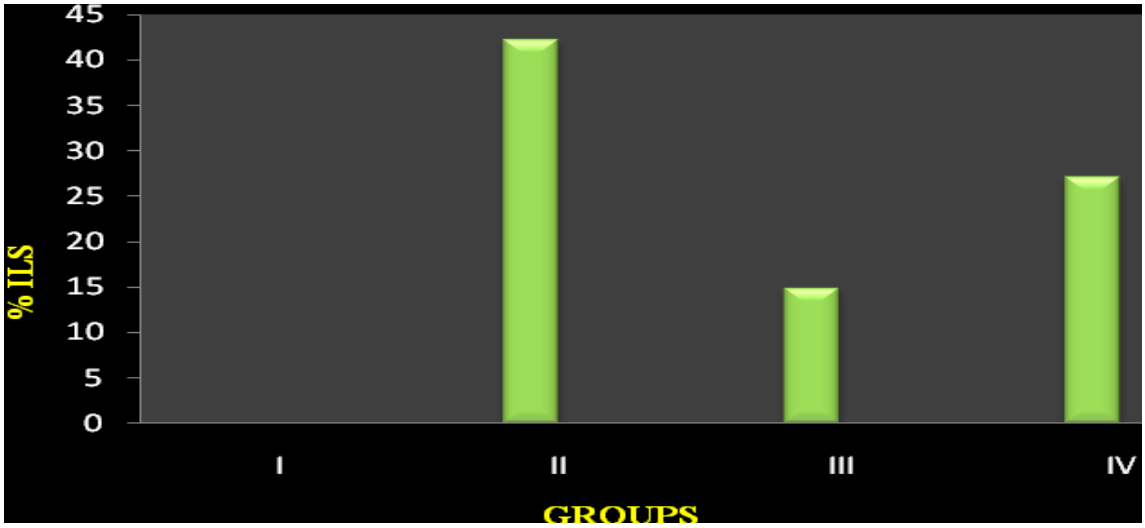


Figure 20 : Percentage increase in life span (% ILS)



Effect of TRM treatment on average life span of ascites tumour bearing mice

The animals of the tumor control group inoculated with EAC cells survived for a period of 19.28± 1.79 days. The animals treated with cyclophosphamide survived for 27.42± 0.97days. The *TRM* at 100 mg/kg body weight slightly increased the average life span of animals by 22.14±1.06 days. But treatment at 200 mg/kg body weight was found to be more inhibiting the proliferation of EAC cells with the percentage increase in life span by 27.04 % and the average life span is 24.5±1.64 days However, in cyclophosphamide treated mice (10 mg/kg), the percentage increase in life span was found to be the highest (42.22 %) (**Table 10 and Figures 19 & 20**)

5.7. DLA INDUCED SOLID TUMOR MODEL SYSTEM

Table 11

Effect of TRM treatment on average solid tumour volume

Groups	Tumor volume on 30 th day (cm^3)	% Inhibition
Control	1.639±0.125	-
Standard	0.412±.006	74.86%
TRM(100mg/kg)	0.823±0.085	49.78%
TRM(200mg/kg)	0.564±0.025	65.58%

Figure 21

Tumour volume on 30th day

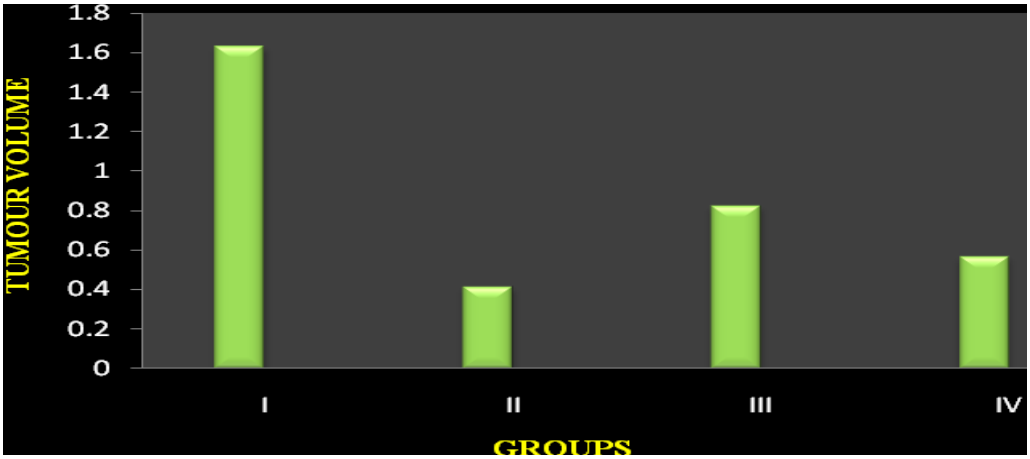
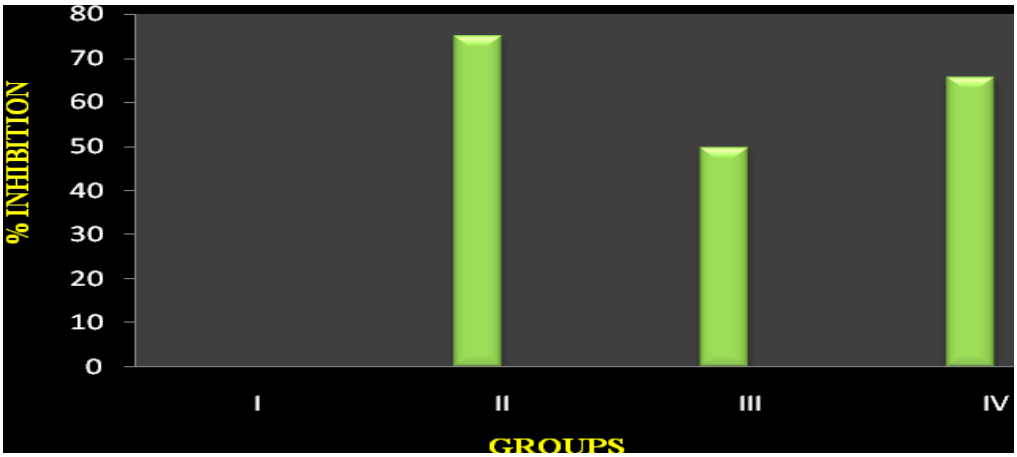


Figure 22

Percentage Inhibition



Effect of TRM treatment on average solid tumour volume

The animals injected with DLA cell lines alone showed marked increase in tumor volume on the 30th day of inoculation in control group. The tumor volume in the mice treated with TRM extract at a dose of 200 mg/kg b.wt on the 30th day of inoculation decreased significantly by 65.58%. The tumor volume had decreased by 74.83 % in cyclophosphamide (10mg/kg) treated group. TRM 200mg/kg has produced excellent activity equal to standard group. (**Table 11 and Figures 21 & 22**)

5.8. DOCKING STUDIES FOR BREAST CANCER

Docking studies were performed to identify the interactions of the compounds with breast cancer target receptors (BRCA1) being retrieved from the Protein Data Bank (id 4Y2G), using MVD (version 2013.6.0.1, installed on an Intel Centrino Machine (Intel Corporation, Santa Clara, CA, USA), probably the most accurate predictive tool of binding geometry, today.

All the ligand structures were constructed using Chem 3D ultra 8.0 software, and then these structures were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL mol format (.mol).

The pre downloaded pdb structure of protein was imported to the workspace. Unwanted water molecules, cofactors, constraints and other ligands were removed.

To obtain better potential binding sites in the protein, a maximum of five cavities were detected using parameters such as molecular surface (expanded Vander Waals), maximum number of cavities (n = 5), minimum cavity volume (10), maximum cavity volume (10000), probe size (1.20), maximum number of ray checks (n = 16), minimum number of ray hits (n = 12), and grid resolution (0.30). The chosen cavity was further refined using side-chain minimization by selecting the add-visible option at a maximum of steps per residue (10000) and a maximum of global steps (10000). The setup for side-chain flexibility by selection of the add-visible option, the setting for the selected

flexible side chain residues of maximum 15 during the docking option, and other parameters, all were kept in default.

All docking calculations were carried out using the grid-based Mol Dock score (GRID) function with a grid resolution of 0.30 A˚. The binding site on the receptor was defined as extending in X=2.89, Y=66.53, and Z=67.62 directions around the Dock molecule with a radius of approximately 15.00 A˚. The Mol Dock optimization search algorithm with a maximum of ten runs was used through the calculations, with all other parameters kept as defaults. One pose per run was retained based on root mean square division clustering using a heavy atom threshold set at 1.0 A˚ and an energy penalty of 100. All the poses were examined manually and the best poses were retained.

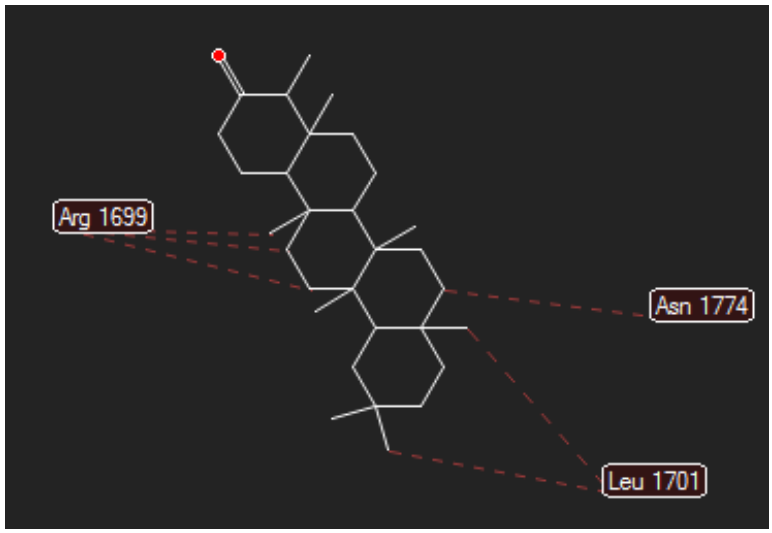


Fig:23 Binding interaction of Friedelin with BRCA 1 protein (4Y2G)

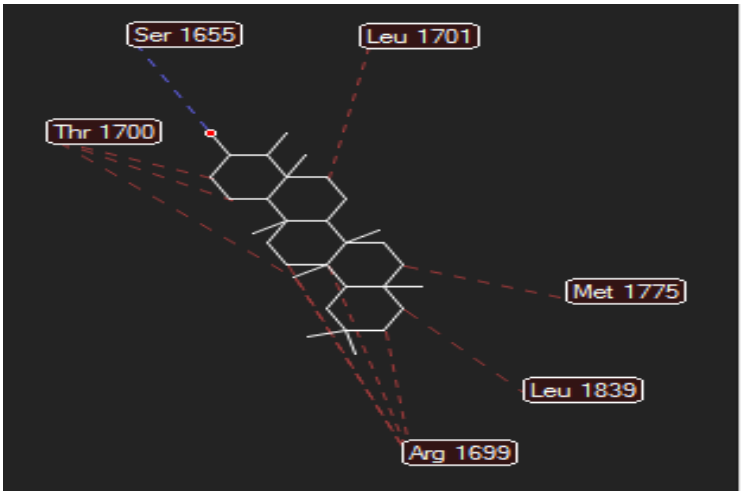


Fig:24 Binding interaction of Friedelinol with BRCA 1 protein (4Y2G)

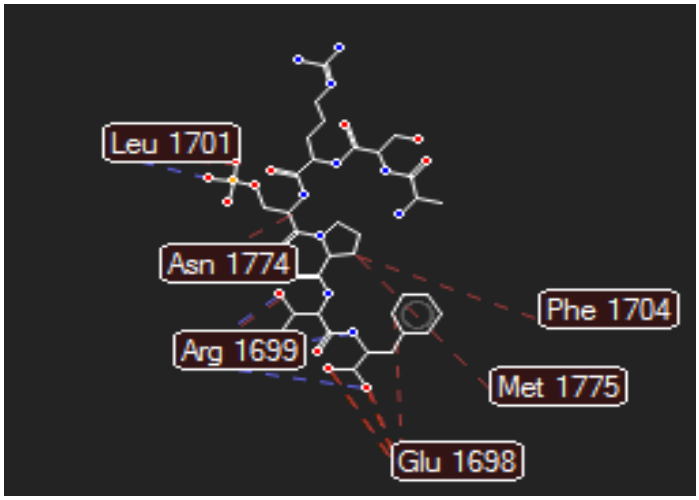


Fig:25 Binding interaction of Co-crystalized ligand with BRCA 1 protein (4Y2G)

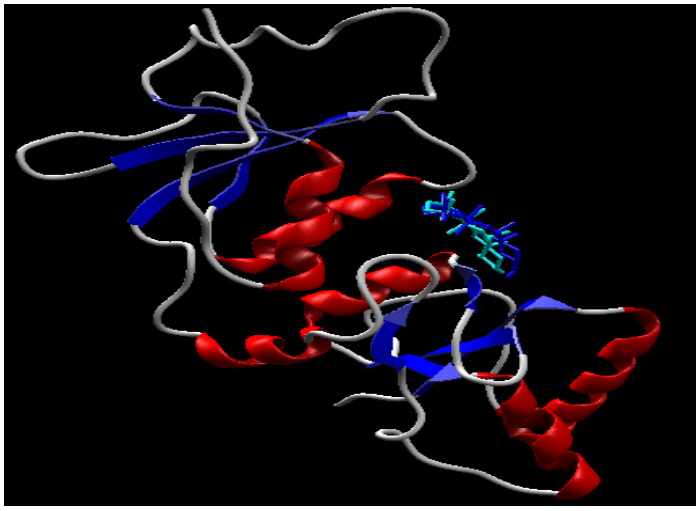


Fig:26 Binding mode of Friedelin and Friedelinol oh the gorge of BRCA 1 protein (4Y2G)

Table:12

Docking results of the compounds with BRCA1 protein (4Y2G)

Name	MolDock Score	Rerank Score	HBond
Co-crystalized ligand	-102.086	-40.001	-0.758
Friedelin	-127.902	-29.632	-2.497
Friedelinol	-130.267	-39.505	-0.752

Name	Interaction with residue
Co-crystalized ligand	Leu 1701, Asn 1774, Arg 1699, Glu 1698, Met 1775, Phe 1704
Friedelin	Arg 1699, Leu 1701, Asn 1774
Friedelinol	Ser 1655, Thr 1700, Leu 1701, Met 1775, Leu 1839, Arg 1699

The newly designed molecules were interacting with the target protein. The MolDock score of the test compounds is comparatively lower, that means the drug-receptor complex is having better binding interaction (the negative values indicating the lower energy conformations).

Between the two compounds, Friedelinol is having the better interaction values due to H-bond formed between the receptor residue (Ser 1655) and compound. Apart from that, the newly docked compounds were interacting with the native binding site of the receptor consisting of residues Arg 1699, Ser 1655, Leu 1701 and Asn 1774. The newly docked polycyclic natural compounds were able to bind with the BRCA1 receptor with more binding interactions (vander wall and steric interactions) than the native ligand (TYR-SER-ARG-SEP-PRO-THR-PHE) present in the crystalized structure of the protein. Results are presented in **Table 12 and Figures 23-26**.

5.9. SCREENING OF CARDIO PROTECTIVE ACTIVITY

Table 13

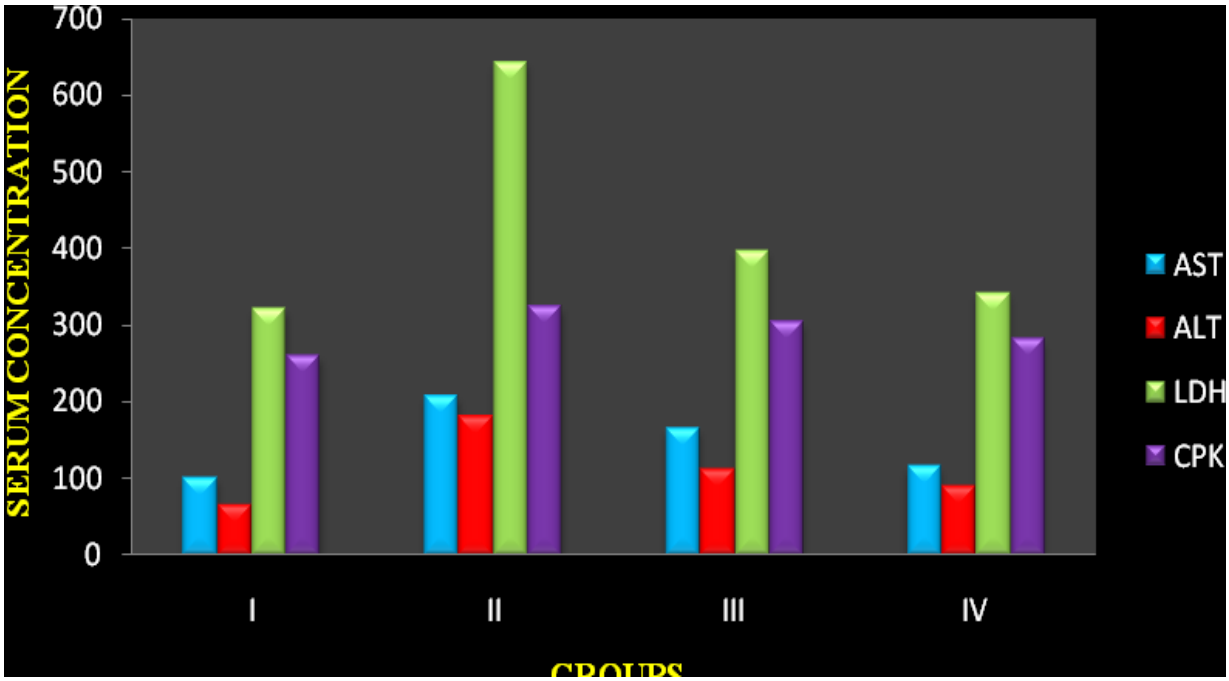
Effect of *Triumfetta rhomboidea* on different biochemical parameters in Isoproterenol induced myocardial infarction in rats.

Groups	Treatment	Aspartate amino transferase (AST)	Alanine amino transferase (ALT)	Lactate dehydrogenase (LDH)	Creatine Phosphokinase (CPK)
I	Control	100.33±3.16	65.42±2.16	324±16	260.07±2.45
II	ISO 200mg/kg	207.01±4.05	180.18±3.03	643.12±3.1	324.57±2.08
III	ISO+TRM (100mg/kg)	165.17±3.63	111.83±3.32	396.18±40	304.05±2.14
IV	ISO+TRM (200mg/kg)	115.12±2.02	89.12±0.03	342.13±1.1	281.37±1.08

Values are mean ± sem of 6 animals in each groups Group II compared with group I. Group III-IV is compared with Group II and I (ONE WAY ANOVA) P<0.001

Figure 27

Effect of *Triumfetta rhomboidea* on different biochemical parameters in Isoproterenol induced myocardial infarction in rats.



Effect of TRM on Isoproterenol induced myocardial infarction in rats

The prior administration of TRM (200 mg /kg) showed significant reduction in ISO induced elevated serum marker enzymes and At a dose of 100mg/kg the effect is only marginal but 200mg/kg effectively prevented Isoproterenol induced cardiac damage (**Table 13 and Figure 27**)

5.10. IN VIVO ANTI-SNAKE VENOM ACTIVITIY

Table 14: In vivo Anti-snake venom activity of the methanolic extract of the leaves of TRM

GROUPS	Treatment	No of animals Survived	% Survival
I	Control	01	16.66
II	Snake Venom Antiserum	06	100
III	TRM 100 mg/kg	03	50
VII	TRM 200mg/kg	05	83.33

Values are mean ± sem of 6 animals in each groups Group II compared with group I. Group III-IV is compared with Group II and I (ONE WAY ANOVA)

Figure 28 : No of Animals Survived in control and extract treated groups

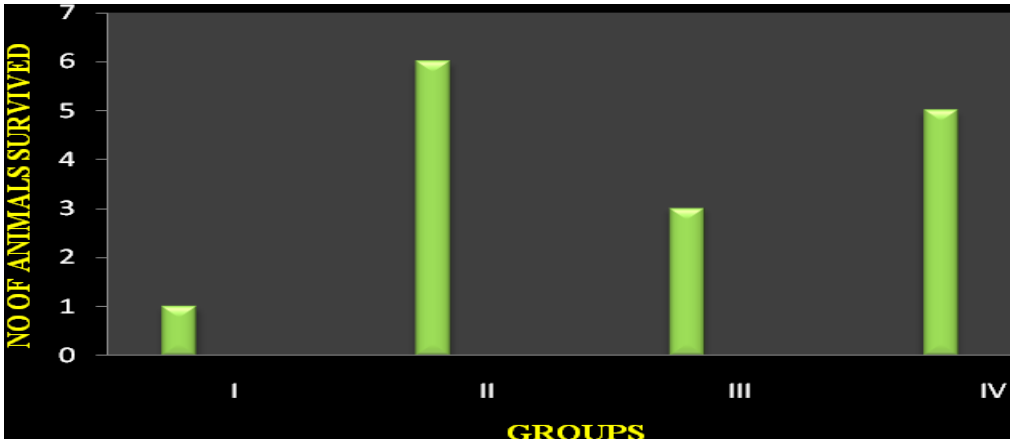
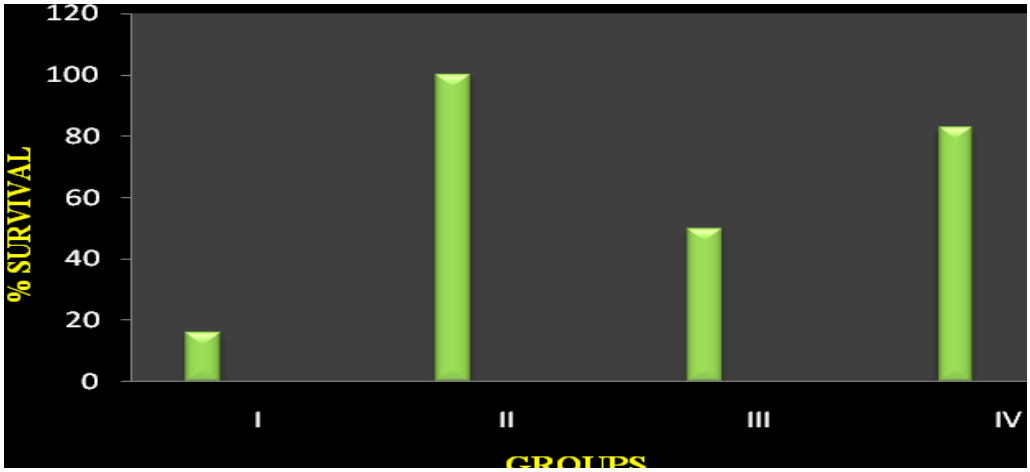


Figure 29 : % Survival in control and extract treated groups



In vivo Anti-snake venom activity of the methanolic extract of the leaves of *Triumfetta rhomboidea*

In-vivo anti-snake venom activity of the methanolic extract of Triumfetta rhomboidea was studied at dose level of 100 and 200 mg/kg body weight respectively. In control group, which was treated with only venom (61mcg/20g) only one mice were survived. Mice treated with 100 mg/kg of methanol extract of TRM recorded 50% survival, 200mg/kg showed 83.33%, and the standard showed 100% survival which is shown in the **Table 14 and Figures 28 &29.**

5.11. ANALGESIC ACTIVITY BY ACETIC ACID INDUCED WRITHING IN MICE

Table 15

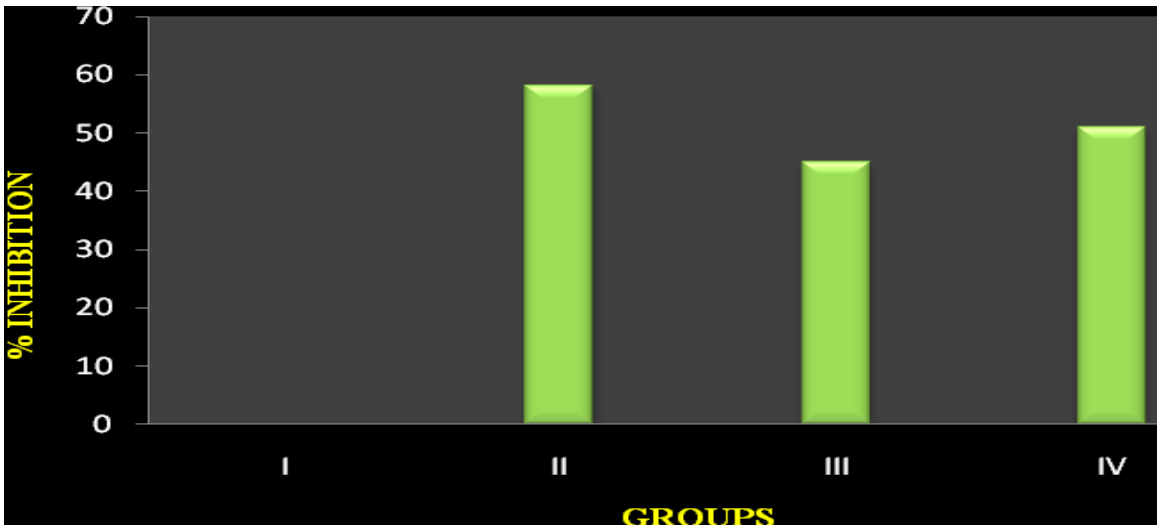
Analgesic activity of the methanolic extract of the leaves of *Triumfetta rhomboidea* by acetic acid induced writhing in mice

GROUPS	DRUG TREATMENT AND DOSE	NO OF WRITHINGS	% INHIIIBITION
I	Control (5ml/kg)	59.32 ±1.69	------
II	Diclofenac (5 mg/kg)	24.52 ±0.45	58.66
III	TRM 100mg/kg	32.45 ±0.85	45.32
IV	TRM 200mg/kg	28.76 ±0.72	51.51

Values are mean ± sem of 6 animals in each groups . Group II compared with group I. Group III-IV is compared with Group II and I (ONE WAY ANOVA) P<0.001

Figure 30

Analgesic activity of the leaves of TRM by acetic acid writhing in mice.



5.11 Analgesic activity of the methanolic extract of the leaves of *Triumfetta rhomboidea* by acetic acid induced writhing in mice

The effect of TRM produced significant reduction in the number of writhing in mice at 200 mg/kg oral dose, the percent reduction of writhing of TRM was 51.51 %, as compared to the control group, whereas the standard drug Diclofenac (5 mg/kg p.o.) showed a reduction of 58.66%.. (**Table 15 and Figure 30)**

5.12. CARRAGEENAN INDUCED PAW EDEMA METHOD

Table 16

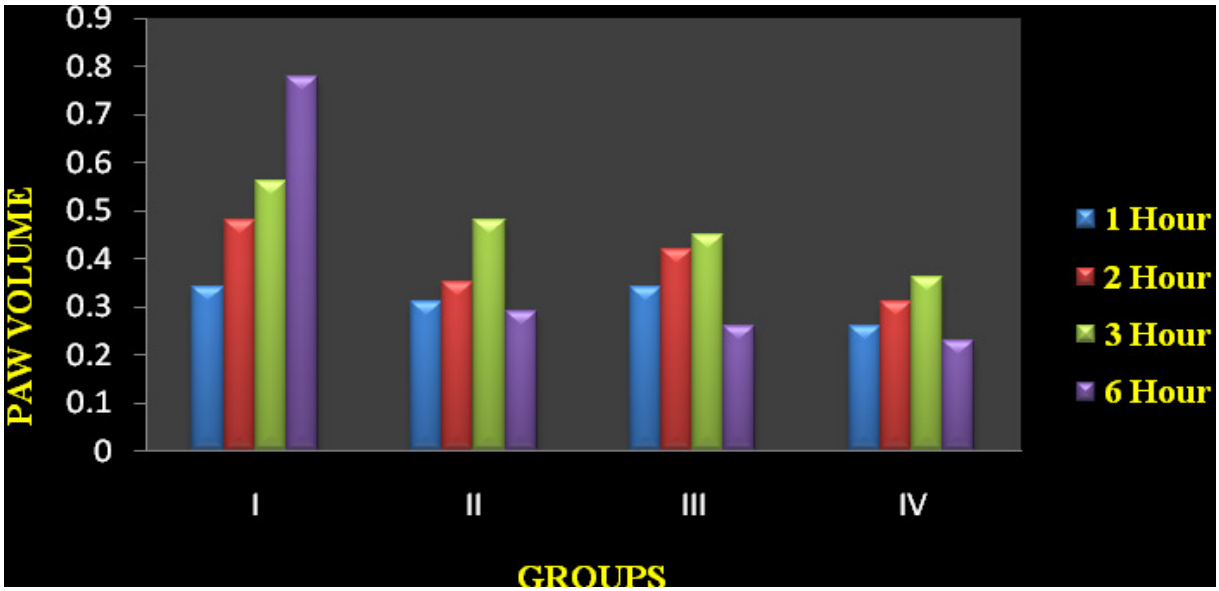
Anti inflammatory activity of *Triumfetta rhomboidea* on Carageenan induced paw edema

Groups	Treatment and Dose	Paw volume Mean ± SEM	Paw volume in ml Mean ± SEM			
			1 hour	2 hour	3 hour	6 hour
I	Control 0.5% tween 80 (0.1ml)	0.25±0.03	0.34±0.01	0.48±0.02	0.56±0.02	0.78±0.03
II	TRM 100mg/kg	0.19±0.03	0.31±0.02	0.35±0,01	0.48±0.03	0.29±0.02
III	TRM 200mg/kg	0.24±0.01	0.34±0.01	0.42±0.02	0.45±0.02	0.26±0.01
IV	Diclofenac sodium 40mg/kg	0.20±0.01	0.26±0.01	0.31±0.01	0.36±0.05	0.23±0.01

Values are expressed as Mean ±Sem for 6 animals in each group .] Group II and III compared with Group I and Group IV.(ONE WAY ANOVA) P<0.001

Figure 31

Anti inflammatory activity of *Triumfetta rhomboidea* on Carageenan induced paw edema in rats



Anti inflammatory activity of *Triumfetta rhomboidea* on carageenan induced paw edema in rats

The carrageenan induced edema develops by release of inflammatory mediators such as histamine, kinin, bradykinin, and prostaglandins .As shown in **Table 16** Diclofenac sodium showed significant inhibition of rat paw edema at 6 hr. The extracts of TRM 100mg/kg and 200mg/kg reduced the inflammation of rat paw edema at 6 hr. At a dose of 200mg/kg effectively reduced inflammation which was comparable with standard(**Figure 31**)

5.13. WOUND HEALING ACTIVITY

Table 17

Effect of of *Triumfetta rhomboidea* treatment on wound contraction (wound excision model)

Groups and Treatment	0 Day	4th Day	8th Day	12th Day	16th Day
Simple ointment base	619±1.02	574±3.12	423±3.09	292±1.65	94±1.33
Povidone iodine ointment	560±2.18	234±2.28	74±2.97	21±2.71	07±2.12
2% w/v of TRM	582±1.07	284±0.98	91±1.17	36±1.56	11±1.09

Figure 32

Effect of of *Triumfetta rhomboidea* treatment on wound contraction (wound excision model)

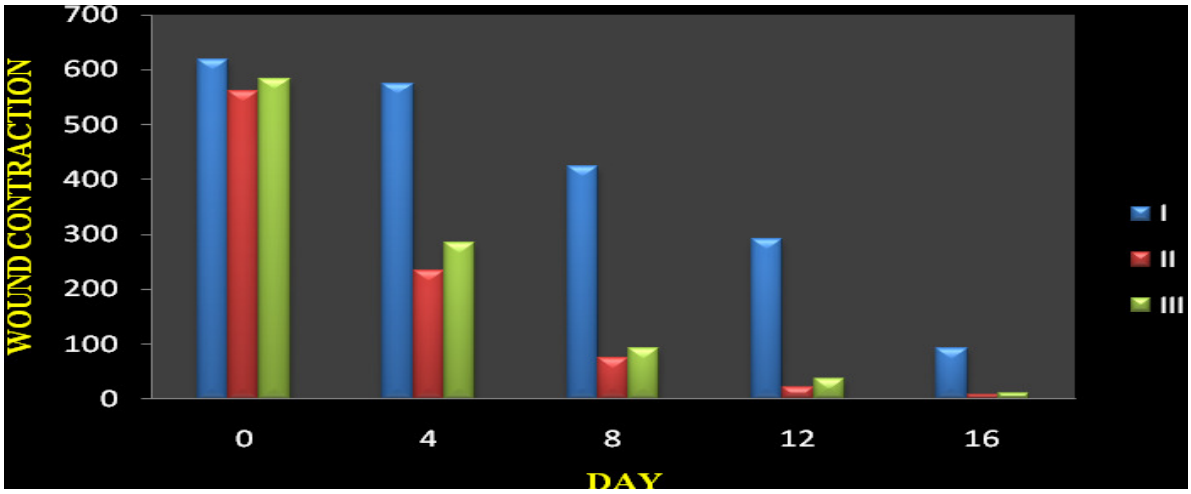
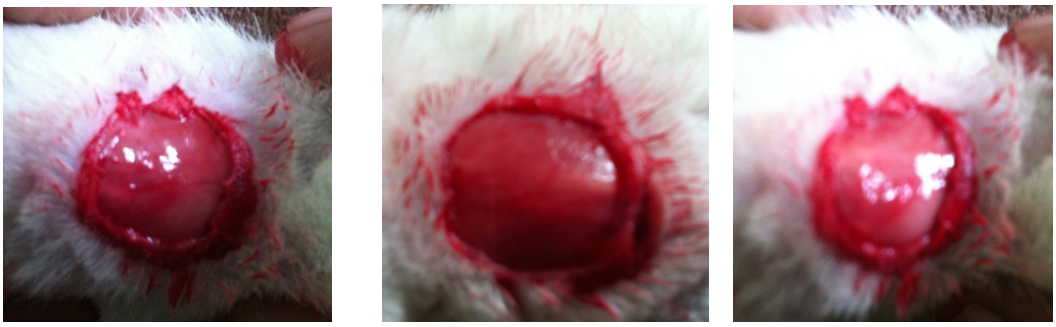


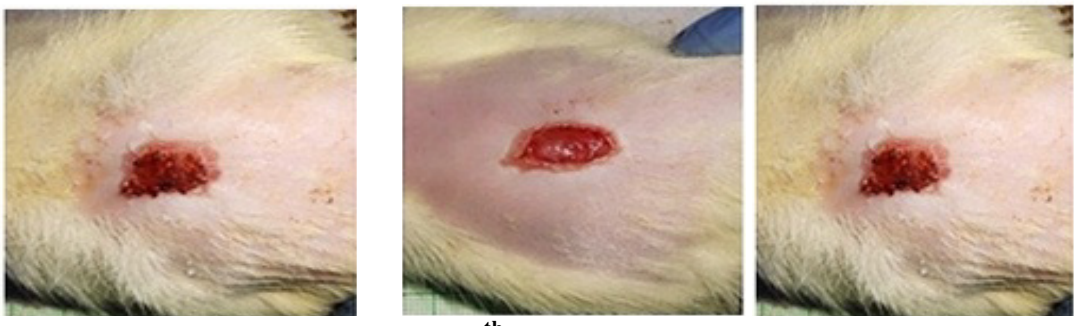
Figure-33

Effect of of *Triumfetta rhomboidea* treatment on wound contraction (wound excision model)

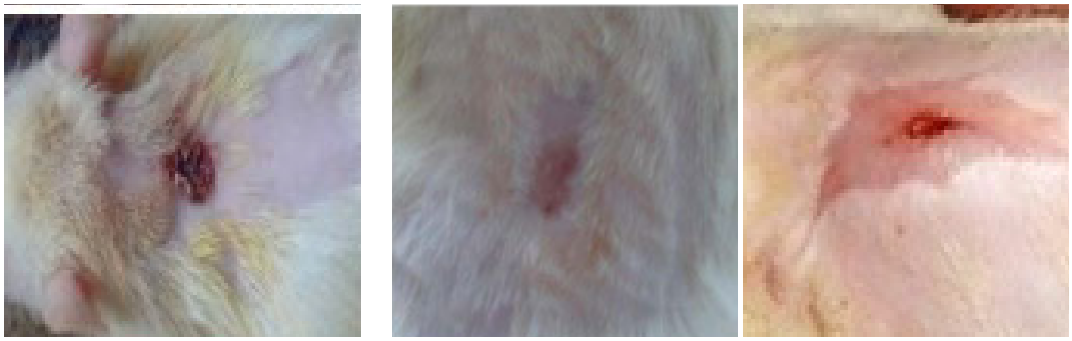
CONTROL **STD** **2% w/v of TRM**



0 day



8th day



16th day

2% w/v of TRM on 16th day (11±1.09) has a positive influence on wound contraction phase of wound healing which is comparable with std Povidone iodine ointment (07±2.12) . Results are presented in **Table 17 and Figures 32 & 33.**

5.14.CHARACTERIZATION OF EFFCTIVE FRACTION OBTAINED FROM TRIUMFETTA RHOMBOIDEA BASED ON INVITRO AND INVIVO PHARMACOLOGICAL ACTIVITY

5.14.1.THIN LAYER CHROMATOGRAPHY

Table 18

The TLC studies of methanol extract of *Triumfetta rhomboidea*

S.No	Name of the Extract	Solvent system	No of spots	R_f Values
1	Methanol (TRM)	Methanol : Ethyl acetate: Water (6:3:1)	04	0.20 0.45 0.52 0.72

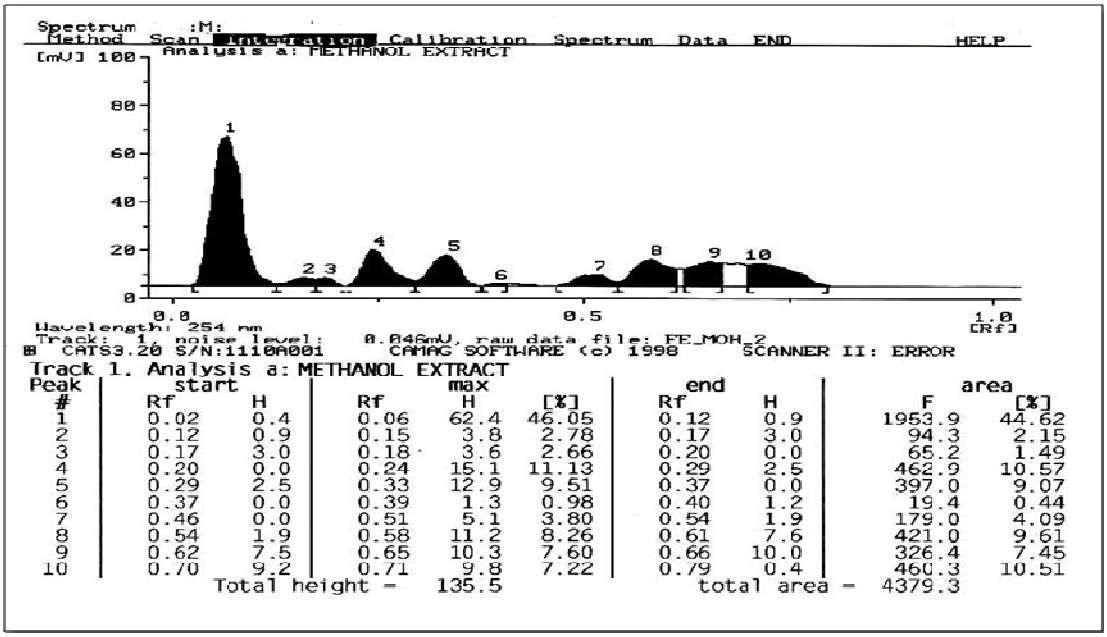
5.14.2.HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Table 19

HPTLC fingerprint of methanol extract of *Triumfetta rhomboidea*

S.No	Name of the Extract	Detection Wavelength	No of spots	R_f Values
1	Methanol (TRM)	254nm	10	0.06 0.15 0.18 0.24 0.33 0.39 0.51 0.58 0.65 0.71

Fig-34. HPTLC fingerprint of methanol extract of *Triumfetta rhomboidea*



Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.02	0.4	0.06	62.4	46.05	0.12	0.9	1953.9	44.62
2	0.12	0.9	0.15	3.8	2.78	0.17	3.0	94.3	2.15
3	0.17	3.0	0.18	3.6	2.66	0.20	0.0	65.2	1.49
4	0.20	0.0	0.24	15.1	11.13	0.29	2.5	462.9	10.57
5	0.29	2.5	0.33	12.9	9.51	0.37	0.0	397.0	9.07
6	0.37	0.0	0.39	1.3	0.98	0.40	1.2	19.4	0.44
7	0.46	0.0	0.51	5.1	3.80	0.54	1.9	179.0	4.09
8	0.54	1.9	0.58	11.2	8.26	0.61	7.6	421.0	9.61
9	0.62	7.5	0.65	10.3	7.60	0.66	10.0	326.4	7.45
10	0.70	9.2	0.71	9.8	7.22	0.79	0.4	460.3	10.51
		Total height =		135.5			total area =	4379.3	

5.15. ISOLATION OF COMPOUNDS FROM *TRIUMFETTA RHOMBOIDEA* BY COLUMN CHROMATOGRAPHY

Table-20

5.15.1.Column Eluted Fractions of *Triumfetta rhomboidea*

List of Column Eluted Fractions of *Triumfetta rhomboidea*			
Fraction No	**Column fractions eluted with following solvent**	**Weight of sample**	**Consistency and color**
1.	Ethyl acetate–hexane (3:2)-	0.176g	Yellow crystal
2.	Ethyl acetate–hexane (3:2)	0.216g	White powder
3.	Benzene–acetone–ethyl acetate (7:5:1)	1g	Yellow needles
4.	Ethyl acetate–*n*-hexane(3:1)	0.074g	Orange needles
5.	Ethyl acetate–hexane (7:3.5)	0.143g	yellow colour needle

Characterization of Compound – I

Yellow needles colour (1g) was obtained which is soluble in methanol and water, the melting point was found to be 210-215^{o} C. The isolated compound answered the test for flavonoids. Spectral data of fraction 3 was depicted in the Figures 35,36,37,38

Fig : 35 Infra red Spectrum of compound I

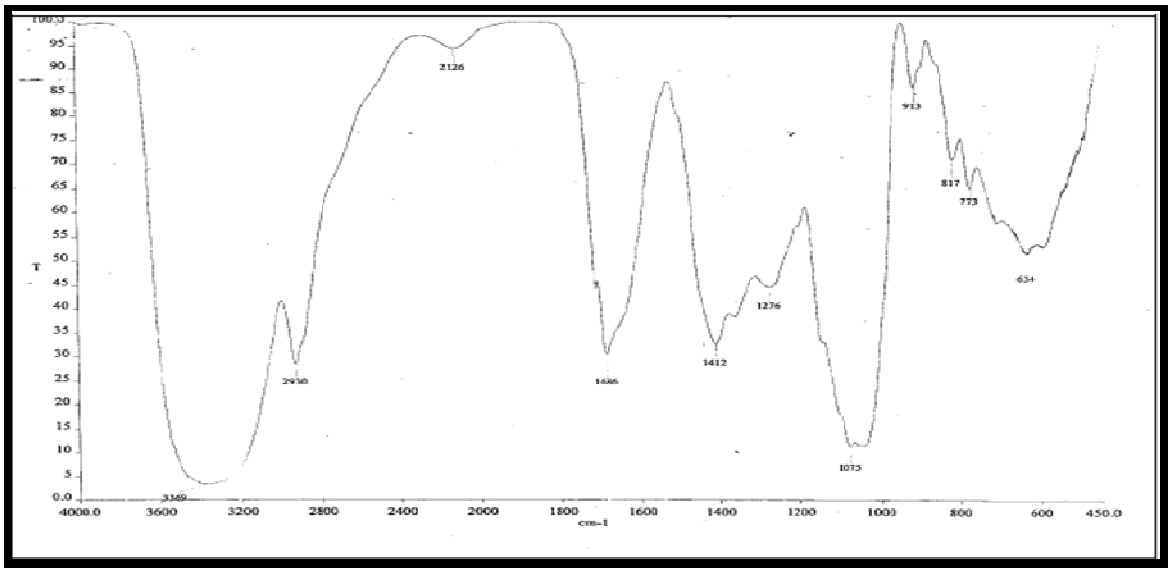


Table-21 Infra red Spectral Data

Wave Numbers (cm-1)	Type
3369	OH
2930	CH3,CH2,CH
1686	C=C
1412	C-O
1276	C-O
1075	C-O
913	C-O
634	(CH)

Fig :36 ^{1}H Nuclear Magnetic Resonance spectral data for Compound 1

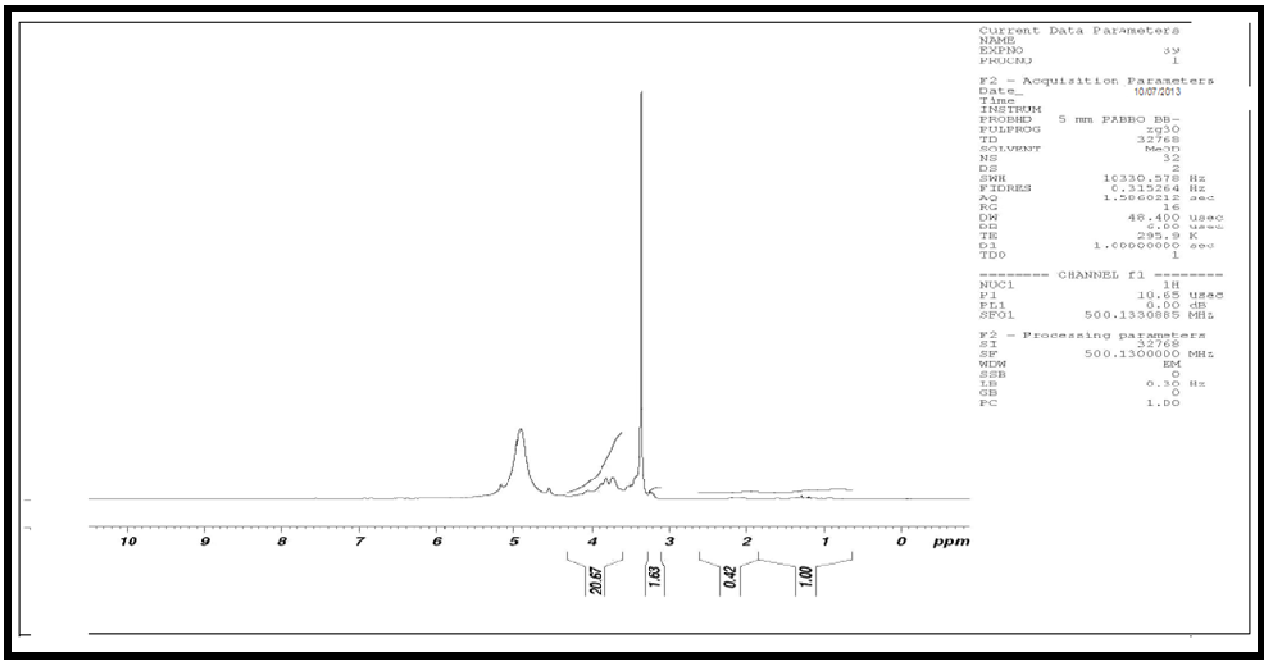


FIG : 37 ^{13}C Nuclear Magnetic Resonance spectral data for Fraction 3 (TRM)

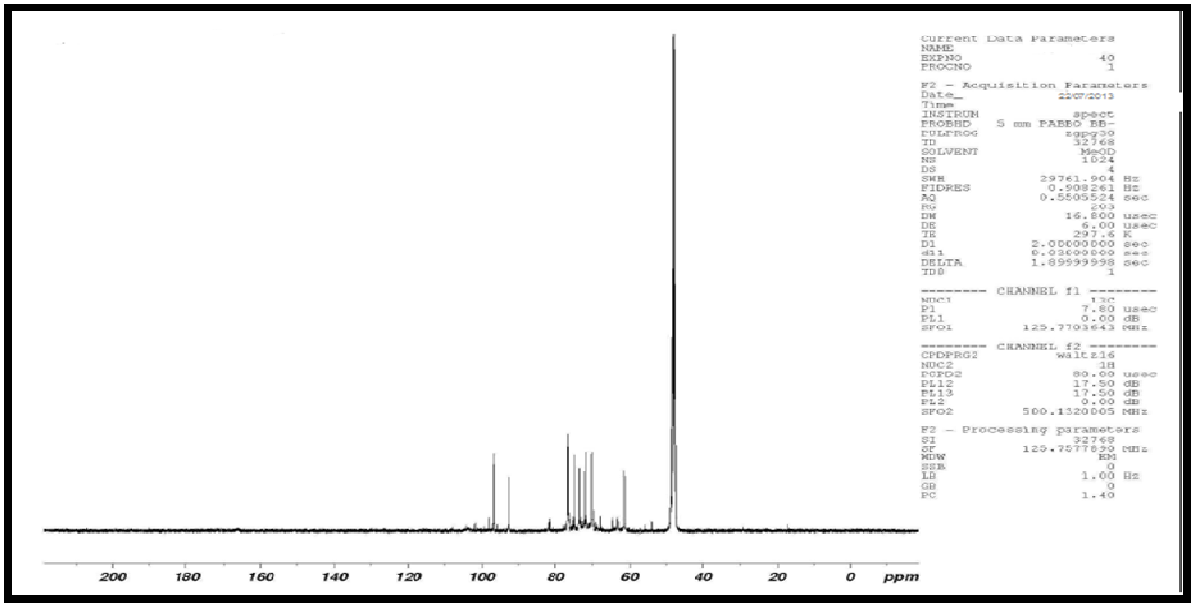
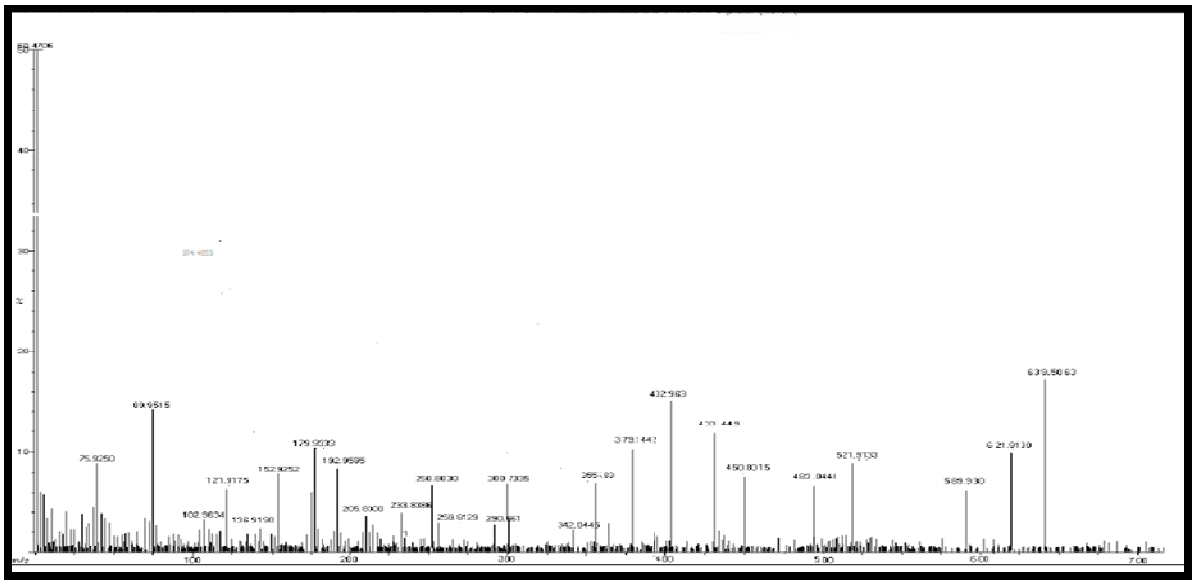


Table-22

HNMR and ^{13}C Nuclear Magnetic Resonance spectral data for Fraction 3 (TRM)

Atom	Carbon, δ , ppm	Hydrogen, δ , ppm
1	73.41	3.36-4.04(m, 1H)
2	73.62	3.36 – 4.04 (m, 1H)
3	101.78	4.91 – 5.17 (m, 1H)
4	64.36	3.36 – 4.04 (m, 2H)
5	75.25	3.36 – 4.04 (m, 1H)
6	71.82	3.36 – 4.04 (m, 1H)
7	72.40	3.36 – 4.04 (m, 1H)
8	76.46	3.36 – 4.04 (m, 1H)
9	97.98	4.91 – 5.17 (m, 1H)
10	71.62	3.36 – 4.04 (m, 1H)
11	76.52	3.36 – 4.04 (m, 1H)
12	61.35	3.36 – 4.04 (m, 2H)

Fig 38 Mass spectrum for Fraction 3 (TRM)



Properties of the Isolated compound

Name - Rhamnetin-3-O- β-D-Galactosyl-4- O-β -D-galactopyranoside

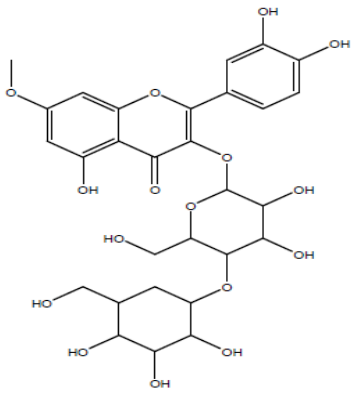
Molecular formula - $C_{28}H_{32}O_{17}$

Molecular weight -640

Mass (m/z) - 639(M)+

Colour -Yellow needles

Structure of the isolated compound



Structure of the fraction 3 (TRM)

6. DISCUSSION

Phytochemical evaluation

Methanolic extract showed high extractive yield of 8.5 %w/w when compared to other extracts of *Triumfetta rhomboidea*. Preliminary phytochemical analysis of *Triumfetta rhomboidea* was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract. Methanolic extract of *Triumfetta rhomboidea* showed the presence of alkaloids, terpenoids, carbohydrates, flavonoids, phenols, saponins, proteins and glycosides.

In Phytochemical evaluation various extracts were prepared and studied for qualitative chemical analysis such as TLC and HPTLC finger print analysis. Qualitative chromatographic analysis (TLC) was performed for the identification of different components in the extracts qualitatively. The HPTLC finger print of methanol extracts of these plants were also studied. HPTLC was scanned at 254nm with the best solvent to detect the maximum number of components and peak abundance qualitatively. HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents.

Invitro free-radical scavenging activity

The therapeutically active extract to carry out the pharmacological studies in animals was selected on the basis of in- vitro studies like in-vitro free radical scavenging activity and phytochemical analysis. Among the four extract the methanolic extract showed significant activity in both plants. The methanol extract of *Triumfetta rhomboidea* showed the dose dependent free radical scavenging activity in all in vitro

assay models. Hence it was selected for the screening of in-vivo pharmacological studies. Pharmacological activity dealt with the screening of acute toxicity study, Chemopreventive, Cardioprotective, Analgesic, Anti-inflammatory, Wound healing activity, Anti-tumor activity and Anti-snake venom activities of methanolic extract of the leaves of *Triumfetta rhomboidea* .

Acute toxicity study

Acute toxicity studies performed on methanol extract of *Triumfetta rhomboidea* (according to OECD guidelines 423). The methanolic extract of *Triumfetta rhomboidea* was found to be very safe up to 2000mg/kg body weight. All the animals were found to be normal and there were no gross behavioral changes till the end of the observation period. Hence from this 1/20th and 1/10th of MTD was selected and the effective doses were fixed as 100 and 200 mg/kg for the further pharmacological studies.

Chemopreventive activity

The present study observed 100% tumor incidence in rats treated with DMBA alone. The tumors were histopathologically confirmed as moderately and poorly differentiated adenocarcinoma. Oral administration of TRM 100mg/kg and 200mg/kg to DMBA-treated rats reduced the tumor incidence and tumors in this group. Histopathologically control Rat shows normal ductal epithelium. DMBA alone treated rats showing ductal carcinoma and abnormal cellular proliferation and TRM 200mg/kg treated rats showed ductal dysplasia.The size of the tumor and tumor volume observed in DMBA+ 200mg/kg treated rats were very small as compared to rats treated with

DMBA alone .Oral administration of TRM 200mg/kg to DMBA-treated rats as well as control rats significantly ($p<0.05$) decreased the level of E2.

The levels of TBARS were significantly increased whereas GSH content and activities of SOD, CAT and Gpx in plasma were decreased in DMBA treated rats as compared to control rats Oral administration of 200mg/kg of TRM to DMBA-treated rats significantly ($p<0.05$) decreased the levels of TBARS and improved the levels of GSH and activities of SOD, CAT and GPx. The levels of TBARS, GSH and activity of GPx were increased whereas the activities of SOD and CAT were decreased in the tumor tissues as compared to normal tissues of control rats. Oral administration of 200mg/kg of TRM to DMBA-treated rats significantly ($p<0.05$) restored the status to near normal. Based on the biochemical analysis and histopathology, administration of TRM prevented the breast cancer caused by the chemotherapy agents. After analysis of the breast tissues the results of this research concluded that the antioxidant properties of this plant and its potentiation effect contributed to the observed protection against breast cancer .

This herbal therapy has proved effective in reducing the toxic effects of breast cancer and have shown that this herbal therapy help get relief from the breast cancer.

Cardioprotective activity

Isoproterenol (ISO) is well known cardiotoxic agent due to its ability to destruct myocardial cells. As a result, cytosolic enzymes such as Lactate Dehydrogenase (LDH), Transaminases (ALT and AST) and Creatine Phosphokinase (CPK) were released into

blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability. In the present study, Isoproterenol treated rats showed significant elevation in the levels of these diagnostic marker enzymes (AST, ALT, LDH and CPK). Moreover, elevated levels of these enzymes are an indicator of the severity of ISO-induced myocardial membrane necrosis. The prior administration of TRM (200 mg /kg) showed significant reduction in ISO induced elevated serum marker enzymes. Higher dose of 200 mg/kg of methanol extract *Triumfetta rhomboidea* effectively prevented Isoproterenol induced cardiac damage.

In the present study, I found that *Triumfetta rhomboidea* leaves methanol extract protected myocardium from Isoproterenol-induced myocardial functional injury via normalization levels of diagnostic marker enzymes, suggesting the beneficial action of *Triumfetta rhomboidea* as a Cardioprotective agent.

In-vivo anti snake venom activity

The methanol extract was screened for in-vivo anti snake venom activity. The extract of *Triumfetta rhomboidea* at 200mg/kg increased the percentage survival which was comparable to that of standard anti venom serum. It was observed that the survival of the mice increased progressively with increasing the dose of the extract in a dose dependant manner. α-Cobratoxin is a substance of the venom of Naja Naja. It is a nicotinic acetylcholine receptor (nAChR) antagonist which binds antagonistically and slowly reversible to muscle-type and neuronal type nAChRs. This bond will block the receptor's ability to bind acetylcholine and thereby inhibits the ion flow through the

postsynaptic membrane, which will lead to paralysation. The probable mechanism of preventing the neurotoxic effect by *Triumfetta rhomboidea* may be by interfering with the acetylcholine receptor sites by antagonizing the actions of the neurotoxic substances in the venom at the acetylcholine receptor sites.

Analgesic activity

Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase PGE_1 and PGE_2 peripherally. NSAIDs can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors

The effect of TRM produced significant reduction in the number of writhing in mice at 200 mg/kg oral dose, the percent reduction of writhing of TRM was increased respectively, as compared to the control group.The mechanism of analgesic activity of TRM could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Diclofenac. Thus, the reduction in the number of writhing indicates that *Triumfetta rhomboidea* might exert anti-nociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandin.

Wound healing activity

Present study clearly demonstrated that methanol extracts *Triumfetta rhomboidea* possessed a definite pro healing action in normal healing as observed by significant increase in the rate of wound contraction. Wound contraction involves a

complex and superbly orchestrated interaction of cells, extracellular matrix and cytokines. In the present study extract treated wounds were found to contract much faster. Increased rate of wound contraction in extract treated wounds might be due to increase in transformation of fibroblast cells into myofibroblast cells. Equal proportion of topical application of methanol extracts of leaves of *Triumfetta rhomboidea* has a positive influence on wound contraction phase of wound healing, has a beneficial in wound healing.

Invivo Anti-tumour activity

The cytotoxic effect of crude methanol extracts of *Triumfetta rhomboidea* on DLA and EAC cells was evaluated .The maximum amount of inhibition is observed in 200mg/kg of TRM. A dose dependent activity was exhibited both by methanol extracts where both DLA and EAC cells were sensitive to methanol extract. Among the various groups tested for anti-cancer activity, 200mg/kg exhibited very good activity which was comparable with standard drug Cyclophosphamide .

Docking Analysis

In this study we have designed a set of 2 compounds of *Triumfetta rhomboidea* molecules and performed docking simulations in order to identify their binding affinity and binding energy towards the BRCA 1 protein .To identify the molecular binding interactions of the analogs with the receptor, two ligands were docked into the active binding site of the enzyme BRCA 1using Glide docking algorithm The newly designed molecules were interacting with the target protein. The MolDock score of the test compounds is comparatively lower, that means the drug-receptor complex is having

better binding interaction (the negative values indicating the lower energy conformations).

Between the two compounds, Friedelinol is having the better interaction values due to H-bond formed between the receptor residue (Ser 1655) and compound. Apart from that, the newly docked compounds were interacting with the native binding site of the receptor consisting of residues Arg 1699, Ser 1655, Leu 1701 and Asn 1774. The newly docked polycyclic natural compounds were able to bind with the BRCA1 receptor with more binding interactions (vander wall and steric interactions) than the native ligand (TYR-SER-ARG-SEP-PRO-THR-PHE) present in the crystalized structure of the protein.

The docking study showed that the leaves of *Triumfetta rhomboidea* is potential for the chemopreventive activity by inhibiting BRCA1 protein. Friedelinol has shown excellent chemopreventive activity compared to Friedelin.

Isolation and Characterization

The structure of the compound isolated from *Triumfetta rhomboidea* was analysed by spectroscopic techniques and the melting point of the compound was found to be 210-2150C. It answered the test for flavanoids. It had IR absorptions at 3369(hydroxyl), 2930 (C-H stretching of alkanes), 1412,1276, 1075 and 913 (C-O vibrations of alcohols) and 634 (C-H bending vibration of alkanes The 1H NMR spectrum showed the presence of 14 protons on saturated carbon atoms. The protons are multiplet with the value of 3.36- 4.04 (M,H) in up field and the value at 4.19- 5.17 (M,H) showed the protons in the down field. The peak appeared in the range between

3.36-4.04 ppm corresponds to carbon connected to oxygen linkage. 1H and 13C NMR spectrum of the compound showed the peaks at 73.41, 73.62, 75.25, 71.82, 72.40, 76.46, 71.62 and 76.52 indicated the carbon connected to oxygen linkage. Mass spectrum of the compound showed the molecular ion peak at m/z 639. From the spectral studies the possible structure of compound may be Rhamnetin-3-O-β-D-Galactosyl-4- O-β-D-galactopyranoside.

7. SUMMARY AND CONCLUSION

SUMMARY

The thesis entitled "Chemo preventive activity of *Triumfetta rhomboidea* in 7, 12 Dimethylbenz (A) Anthracene induced Breast cancer in Sprague –Dawley rat model." deals with phytochemical and pharmacological investigation of traditionally used medicinal plants. A perusal of the literature revealed that only fragmentary information was available on these plant species regarding pharmacological activity by any other researchers. Plants are becoming potential source for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance.

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents, some of which are necessary for life. In the status of normal metabolism, levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins. Many synthetic drugs protect against oxidative damage but they have adverse side effects. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counterparts. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances called ‘‘plant chemicals’’ or ‘‘phytochemicals’’ that possess

antioxidant activity Since, secondary metabolites are responsible for biological activity, this study would be the leading path way of information for selection of the extract for pharmacological activity and isolation of constituents responsible for the activity**.**

The various pharmacological activities such as Cardioprotective, Analgesic, Antiinflammatory, Wound healing, Anti-tumor and Anti-snake venom activity were established by this test extract can be investigated further in future to get their meaningful extension in clinical use. The therapeutic activities were carried out scientifically and reported for the first time in this plant

Methanol extracts of *Triumfetta rhomboidea* was selected for column chromatographic separation in an attempt to isolate the most effective fraction responsible for the therapeutic activity. The effective fraction of *Triumfetta rhomboidea* was selected based on the pharmacological activity and the structure of the compound was confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The compound was found to be Rhamnetin-3-O-β-D-Galactosyl-4- O-β-D-galactopyranoside and it was first time isolated from the leaves of *Triumfetta rhomboidea* .The interest in studying herbal products is further supported by the observations that many herbal extracts show superior effect when compared to single chemical constituents at the equivalent dose (or concentration).

With the support of phytochemical studies and in-vitro pharmacological activities the methanolic extract was selected and subjected to in-vivo pharmacological studies.

The dose level of 200mg/kg showed promising Chemopreventive activity against breast cancer. Which is equal to standard drug Tamoxifen. The in vitro free radical scavenging assays indicate that these plant extracts provided significant source of natural antioxidant which is mainly responsible for its anticancer activity

Based on the biochemical analysis and histopathology, the plant *Triumfetta rhomboidea* leaves prevented breast cancer caused by the chemotherapy agents. After analysis of the breast tissue , the results of this research concluded that the antioxidant properties of this plant contributed to the observed protection against breast cancer .

The docking study concluded that the leaves of *Triumfetta rhomboidea* is potential for the breast cancer by inhibiting BRCA1 Receptor. Friedelinol has shown excellent activity against breast cancer..

CONCLUSION

In my research I found the effective herbal therapy for breast cancer. *Triumfetta rhomboidea* leaves exhibited stongest chemopreventive activity especially for treating the Breast cancer

8. IMPACT OF THE STUDY

In my research I found the effective herbal therapy for breast cancer. *Triumfetta rhomboidea* leaves exhibited stongest chemopreventive activity especially for treating the Breast cancer.

Future studies

Use of this herbal therapy according to Indian medicine concepts has not been systematically explored to benefit modern pharmacotherapy. The therapeutic effect must be carefully evaluated by a well-designed, randomized, double-blinded, placebo-controlled clinical trial involving a significant number of subjects (*i.e.*, with sufficient power). , clinical trials following good clinical practice (GCP) should be implemented to demonstrate clinical efficacy of this plant in breast cancer. In future this effective herbal therapy of *Triumfetta rhomboidea* must be evaluated clinically to benefit the patients suffering breast cancer.

BIBLIOGRAPHY

1. Cohen J.H. Et Al "Fruits and Vegetable Intakes And Prostate Cancer Risk" ***Journal of National Cancer Institute***, 2002, 92,61-8
2. Bushman J.L., Green Tea and Cancer in Humans; A Review of Literature. ***Nutr Cancer***, 1998, 31, 151-159.
3. Macho A, Lucena C., Sancho R., Daddario N., Minassi A., Munoz E. Appendino G. Nonpungent Capsaicinoids from Sweet Pepper Synthesis and Evaluation of Chemoprotective and Anticancer Potential. ***Eur. J. Nutr***., 2003, 42(1),2-9
4. Powell JM, McCrory DF, Jackson-Smith DB, Saam H Manure collection and distribution on Wisconsin dairy farms. ***J Environ Qual*:** 2005 ;34: 2036-2044.
5. Cerutti PA, Oxy-radicals and cancer. ***Lancet*** :1994:344: 862- 863.
6. Cook PLM, Wenzhöfer F, Rysgaard S, Galaktionov OS, Meysman FJR, et al. (2006)Quantification of denitrification in permeable sediments: Insights from a two dimensional simulation analysis and experimental data. ***Journal of Diseases and Medicinal Plants*** 2015; 1(1): 19-23 23
7. Vickers A Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. ***Cancer Invest*** 2002;20: 1069-1079.
8. Bonham M, Arnold H, Montgomery B, Nelson PS Molecular effects of the herbal compound PC-SPES: identification of activity pathways in prostate carcinoma. ***Cancer Res*:**2002 62: 3920-3924.
9. Hu H, Ahn NS, Yang X, Lee YS, Kang KS Ganoderm lucidum extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. ***Int J Cancer*** 2002;102: 250-253.
10. El-Shemy HA, Aboul-Enein AM, Aboul-Enein MI, Issa SI, Fujita K The effect of willow leaf extracts on human leukemic cells in vitro. ***J Biochem Mol Biol*** :2003;36: 387-389.
11. Kao ST, Yeh CC, Hsieh CC, Yang MD, Lee MR, et al. The Chinese medicine Bu-Zhong-Yi-Qi-Tang inhibited proliferation of hepatoma cell lines by inducing apoptosis via G0/G1 arrest. ***Life Sci*** ;2001;69: 1485-1496.

12. Meyers KJ, Watkins CB, Pritts MP, Liu RH Antioxidant and antiproliferative activities of strawberries. ***J Agric Food Chem*** 2003;51: 6887-6892.
13. Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, et al. (1994) The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. ***Cancer Res*** :1994;54: 448-454.
14. Wang X, Wei L, Ouyang JP, Muller S, Gentils M, et al. Effects of an angelica extract on human erythrocyte aggregation, deformation and osmotic fragility. ***Clin Hemorheol Microcirc*** ;2001;24: 201-205.
15. Xie F, Li X, Sun K, Chu Y, Cao H, et al. An experimental study on drugs for improving blood circulation and removing blood stasis in treating mild chronic hepatic damage. ***J Tradit Chin Med*** :2001;21: 225-231.
16. Poma A, Miranda M, Spanò L Differential response of human melanoma and Ehrlich ascites cells in vitro to the ribosome-inactivating protein luffin. ***Melanoma Res*** 1998;8: 465-467.
17. Ma H, Carpenter CL, Sullivan-Halley J, Bernstein L The roles of herbal remedies in survival and quality of life among long-term breast cancer survivors-results of a prospective study. ***BMC Cancer*** 2011; 11: 222.
18. Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, et al. Benign breast disease and the risk of breast cancer. ***N Engl J Med.*** 2005;353:229–237.
19. Buist DS, Abraham LA, Barlow WE, Krishnaraj A, Holdridge RC, Sickles EA, Carney PA, Kerlikowske K, Geller BM. Diagnosis of second breast cancer events after initial diagnosis of early stage breast cancer. ***Breast Cancer Res Treat***. 2010;124:863–873.
20. Dupont WD, Parl FF, Hartmann WH, Brinton LA, Winfield AC, Worrell JA, Schuyler PA, Plummer WD. Breast cancer risk associated with proliferative breast disease and atypical hyperplasia. ***Cancer***. 1993;71:1258–1265.

21. Boon H, Stewart M, Kennard MA, Gray R, Sawka C, Use of complementary/alternative medicine by breast cancer survivors in Ontario: prevalence and perceptions. ***Journal Clinical Oncology*** ;2000;18: 2515-2521.
22. Murad TM, von Haam E. Studies on mammary carcinoma induced by 7,12-dimethylbenzanthracene administration. ***Cancer Res*****:** 1972;32:1404-15.
23. Burstein HJ, Gelber S, Guadagnoli E, Weeks JC Use of alternative medicine by women with early-stage breast cancer. ***The New England Journal of Medicine***; 1999; 340: 1733-1739.
24. Ma H, Carpenter CL, Sullivan-Halley J, Bernstein L The roles of herbal remedies in survival and quality of life among long-term breast cancer survivors-results of a prospective study. ***BMC Cancer*** ; 2011;11: 222.
25. Grubbs CJ, Juliana MM, Hill DL, Whitaker LM. Suppression by pregnancy of chemically induced preneoplastic cells of the rat mammary gland. ***Anticancer Res*** 1986;6:2395-401.
26. Halliwell B, Gutteridge J (2007) Free radicals in biology and medicine. (4th Edn), Oxford University Press, Oxford, USA.
27. Clarkson PM, Thompson HS (2000) Antioxidants: what role do they play in physical activity and health ***Am J Clin Nutr*** 72: 637S-646S.
28. Feher J, Csomos G, Vereckei A (1987) Free radical reactions in medicine. Springer-Verlag, Berlin-Heidelberg, USA 40-43.
29. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP (1999) Biomarkers of free radical damage applications in experimental animals and in humans. ***Free Radic Biol Med*** 26:202-226.
30. Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. ***J Biol Chem*** 272: 20313-20316.
31. Dean RT, Fu S, Stocker R, Davies MJ (1997) Biochemistry and pathology of radical-mediated protein oxidation. ***Biochem J*** 324 : 1-18.
32. Morabito MA, Sheng M, Tsai LH (2004) Cyclin-dependent kinase 5 phosphorylates the N-terminal domain of the postsynaptic density protein PSD-95 in neurons**.** ***J Neurosci*** 24: 865-876.

33. Benderitter M, Maupoil V, Vergely C, Dalloz F, Briot F, et al. (1998) Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: effects of iron chelators. ***Fundam Clin Pharmacol*** 12: 510-516.
34. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, et al. Free radicals and antioxidants in normal physiological functions and human disease. ***Int J Biochem Cell Biol*** ; 2007:7 : 44- 84.
35. Chopra R., Nayar S., Chopra, I., In; Glossary of Indian Medicinal Plant, 3rd Edn., Council of Scientific and Industrial Research, New Delhi, 1986, 249.
36. Mukharjee P.K. In, Quality Control of Herbal Drugs, 1st Edn, Business Horizons, Pharmaceutical Publishers, 2002, 40.
37. Barnes, An Introduction to Herbal Medicinal Products, ***The Pharmaceutical Journal****:* 2002:268-804.
38. Chattergee A, Chandra Prakash S., The Treatise of Indian Medicinal Plant, Vol 2, National Institute of Science and Communication., CSIR, New Delhi, 1992, 170-171.
39. Basu B.D, Indian Medicinal Plant, Part-I, Dehradun, 1997, 159.
40. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol-I, International Book Distributors, India, 2005, 76-80.
41. Fleischauer AT, Arab L Garlic and cancer: a critical review of the epidemiologic literature. ***Journal of Nutrition*** ; 2001;131: 1032S-40S
42. Osore H., Ecbolic properties of Triumfetta rhomboidea Jacq on the gravid mammalian uterus, **East Afri. Medi. J**., 1982, 59(11), 733.
43. Maksoud S, Hosni A, Hanaa H.A., The distribution and concentration of urease in the seeds of Triumfetta rhomboidea Jacq., ***Egyp. J. Physio. Sci***., 1998, 21(2), 209.
44. Nair A.G.R., Seetharaman T.R., Voirin, B., Bonvin, J.F., The true structure of Triumboidin, a flavone glycosides from the leaves of Triumfetta rhomboidea Jacq. ***Phytochemistry*****.,** 1986, 25(3), 786.

45. Kusmi, T., Chang C.C., Wheelar M., Kubo, I., Nakanishi, K., isolation of Triumferol and structural determination by standard spectral method and reported the antigermination activity against lecture seeds from the Triumfetta rhomboidea Jacq ***Tetra. Lett***., 1981, 22(36), 786.
46. Pradhan, D., Panda P.K. and Kar D.M., Study of antiulcer activity of roots of Triumfetta rhomboidea., ***Journal of Science and Pharmacy***,2003, 5, 18-21
47. Chiranjibi Pattanaik C. Reddy S and Murthy M.S.R., An ethnobotanical survey of medicinal plants used by the Didayi tribe of Malkangiri district of Orissa, India, ***Fitoterapia***, 2008, 79(1), 67-71
48. Mevy J.P., Bessiere J.M., Rabier J, Dherbomez M, Ruzzier M, Millogo J, Viano J, Composition and antimicrobial activities of the essential oil of Triumfetta rhomboidea Jacq. ***Flavour and Fragrance Journal***, 2005, 21 (1), 80 – 83.
49. Sakarkar DM, Deshmukh VN Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity. ***International Journal of PharmTech Research*** 2011;3: 298- 308.
50. Boon H, Stewart M, Kennard MA, Gray R, Sawka C, et al. Use of complementary/alternative medicine by breast cancer survivors in Ontario: prevalence and perceptions. ***J Clin Oncol*** ;2000;18: 2515-2521.
51. Burstein HJ, Gelber S, Guadagnoli E, Weeks JC Use of alternative medicine by women with early-stage breast cancer. ***N Engl J Med*** ;1999;340: 1733-1739.
52. Michael JW, Cynthia W, Destiny MH, Mary EZ Herbals, Cancer Prevention and Health. American Society for Nutritional Sciences, ***Journal of Nutrition*** 2001;131: 3034-3036.
53. Kwak MK, Egner PA, Dolan PM, Ramos GM, Groopman JD, et al. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. ***Mutat Res*** ;2001;480-481: 305-315.
54. Devmurari V.P., Ghodasara T.J., Jivani N.P .AntibacteriaL Activity and Phytochemical Study of Ethanolic Extract of Triumfetta rhomboidea Jacq. ***International Journal of Pharm Tech Research*** : 2005;2 (2); 1182-186

55. CD Odimugwu, IF Uche, CL Ozioko, CE Ogbuanya, HT Gugu, OC *Esimone* In vitro antimicrobial evaluation of methanol extract of Triumfetta rhomboidea leaves against some clinical bacterial isolates ***International Journal of Biological and Chemical Sciences;***2015;5(5);245-256
56. Uche, F. I.; Okunna, B. U Phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Triumfetta rhomboidea* leaves in animal models. ***Asian Pacific Journal of Tropical Medicine*** :2009: 2(5); 26-29
57. Prasad Ekanath Funde Antibacterial activity of water extract and active constituents were isolated of butanol from a Triumfetta rhomboidea and analytical study by HPLC ***Der Chemica Sinica***;2011; 2 (1): 8-14
58. Rozina Parul, Sukalayan Kumar Kundu and Pijush Saha In Vitro Nitric Oxide Scavenging Activity Of Methanol Extracts Of Three Bangladeshi Medicinal Plants ***The Pharma Innovation - Journal*** :2012; 1: 12:100-105
59. P Sivakumar*, Sunil Mengani, M Vijayabaskaran, R Sambath Kumar, P Perumal, B Jayakar‘ Antitumor and Antioxidant Activities of Triumfetta rhomboidea against Ehrlich Ascites Carcinoma bearing Swiss Albino mice. ***Research Journal of Pharmaceutical, Biological and Chemical Sciences****:* 2016;1(4): 486-494
60. K.P. Lissy,[*] Thara K. Simon,[a] and M.S. Lathab Antioxidant potential of *Sida retusa, Urena lobata* AND *Triumfetta rhomboidea* **Ancient Science of Life**: 2006; 25(3-4): 10–15
61. N Suganath*, D Rama Krishna , Deepak Reddy G , B Sudheera , M Mallikarjun , Pavani Beesetty2 Evaluation of anti-diabetic activity of Triumfetta rhomboidea in alloxan induced Wistar rats ***Research Journal of Pharmaceutical, Biological and Chemical Sciences***;2011;2(1);475-77
62. .P. Mevy a,*, J.M. Bessiere b , M. Dherbomez c , J. Millogo d , J. Viano aChemical composition and some biological activities of the volatile oils of a chemotype of Lippia chevalieri Moldenke***; Food Chemistry*** :2007;101: 682–685

63. Natla Sashidhar Reddy, Parthasarathy, Nirmala Quercetin in Dimethyl Benzanthracene Induced Breast Cancer in Rats, ***American Journal of Pharmacology and Toxicology*** 2012;7 (2): 68-72.
64. Thamaraiselvan Rengarajan, Natarajan Nandakumar, Maruthaiveeran Periyasamy Balasubramanian D-Pinitol preventsrat breast carcinogenesis induced by 7,12- Dimethylbenz(a) anthracene through inhibition of Bcl-2 and induction of P53, caspase-3 proteins and modulation of hepatic biotransformation enzymes and antioxidants ***Biomedicine and aging pathology****:*2012;2;3;85-93
65. Margarida F Dias Chemoprevention of DMBA-Induced Mammary Tumors in Rats by a Combined Regimen of Alpha-Tocopherol, Selenium, and Ascorbic Acid ***The Breast Journal*** 2000;6(1):14-19.
66. Jyoti Parmar, Priyanka Sharma, and Pradeep K Goyal Role of Syzygium Cumini Seed Extract in Preventing the 7, 12-Dimethylbenz (a) anthracene Induced Skin Carcinogenesis ***Research & Reviews: Journal of Zoological Sciences : 2013;2347-2394***
67. Veena Sharma and Ritu Paliwal Potential Chemoprevention of 7,12 Dimethylbenz[a]anthracene Induced Renal Carcinogenesis by *Moringa oleifera* Pods and Its Isolated Saponin ***Indian J Clin Biochem*** 2014;29(2):202-209
68. Jyoti Parmar, Priyanka Sharma, and Pradeep K Goyal Role of Syzygium Cumini Seed Extract in Preventing the 7, 12-Dimethylbenz (a) anthracene Induced Skin Carcinogenesis ***Research & Reviews: Journal of Zoological Sciences : 2013;2347-2394***
69. Takeda, Y Matsumoto, T Terao, H Shingu, T, ***Phytochemistry***;1993; 33; 411
70. MacDiarmid DM, Khodarev NN, Pitroda SP, Edwards DC, Pelizzari CA, Huang L, Kufe DW, Weichselbaum RR, MUC1-associated proliferation signature predicts outcomes in lung adenocarcinoma patients, ***BMC Med Genomics***, 2010, 3;1-12.

71. Zhang Li, Xiang ZM. Determination of the flavonoids from *Ginkgo Biloba* extract by High Performance Liquid Chromatography. ***Chinese Chemical Letters*** 2002; 13: 968–970
72. Dudonne S, Vitrac X, Coutiere P, Woillez M, Merillon J . Comparative study of antioxidant properties and total M phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays***. J Agric Food Chem;*** 2009***;*** 57:1768-1774.
73. Blois, M.S. Antioxidant determinations by the use of a stable free radical; ***Nature*** 1958: 181: 1199- 1200.
74. Zhu, Q.Y., Hackman, R.M., Ensunsa, J.L., Holt, R.R. and Keen, Antioxidative activities of oolong tea, ***J. Agric. Food Chem***.;2002;50: 6929-6934.
75. Sa ′ nchez-Moreno, C., Larrauri, J.A. and Saura-Calixto, F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents, ***Food Res. Int***., 1999; 32: 407-412
76. Ilhami Gulcin. Haci Ahmet Alici. and Mehmet Cesur., Determination of in vitro antioxidant and radical scavenging activities of propofol. ***Chem Pharm Bull***. 2005; 53, 281 – 85.
77. Akowuah, G. A.; Zhari, I.; Norhayati, I.; Sadikun, A.; Khamsah, S. M. ***Food Chemistry***, 2004;87; 559.
78. Blois, M.S. Antioxidant determinations by the use of a stable free radical, ***Nature***; 1981; 181: 1199- 1200.
79. Samak, G., Shenoy, R.P., Manjunatha, S.M. and Vinayak, K.S. Superoxide and hydroxyl radical scavenging actions of botanical extracts of Wagatea spicata. ***Food Chemistry*** ;2009;115: 631-634
80. Takeda, Y Matsumoto, T Terao, H Shingu, T, ***Phytochemistry***;1993; 33; 411
81. Ecobichon DJ. The basis of toxicology testing. 2nd ed, CRC Press: New York; 1997, pp. 43-60.
82. Lorke, D. A new approach to practical acute toxicity testing. ***Arch. Toxicol.,*** 1983;54;275-287

83. Russo J, Russo IH. Experimentally induced mammary tumors in rats. Breast Cancer ***Res Treat*** 1996;39:7-20

84. Dias MF, Sousa E, Cabrita S, Patricio J, Oliveira CF. Chemoprevention of DMBA induced mammary tumors in rats by a combined regimen of alpha tocopherol, selenium, and ascorbic acid. ***Breast J*** 2000;16:14-19

85. Giullino PM, Pettingrew HM, Grantham FIT. N-nitrosomethylurea as mammary gland carcinogen in rats. ***J Natl Cancer Inst*** 1975;54:401-14.

86. Russo IH, Russo J. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenzan(a)thracene. ***J Natl Cancer Inst*** 1978;61:1439-42.

87. Yagi, K. (1998) Free Rad. Antiox. Prot., 108; 101-106.

88. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction**.** ***Anal Biochem***. 1979 ;95(2):351-8.

89. Kakkar P, Das B, Viswanathan P. ***Ind J Biochem Biophys*** 1984; 21, 131-132.

90. Sinha AK,Colorimetric assay of catalase, ***Analytical Biochemistry***, 1972, 47:2:389-394.

91. Rotruck J.T, Pope A.L, Ganther H.E, Swanson A.B, Hafeman D.G, and Hoekstra W.G, ***Scienc*e**, 1973;179:588-590.-590.

92. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–890

93. Gupta M, Mazumder UK, Sambath Kumar R, Sivakumar T, et al Antitumor activity and antioxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in Swiss albino mice. ***J Pharmacol Sci*** ; 94; 2004 ; 177-84.

94. Rajkapoor B, Jayakar B, Murugesh N et al Antitumor activity of Indigofera aspalathoides on Ehrlich ascites carcinoma in mice. ***Indian J Pharmacol*:** 2004; **36**, 38-40.

95. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. et al Antitumor activity of methanolic extract of Cassia fistula L. seed against Ehrlich ascites carcinoma ***J Ethnopharmacol*** ; 2000; 72: 151- 6.

96. Ramalingam Radha, Subramaniyam Kavimani ,Velayudham Ravichandran et al Antitumour Activity of Methanolic Extract of *Plumeria alba L.* Leaves Against Dalton Lymphoma Ascites in Mice ***Int J Health Res*** ; 2008; 1(2):79-85
97. Vijayan P, Vinod Kumar S, Dhanaraj SA, Mukherjee PK, Suresh B In vitro cytotoxicity and antitumor properties of Hypericum mysorense and Hypericum patulum. ***Phytother Res;*** 2003; 17; 952-56
98. Perola E, Walters WP,Charifson P.S.A detailed comparision of current docking & scoring methods on Pharmaceutical relevance ; ***Proteins*** ; 2004 ; 56 ; 235-249
99. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening. ***J. Med. Chem***. 2004; 47; 1750-1759.
100. Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects. ***J. Med. Chem***;2006; 49; 534-55.
101. Golke, H.; Hendlich, M.; Kelbe, G. Knowledge-Based Scoring Function to Predict Protein-Ligand Interactions***. J. Mol. Biol***; 2000; 295; 337-356 .
102. Sabeena Farvin, K.H., R. Anandan, S.H. Kumar, K.S. Shiny, T.V. Sankar and T.K. Thankappan,. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. ***Pharmacol. Res***., 2004; 50: 231-236.
103. Gomes, A., Saha, A., Chatterjee, J., Chackravarty, A. K: Viper and Cobra Venom neutralization by beta silosterol and stigmasterol isolated from the root extract of pluchea indica less (Asteracea). ***Phytomedicine***. 2007; 14 (9): 637 – 643.
104. Sakai, A Clinical features of envenomation by the snake, Yamakagashi rhabdophis tigrinus). ***J. Chudaku. Kenkyu.*** 2007;20 (3): 235 – 243.
105. Gold, B. S., Dart, R. C Bites of venomous snakes N. ***Engl. J. Med***; 2002; 47:347– 56
106. Koster R, Anderson M, De Beer EJ. Fed Proc 1959; 18:412.

107. Turner RA. Screening Methods in Pharmacology. New York, Academic Press ;1965; 63

108. Coruzzi G, Adami M, Guaita E et al . Antiinflammatory and antinociceptive effect of the selective histamine H4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. ***Eur J Pharmacol***. 2007; 563:240-244

109. Crunkhon P and Meacock SER, Mediators of the inflammation induced in the rat paw by carrageenan. ***Bri. J. Pharmacol***., 1971; 292 : 392-402.

110. Di Rosa M, Girround JP and Willoughby DA, 1971. Studies on the acute inflammatory response induced in rats in different sites by carrageenan and turpertine. ***J. Pathology*****;** 1971**;** 104: 15-29.

111. Christholm CM. Wound evaluation and cleaning. ***Emerg Med Clin North Am*** 1992, 10, 665.

112. Robson MC. Disturbances in wound healing. ***Annals of Emergency Medicine*** 1994, 17, 1274.