PARTIAL PURIFICATION OF ANTI-TUMOR AND ANTIOXIDANT COMPONENTS FROM UVARIA NARUM (DUNAL) WALL SEED

Thesis Submitted to

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

In partial fulfillment of the requirements

for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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Reg. No: 26116393

Under the guidance of

Institutional Guide

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TAMIL NADU.

OCTOBER – 2013

"PARTIAL PURIFICATION OF ANTI-TUMOR AND ANTIOXIDANT COMPONENTS FROM UVARIA NARUM (DUNAL) WALL SEED"

DEGREE DISSERTATION WORK SUBMITTED TO THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY, CHENNAI IN PARTIAL FULFILLMENT FOR THE AWARD OF

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CERTIFICATE

This is to certify that this project work entitled "**PARTIAL PURIFICATION OF ANTI-TUMOR AND ANTIOXIDANT COMPONENTS FROM UVARIA NARUM (DUNAL) WALL SEED"** submitted in partial fulfilment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmacology** to **The Tamilnadu Dr. M.G.R Medical University, Chennai** is a bonafied work carried out by **Ms. ANSA P.U (Reg. No: 26116393)** at **Amala Cancer Research Centre, Thrissur, Kerala**, and completed the work at **Department of Pharmacology, R.V.S College of Pharmaceutical Sciences**, **Sulur, Coimbatore-641402** under the supervision and guidance of **Mrs. C. Maheswari, M.Pharm, (Ph.D).,**(Institutional Guide) and **Dr. Achuthan C. Raghavamenon** (Industrial Guide) during the academic year 2012-2013.

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DECLARATION

I **ANSA P.U,** hereby declare that the dissertation work entitled **"Partial purification of anti-tumor and antioxidant components from *Uvaria narum* (Dunal) Wall seed"** submitted by me, in partial fulfillment of the requirements for the degree of **MASTER OF PHARMACY** in **PHARMACOLOGY** to **The Tamilnadu Dr.M.G.R Medical University, Chennai** is the result of my original and independent research work carried out under the guidance and supervision of **Dr. ACHUTHAN C.RAGHAVAMENON, Assistant Professor of Amala Cancer Research Centre, Thrissur** and under the internal supervision of **Mrs. C. MAHESWARI,** M.Pharm, (Ph.D)., during the academic year 2012-2013. This has not formed the basis for the award of any Degree/ Diploma/ Fellowship or similar title to any candidate of any university.

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EVALUATION CERTIFICATE

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Examination centre:

Date:

Internal Examiner **External Examiner**

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(ANSA P.U)

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	:	Percentage sign
µl	:	micro litre
^{0}C	:	Degree celsius
ANOVA	:	Analysis of variation
cm^3	:	Cubic centimetre
Fig	:	Figure
Tab	:	Table
gm	:	Gram
U. narum	:	*Uvaria narum*
hr	:	Hour
i.p	:	Intraperitonially
m mole	:	millimoles
mg/dl	:	milligrams per decilitre
mg/kg b.wt	:	milligram per kilogram body weight
mg/kg	:	1 milligram per kilogram
mg/m^2	:	1 milligram per square meter
min	:	minute
sec	:	seconds
ml	:	Milli liter
mm	:	Millimeter
mM	:	millimolar

mm^3	:	Cubic millimetre
nm	:	nanometer
OD	:	Optical density
T.vol	:	Tumor volume
Hb	:	Hemoglobin
ROS	:	Reactive oxygen species
SD	:	Standard deviation
WBC	:	White blood cells
SOD	:	Superoxide dismutase
DPPH	:	2, 2- Diphenyl-1-picryl hydrazyl
AAPH	:	2,2'-azobis-2-amidino propane hydrochloride
FRAP	:	Ferric reducing antioxidant power activity

INTRODUCTION

Cancer is a group of diseases which is characterized by uncontrolled growth and spread of abnormal cells. If the spread of abnormal cells is not controlled, it can result in death. Cancer is supposed to cause by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, immune conditions, hormones, and other mutations that occur from metabolism). These causal factors may act either together or in sequence to initiate or promote carcinogenesis. Most cancers requires multiple steps for their development which occur over many years.

According to the estimates from the International Agency for Research on Cancer (IARC) (Ferlay *et al*., 2008), there were 12.7 million recent cancer cases in 2008 worldwide, of which 5.6 million occured in economically developed countries and 7 million in economically developing countries.

Nowadays, there is an increasing demand for medicinal plants in chemotherapy. This is because of their ability to produce the significant therapeutic effect without causing much side effects. Hence the studies for evaluating the medicinal properties of various plants have prominent role in medicine.

TUMOR

A tumor is commonly used as a synonym for a neoplasm (Saunders., 2007). It is a solid or fluid filled (cystic) lesion that may or may not be formed by an abnormal growth of neoplastic cells that appears enlarged in size. However tumor is not synonymous with cancer. By definition cancer is malignant, while a tumor can be benign, pre-malignant or malignant, or it can represent a lesion without any cancerous potential so ever. Cancer stem cells may play a major role in tumor growth. Scientists believe cancer might have its own stem cells that result in the regrowth of tumors.

According to Medilexicon's medical dictionary (Nordqvist *et al.*, 2012), a Tumor is:

1. *Any swelling or tumefaction.*

2. *One of the four signs of inflammation enunciated by Celsus.*

CANCER CAUSES: THEORIES THROUGHOUT HISTORY:

From the earliest times, physicians have puzzled over the causes of cancer. However the ancient Egyptians blamed cancers on the gods.

- **Humoral theory**

  Hippocrates believed that the body had 4 humors (body fluids) - blood, yellow bile, phlegm and black bile. When the four humors were balanced, a person was healthy. They believed that too much or too little of any of the humors caused disease.

- **Lymph theory**

  Stahl and Hoffman theorized that cancer was composed of fermenting and degenerating lymph, varying in density, alkalinity and acidity. This theory gained rapid support. John Hunter, from the 1700s, supported that tumors grow from lymph constantly thrown out by the blood.

- **Blastema theory**

  In 1838, Johannes Muller proposed that cancer is made up of cells and not lymph, but he believed that a cancer cell does not originate from normal cells. Also he proposed that cancer cells developed from budding elements (blastema) between normal tissues. Rudolph Virchow demonstrated that all cells including the cancer cells are derived from other cells.

- **Chronic irritation theory**

  Virchow determined that chronic irritation was the cause for cancer, but he believed incorrectly that cancers spread like a liquid. In the 1860s, Karl Thiersch, showed that cancers metastasize through the spread of malignant cells and not through some unidentified fluid.

- **Trauma theory**

  Despite the advances in the understanding of cancer, from the late 1800s to 1920s, trauma was thought by some to cause cancer. This belief was rejected as injury failed to cause cancer in experimental animals.

- **Infectious disease theory**

  Zacutus Lusitani (1575−1642) and Nicholas Tulp (1593−1674), two doctors in Holland, concluded at the same time, that cancer was contagious. They proposed that cancer patients should be isolated, preferably outside from the cities and towns, so as to prevent the spread of cancer.

CAUSES OF TUMOR:

In general, tumors (Moscow *et al.,* 2011) occur when cells divide excessively in the body. Typically, the cell division is strictly controlled. New cells are formed to replace older ones or to perform new functions. Cells which are damaged or no longer needed would die to make room for healthy replacements. The common causes of tumor can be:

– If the balance between cell division and death is disturbed, a tumor may form.

– Problems with the body's immune system can also lead to tumors. Tobacco causes more deaths as cancer than any other environmental pollutants. Other causes include:

- Benzene, other chemicals and toxins
- Drinking too much alcohol
- Environmental toxins, such as certain poisonous mushrooms and a type of poison that can grow on peanut plants (aflatoxins)
- Excessive sunlight exposure, obesity, radiation, viruses and genetic problems

Tumors which are known to be caused by viruses are:

- Cervical cancer (human papilloma virus)
- Hepatocellular carcinoma (hepatitis B virus)

Some tumors are more common in one gender compared to the other. Some are more common among children than elderly and viceversa. Others causes are related to diet, family history and environment.

SYMPTOMS OF TUMOR:

Symptoms depend on the type and location of tumor. For instance, lung tumors may cause shortness of breath, coughing or chest pain and tumors of the colon may cause diarrhea, weight loss, constipation, blood in the stool or iron deficiency anaemia.

Some tumors may not cause any symptoms until the advanced stage. In certain tumors, such as pancreatic cancer, symptoms do not start until the disease has reached the chronic stage. The common symptoms with most tumors are chills, fatigue, fever, weight loss, night sweats and loss of appetite.

TYPES OF TUMOR

Tumors are groups of abnormal cells that may form lumps or growths. Tumors grow and behave differently depending on whether they are non-cancerous (benign) or cancerous (malignant). Precancerous conditions may have the potential to develop into a cancer. Tumor is classified as:

- Benign Tumor
- Premalignant Tumor
- Malignant Tumor

Benign Tumor:

Benign tumors (Mazumdar *et al*., 2001) grow slowly, and do not spread into other tissues. They are non-cancerous and are not usually life-threatening. They cause no harm if they are left alone. But, some benign tumours can cause problems. Most benign tumors are not harmful to human health. Some may press against nerves or blood vessels and cause pain or other adverse effects. Benign tumors of endocrine tissues may result in the excessive production of some hormones. Examples of benign tumors include:

- **Adenomas** - tumors that arise from glandular epithelial tissue. Examples include hepatocellular adenoma, pituitary adenoma, bile duct adenoma, adrenocortical adenoma, basal cell adenoma, follicular adenoma, chromophobe adenoma, and nipple adenoma. Although adenomas are not cancerous, they can change and become cancerous, then they are called adenocarcinomas.

- **Fibroids** (fibromas) - benign tumors that grow on fibrous or connective tissue of any organ in the body. Uterine fibroids are common. It is of two types- hard fibroma, which is made up of many fibers and few cells; and soft fibroma which is made up of several loosely connected cells and less fibroid tissue. Usually soft fibroma is found in the armpits, groin, neck and eyelids. Other types of fibromas are angiofibroma, cystic fibroma, myxofibroma, nonossifying and ossifying fibroma, cemento-ossifying fibroma, pleomorphic fibroma, etc. Some fibromas may cause symptoms and require surgical removal. Rarely, fibroids that change and eventually become cancerous are called fibrosarcomas.

- **Hemangiomas** - are benign tumors which consist of many blood cells. Sometimes they may be seen on the surface of the skin and are called *strawberry marks*. The majority of them appear at birth and they gradually goes with time. Usually they do not require any treatment. If they affect the patient's sense organs, the doctor may recommend treatment with corticosteroids. If the patient is above 10 years of age, they are commonly removed using laser surgery.

- **Lipomas** -are soft-tissue tumors. Lipomas consist of adipose tissue (fat cells). Most of them are very small, painless, movable and often soft to the touch. They are more common among people above 40 years. Experts disagree on whether lipomas change and become cancerous (malignant). Examples are angiolipoleiomyoma, angio lipoma, chondroid lipoma, intradermal spindle-cell lipoma, neural fibrolipoma, pleomorphic lipomas and superficial subcutaneous lipoma (most common type which is found just below the skin's surface).

Premalignant Tumor:

A premalignant (Ambrosi *et al*., 2002) or precancerous tumor is one that is not yet malignant or cancerous, but is about to become so. Examples of premalignant growths include:

- **Actinic keratosis** - also known as senile keratosis or solar keratosis, this is a premalignant growth consisting of crusty, scaly and thick patches of skin. Fair people are more susceptible to these types of growths, especially people who are exposed to sunlight. They are potentially premalignant because a number of them progress to squamous cell carcinoma. There is about 20% risk that untreated lesions may eventually become cancerous. However continuous sun exposure increases the risk of malignancy.
- **Dysplasia of the cervix** - the normal cells lining the cervix of the uterus change. The growth can be premalignant and may result in cervical cancer. Cervical dysplasia is usually diagnosed with PAP smear. According to the National Institutes of Health, USA, about 5% of PAP smears detect the presence of cervical dysplasia. They are common in women age group 25 to 35. They may be removed with cryotherapy (freezing), or conization (the cone of tissue from the cervix is removed).
- **Metaplasia of the lung** - the growths occur in the bronchi. The bronchi which are grandular can change and become squamous cells. Its major cause is smoking.

- **Leukoplakia** - thick, white patches on the gums, bottom of the mouth, inside cheeks and on the tongue (less commonly). They cannot be scraped off easily. Experts believe tobacco smoking or chewing is the main cause. As leukoplakia is rarely dangerous, a small percentage is premalignant and can eventually become cancerous. Many mouth cancers occur in the areas next to leukoplakia.

Malignant Tumor:

Malignant tumors (Mazumdar *et al*., 2001) are cancerous tumors, they tends to become progressively worse, and can result in death. Unlike benign tumors, malignant grows fast, and they spread (metastasize).Metastasis is the process by which cancer cells spread from their primary site to distant locations in the human body. For instance, a patient may have started off with melanoma (skin cancer) which metastasized in their brain. There are many types of tumors, which are made up of specific types of cancer cells:

- **Carcinoma** - these tumors are derived from the skin or tissues that line body organs (epithelial cells). For example, carcinomas can be of the lung, liver, stomach, pancreas, colon, prostate or breast. Many of the most common tumors are of this type, especially among the older patients.

- **Sarcoma** - these are tumors that start off in connective tissue, such as bones, cartilage, fat and nerves. They originate from the mesenchymal cells outside the bone marrow. Majority of them are malignant. They are so called after the cell, tissue or structure they originate from, for instance osteosarcoma, angiosarcoma, liposarcoma, chondrosarcoma and fibrosarcoma.

- **Lymphoma/Leukemia** - cancer arises from the blood forming (hematopoietic) cells that originate in the marrow and generally mature in the blood or lymph nodes. Leukemia accounts for 30% of childhood cancers. Leukemia is the only cancer where tumors are not formed.

- **Germ cell tumor** - these are tumors that arise from germ cells or pluripotent cells. They are most commonly present in the ovary or testicle. The majority of testicular tumors are of germ cells. Less commonly, germ cell tumors may also appear in the brain, abdomen and chest.
- **Blastoma** - tumors derived from embryonic tissue or immature precursor cells. They are more common in children than adults. For example, medulloblastoma and glioblastoma are kinds of brain tumors, retinoblastoma is a tumor within the retina of the eye, an osteoblastoma is a type of bone tumor, while a neuroblastoma is a tumor mostly found in children of neural origin.

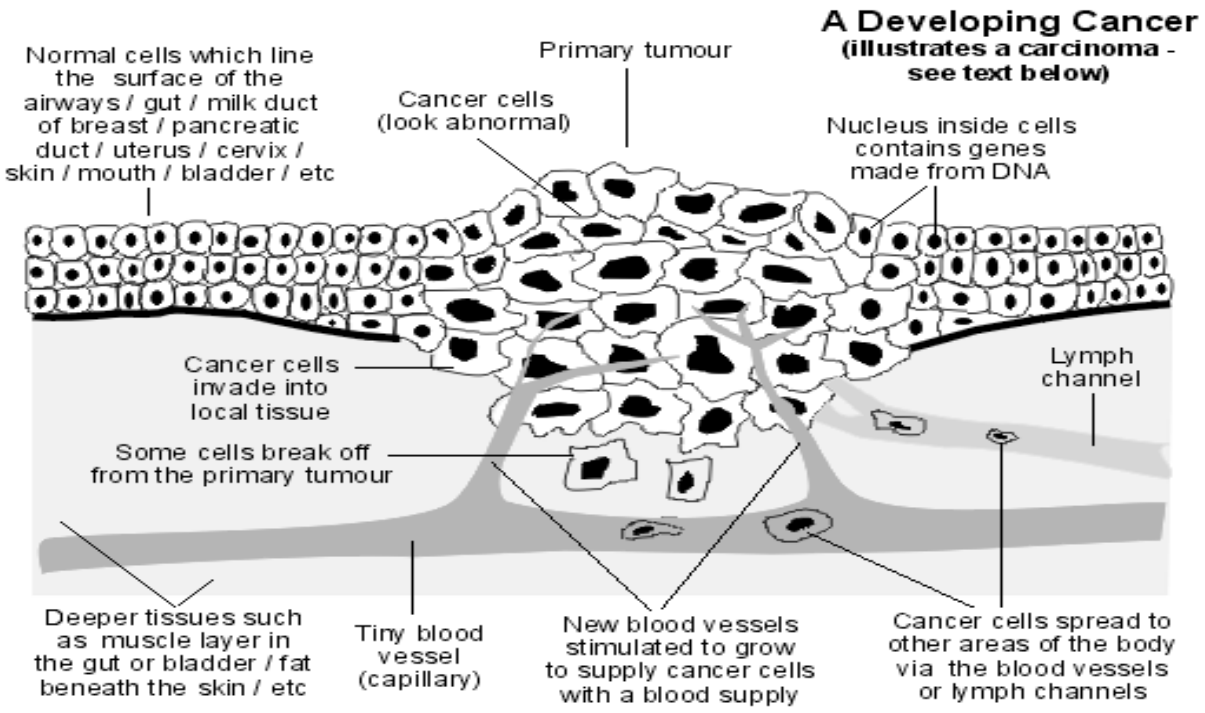


Fig.1: Stages of development of cancer

EXAMINATIONS AND TESTS FOR TUMOR:

When a tumor is found, a biopsy is performed to determine if the tumor is noncancerous (benign) or cancerous (malignant). Depending on the location of the tumor, the biopsy can be a simple procedure or a serious operation. Most patients with tumors have CT or MRI scans to determine the exact location of the tumor and how far it has spread. Recently, positron emission tomography (PET) scans have been used to find certain tumor types.

Other tests include:

- Biopsy of the tumor
- Blood tests (to look for chemicals such as tumor markers)
- Bone marrow biopsy (most often for lymphoma or leukemia)
- Chest X-ray
- Complete blood count (CBC)

TREATMENT OF TUMOR:

Treatment varies based on:

- The type of tumor
- Whether it is noncancerous or cancerous
- Its location

If the tumor is benign (meaning it has no potential to spread) and is located in a safe area where it will not cause symptoms or affect the function of the organ, sometimes no treatment is needed. Benign tumors of the brain may be removed because of their location or harmful effect on the surrounding normal brain tissue.

If a tumor is cancerous, possible treatments include:

- Chemotherapy
- Radiation
- Surgery
- A combination of these methods

If the cancer sticks to one location, the goal of treatment is usually to remove the tumor with surgery. If the tumor has spread only to local lymph nodes, sometimes these can also be removed. If the cancer cannot be removed with surgery, the options for treatment include radiation and chemotherapy or both. Some patients need a combination of radiation, surgery, and chemotherapy. Lymphoma is rarely treated with surgery. Radiation therapy and chemotherapy are most often used for treating lymphoma.

CHEMOTHERAPY

Chemotherapy (Tripathi., 2008) is an important modality in cancer treatment. Chemotherapy involves administering powerful chemical agents (drugs) to destroy cancer cells in the entire organism. It causes a greater proportion of cell death among neoplastic as opposed to normal cells. However damage to normal cells result in chemotherapy toxicities and side effects, it can be seen that those actively dividing cells are most vulnerable.

Antineoplastics are general category of drugs used in chemotherapy and they are classified as:

1. **Alkylating Agents** :
    - Nitrogen mustards -

      Cyclophosphamide (Cytoxam)

Chlorambucil (Leukeran)

Melphalan (Alkeran)

Mechlorethamine hydrochloride (Mustargen)

Ifosfamide (Ifex)

- Alkyl sulphonates -

  Busulphan (Myleran)

- Nitrosoureas -

  Carmustine (BCNU)

  Lomustine (CCNU)

- Ethylenimines -

  Thiotepa

- Triazenes -

  Dacarbazine (DTIC-Dome)

2. **Antimetabolites**
   - Folate antagonist -

     Methotrexate (Folex, Mexate)

   - Purine analogues -

     Mercaptopurine (Purinethol)

     Pentostatin (Nipent)

   - Pyrimidine analogues -

     Cytarabine (Cytosar-U)

     Fluorouracil

3. **Anthracycline Antibiotics**

   Doxorubicin, Daunorubicin

   Mithramycin, Actinomycin D

4. **Vinca Alkaloids**

   Vinblastin (Velban)

   Vincristine (Oncovin)

5. **Epipodophyllotaxins**

   Etoposide, Teniposide

6. **Taxanes**

   Paclitaxel, Docetaxel

7. **Camptothecins**

   Irinotecan, Topotecan

8. **Enzymes**

   L-Asparaginase

9. **Hormone derivatives**

   Tamoxifen (Nolvadex), Prednisolone

   Ethinyl estradiol

CYCLOPHOSPHAMIDE

Cyclophosphamide is a nitrogen mustard alkylating agent from oxazophorines group (McEvoy., 2007). It is a medicine used to treat severe inflammatory illnesses such as complicated systemic lupus erythematosus (SLE/lupus), polymyositis (muscle inflammation), scleroderma and vasculitis (inflamed blood vessels) like Wegener's granulomatosis.

Brand name : Cycloblastin, Endoxan, Cytoxan, cytophosphane

Molecular Formula : $C_7H_{15}Cl_2N_2O_2P$

Molecular Mass : 261.1

Mechanism of Action :

Cyclophosphamide

⇓ P_{450} in liver

Hydroxylated metabolite

⇓

Phosphoramide mustard

⇓ + DNA

Alkylated DNA

Side effects:

Nausea and vomiting, alopecia, mouth ulcers and skin rash, bone marrow depression, bladder inflammation, Infections, neurotoxicity and reduce fertility.

Drug Interactions:

Cyclophosphamide (Takimoto *et al.*, 2008) should be used carefully if taken at the same time as:

- anti-epileptic medicines such as phenytoin and carbamazepine
- heart and blood pressure medicines such as verapamil and diltiazem

- anti-fungal treatments such as ketoconazole, voriconazole and posiconazole
- anti-HIV medicines such as efavirenz, ritonavir, nevirapine, tipranavir, atazanavir
- other antibiotics such as erythromycin, ciprofloxacin, rifampacin, clarithromycin and norfloxacin
- warfarin, anticoagulant
- Avoid eating grapefruit or drinking grapefruit juice while taking this medicine, as it can affect the level of cyclophosphamide in the body.
- If cyclophosphamide is taking, it is recommended not immunized with live vaccines such as MMR (measles, mumps and rubella), OPV (oral polio virus) or yellow fever.

Dosage:

Cyclophosphamide (Novack SN *et al.*, 1971) can be taken by mouth as tablets or it can be given via a vein as an infusion or injection. For long-term treatment it is normally taken in tablet form. For adults and children, usual dose is 1 to 5mg/Kg body wt/day.

Uses:

Cyclophosphamide is used in the treatment of Hodgkin & Non-Hodgkin lymphoma, Multiple myeloma, Leukaemia, cutaneous T-cell lymphoma, neuroblastoma, retinoblastoma, cancer of the ovary and breast, small cell cancer of the lung and sarcoma.

BIOPSY:

To decide whether a tumor is malignant or not, a sample is taken by a surgeon or an interventional radiologist and sent to the laboratory and examined under a microscope by a pathologist - the sample is called a biopsy (Nordqvist *et al*., 2012). There are three different types of biopsies:

- **Excisional biopsy** - the entire lump or suspicious area is surgically removed.
- **Incisional (core) biopsy** - a sample is surgically removed from the tumor
- **Needle aspiration biopsy** - fluid or a sample of tissue is removed with a needle

PREVENTION OF TUMOR:

The risk of cancerous (malignant) tumors can be reduced by:

- Eating a healthy diet
- Exercising regularly

- Limiting alcohol
- Maintaining a healthy weight
- Minimizing the exposure to radiation and toxic chemicals
- Not smoking or chewing tobacco
- Reducing sun exposure, especially if you burn easily

TUMOR MARKERS:

These are usually glycoproteins (soluble molecules) in the blood, which can be detected by monoclonal antibodies. Highly raised levels of a tumour marker (Sturgeon *et al*., 2009) can provide helpful information but inappropriate use can have economic implications, cause patients additional anxiety and distress, and unnecessary investigations may be associated with side-effects and may delay correct diagnosis and treatment.

Uses

Each tumour marker has a variable profile of uses (Perkins *et al*., 2003)

- **Screening -** Screening tests require high sensitivity to detect early stage disease.
- **Disease staging -** For diagnosis and prognosis.exa summary to use in appraisal
- **Monitoring for cancer recurrence -** When monitoring these patients, tumour marker levels should be determined only when there is a potential for meaningful treatment
- **Assessing response to therapy**
  - Tumour marker values returning to normal may indicate cure despite radiographic evidence of persistent disease. In this circumstance, the residual tumour is often non-viable.
  - However an increase in tumour marker levels, coupled with lack of clinical improvement, may indicate treatment failure.
  - Residual elevation after specific treatment usually indicates persistent disease.
  - Tumour marker response is particularly useful in cases where other evidence of disease is not readily accessible.

SL.NO:	TUMOR MARKER	ASSOCIATED PRIMARY TUMOR
1	CA 27.29	Breast cancer
2	CEA	Colorectal cancer
3	CA 19-9	Pancreatic and biliary tract cancers
4	AFP	Hepatocellular carcinoma, Nonseminomatous germ cell tumors
5	b-hCG	Non-seminomatous germ cell tumors and Gestational trophoblastic disease
6	CA-125	Ovarian cancer
7	PSA	Prostate cancer

Table.1: Clinically important Tumor markers

FREE RADICALS

The free radical theory of aging states that organisms age because cells accumulate free radical (Herrling *et al*., 2008) damage over time. Free radicals are molecular species which contain an unpaired electron (represented as R·). Free radicals are some of the most chemically reactive molecules that need to pair its single electron, thus it must abstract a second electron from a neighbouring molecule. This results in the formation of yet another free radical and self-propagating chain reaction ensues.Chain reactions involving free radicals (Halliwell *et al*., 2012) can usually be divided into three distinct processes such as initiation phase, propagation phase and termination phase.

- **Initiation** reactions are those that result in a net increase in the number of free radicals. They involve in the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals.
- **Propagation** reactions are those in which the total number of free radicals remains the same.
- **Termination** reactions are those reactions resulting in a net decrease in the number of free radicals. However, two free radicals combine to form a more stable species, for example: $2Cl\cdot \rightarrow Cl_2$

Free radicals in human body can arise from fatty food, alcohol, smoking, environmental pollutants, ozone, hydrogen peroxide, ionisation, toxins, carcinogen toxins etc. The vast majority of free radicals originates within the body, an unavoidable byproduct of living system. Under normal conditions free radical intermediates are produced in living systems, the body handles free radicals formed by the breakdown of compounds through the process of metabolism. The major sources of free radicals (such as O_2^- and $HO_2\cdot$) are modest leakages from the electron transport chains in mitochondria, endoplasmic reticulum and chloroplasts.

DANGER OF FREE RADICALS:

There are four types of free radicals damages (Morrison *et al*., 1992):

1. **Damage to fat compounds:** The fatty membranes surrounding the cells being the prime target of free radicals attacks, these damaged membranes then loose its ability to transport oxygen, water or nutrients to the cells.
2. **Damage to protein molecules:** Free radicals also attack the nucleic acid which comprises the genetic code in every cell. The function of nucleic acids is to regulate the normal cell function, growth and repair the damaged tissues.
3. **Cell damage:** Damages done to the chromosomes and nucleic acids might initiate the growth of abnormal cells in the body, which was the first step in cancer development.
4. **Lysosomes damages:** Lysosomes are little sacs in the cell that contain degenerative enzymes. These enzymes leak out when the membrane cell breaks and they start digesting their own cell and also spreads to nearby cell causing a chain reaction of destruction which will lower the immune system resistance.

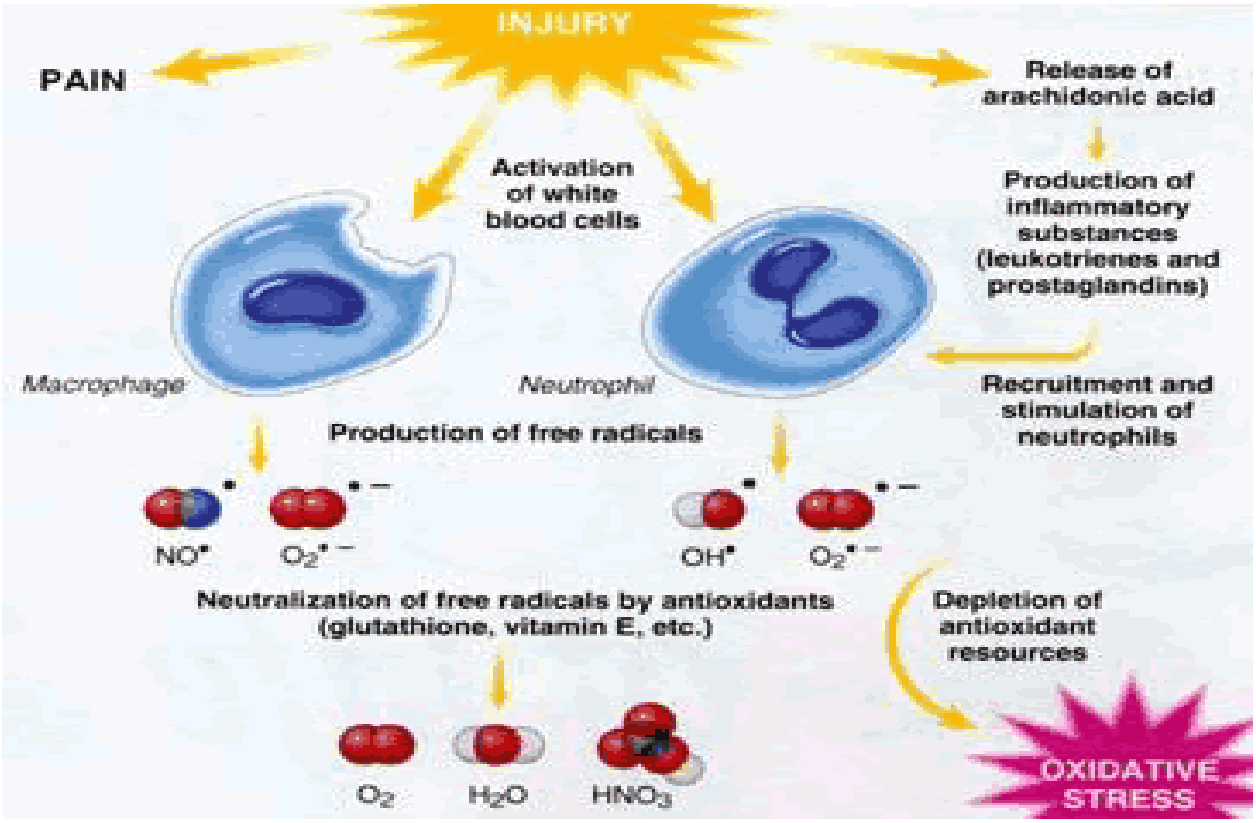


Fig.2: Complications produced by free radicals in the body

EFFECTS OF FREE RADICAL OXIDATION STRESS:

Production of reactive oxygen species and other free radicals (Acworth *et al.,* 1997) and theirs damages to various molecules and cells may result not only in the toxicity of xenobiotics but also results in ageing and various age-related diseases, including :

- **Cardiovascular**: Heart Attack, High Blood Pressure, Angina, Stroke, Atherosclerosis.
- **Lungs** **:** Asthma, Allergies, COPD, Cancer, Chronic Bronchitis
- **Brain** : Alzhemer's, Parkinson's, Migraine, Insomnia, Lack of Mental Clarity, Depression, Bipolar, Dementia
- **Joints** : Osteoarthritis and Rheumatoid arthritis
- **Eyes** **:** Cataracts, Macular & Retinal Degeneration
- **Skin** : Wrinkles, Acne, Skin Aging, Eczema, Cancer, Psoriasis
- **Immune** **:** Cold and Flu, Autoimmune, HIV, Herpes, Cancer, Ulcerative colitis, Chronic Viral Disease, Crohn's, Lupus, Hepatitis
- **Multiorgan** **:** Diabetes, Chronic Fatigue, Fibromyalgia, Heavy metal Toxicity, Aging.

SOURCES OF FREE RADICALS :

Free radicals (Dillard *et al.,* 1978) are produced during the various metabolic activities carried out inside our body and also get exposed to free radicals present in the environment. For example:

Exogenous sources: Sunlight, Automobile Exhaust, Cigarette Smoke, Alcohol Consumption, Emotional Stress, Pollutants, UV light, Xenobiotics, Ionising radiation and Exposure to Heavy metals like Mercury, Lead, Cadmium etc

Endogenous sources: Mitochondrial leak**,** Respiratory burst and Enzyme reactions

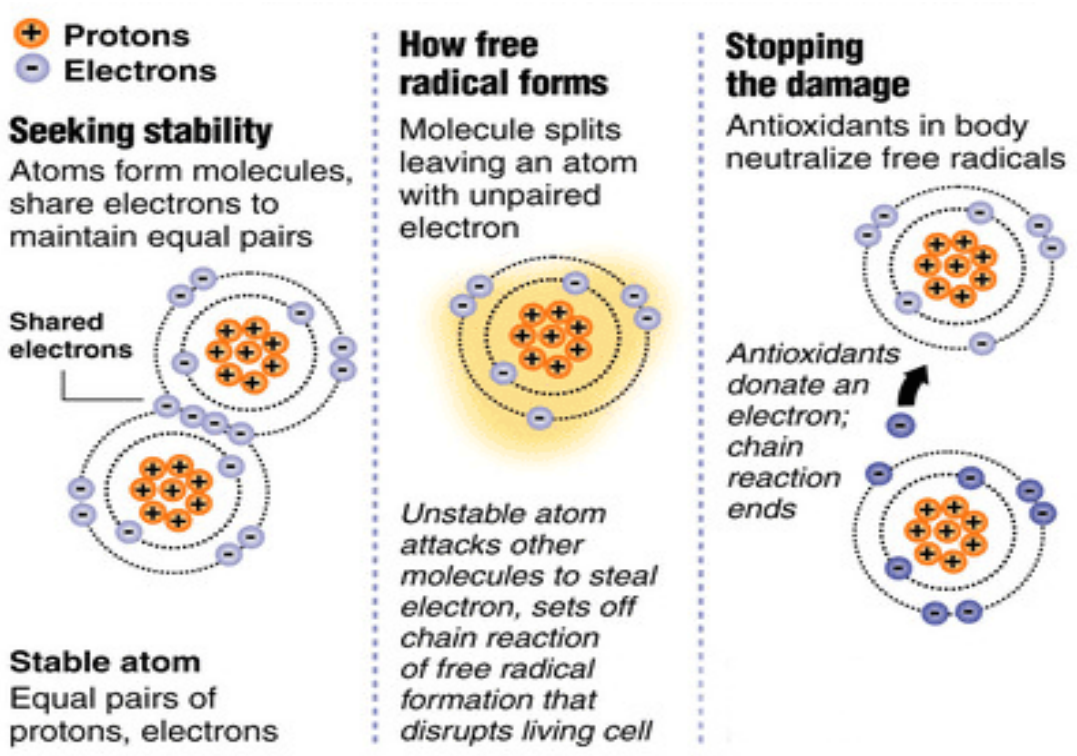


Fig.3: Formation of free radicals and its neutralisation by antioxidants

TYPES OF FREE RADICALS IN THE BODY:

The most important free radicals (Goldfarb., 1999) in the body are the radical derivatives of oxygen better known as reactive oxygen species .These include oxygen in its triplet state or singlet state, superoxide anion, hydroxyl radical , nitric oxide, peroxynitrite, hypochlorous acid, hydrogen peroxide, alkoxyl radical, and the peroxyl radical. Others are carbon-centered

free radical that arises from the attack of an oxidizing radical on an organic molecule. Hydrogen-centered radicals result from attack of the H atom. Another form is the sulfur-centered radical produced in the oxidation of glutathione resulting in the thiyl radical. A nitrogen-centered radical also exists for example the phenyl diazine radical.

Reactive Oxygen Species	
$O_2?^-$	Superoxide radical
?OH	Hydroxyl radical
ROO?	Peroxyl radical
H_2O_2	Hydrogen peroxide
$^1O_2$	Singlet oxygen
NO?	Nitric oxide
$ONOO^-$	Peroxynitrite
HOCl	Hypochlorous acid

Table.2: Biologically significant free radicals.

ANTIOXIDANTS

The body also has several natural chemical means or systems for neutralizing free radicals. These agents that counteract and minimize free radical damage, donate or provide unpaired electrons to which free radicals can attach without causing harm. Antioxidants (Singh *et al*., 2008) are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when body breaks down food or by environmental exposures like radiation and tobacco smoke. Antioxidants are **nutrients** (vitamins and minerals) as well as **enzymes** (proteins in your body that assist in chemical reactions). They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, stroke,

Alzheimer's disease and cataracts. Antioxidant substances include Beta-carotene, Lycopene, Lutein, Selenium and Vitamins A,C,E.

Antioxidants get their name because they prevent oxidation. Oxidation is a reaction in which a molecule looses its electron. The two major sources of antioxidants are:

- Those that you get from food or food supplements
- Those produced within your own body.

TYPES OF ANTIOXIDANTS

- **Antioxidant Nutrients**

  Antioxidants (Karlsson., 1997) from our diet appear to be of great importance in controlling damage by free radicals. Each nutrient is unique in terms of its structure and antioxidant function.

Vitamin E is actually a generic term that refers to all entities (eight found so far) that exhibit biological activity of the isomer tocopherol. Alpha-tocopherol has the highest biopotency, or strongest effect in the body. Because it is fat-soluble, it safeguard cell membranes from damage by free radicals. Alpha-tocopherol also protects the fats in low-density lipoproteins (LDLs or bad cholesterol) from oxidation.

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. As such, it scavenges free radicals that are in an aqueous environment, such as inside your cells. Vitamin C works synergistically with vitamin E to quench free radicals and regenerates the reduced (stable) form of vitamin E.

Beta-carotene, also a water-soluble vitamin, is the most widely studied of the 600 carotenoids identified to date. It is thought to be the best quencher of singlet oxygen. Beta-carotene is also especially excellent at scavenging free radicals in low oxygen concentration.

Selenium is a trace element but without which we could not survive and forms the active site of several antioxidant enzymes including glutathione peroxidase. Similar to selenium, the minerals **zinc** and **manganese** are trace elements that form an essential part of various antioxidant enzymes (Harman., 1956).

- **Antioxidant Enzymes**

  The antioxidant enzymes **superoxide dismutase** (SOD), **catalase (**CAT) and **glutathione peroxidase** (GPx) serve as your primary line of defense in destroying free radicals.

SOD first reduces (adds an electron to) the radical superoxide ($O2^-$) to form hydrogen peroxide (H2O2) and oxygen (O2).

$$2O_{2^-} + 2H \text{ ----SOD--> } H_2O_2 + O_2$$

Catalase and GPx then work simultaneously with the protein glutathione to reduce hydrogen peroxide and ultimately produce water (H_2O).

$$2H_2O_2 \text{ ---CAT---> } H_2O + O_2$$

$$H_2O_2 + 2\text{glutathione } \text{---GPx---> oxidized glutathione} + 2H_2O$$

(The oxidized glutathione is then reduced by another antioxidant enzyme -- glutathione reductase.)

Together, they repair oxidized DNA, destroy oxidized lipids and degrade oxidized protein. Various other enzymes act as a secondary antioxidant defense mechanism to protect you from further damage.

- **Other Antioxidants**

In addition to enzymes, minerals and vitamins, there are many other nutrients and compounds that have antioxidant properties. Among them is **coenzyme Q10** (CoQ10, or ubiquinone), which is essential to energy production and can also protect the body from destructive free radicals. Also product of DNA metabolism, **uric acid**, has become increasingly recognized as an important antioxidant. Additionally, **phytochemicals** are being investigated for their antioxidant activity and health-promoting potential.

ANTIOXIDANT PROCESS

Antioxidants (Kaczmarski *et al.*,1999) block the process of oxidation by **neutralizing** free radicals and themselves become oxidized. Thus there is a constant need to replenish our antioxidant resources. Their work can be classified in one of two ways:

- **Chain-breaking** - When a free radical releases or captures an electron, a second radical is formed. This molecule then turns around and does the same thing to a next molecule, thus generating more unstable products. This process continues until termination occurs, either the radical is neutralised by a chain-breaking antioxidants such as beta-carotene and vitamins C, E or simply it decays into a harmless product.
- **Preventive** - Antioxidant enzymes like catalase, superoxide-dismutase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation by scavenging initiating radicals, such antioxidants can stabilise oxidation chain from ever setting in motion. It also prevent oxidation by stabilizing transition metal radicals such as copper and iron.

The effectiveness of any given antioxidant in the body depends on type of free radical involved, its generation and the target of damage. Thus, in one particular system an antioxidant may protect against free radicals, while in other systems it could have any effect at all. In certain circumstances, an antioxidant may even act as a **pro-oxidant** that generates toxic oxygen species.

ROLE OF MEDICINAL PLANTS IN CHEMOTHERAPY

India is the largest producer of medicinal plants and is rightly called the Botanical garden of the World. Medical information in the old Indian literatures includes several medicinal herbs, which have been in the use for thousands of years, under the indigenous system of medicine. In India, about 45 thousand plant species have been identified, out of which about 15-20 thousand plants are having good medicinal value. However, among these plants, traditional communities use only about 7000-7500 plants for medicinal purposes. The Ayurvedic system of medicine uses about 700, Siddha 600, Unani 700 and the modern medicine uses about 30 medicinal plants for treating a variety of diseases in both man and animal. Only few medicinal plants are attracted by the scientists for investigating them as a remedy for tumour.

More than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various tumour cells. According to the World Health Organization (WHO) estimates, more than 80% of the people in developing countries depend on traditional medicine for their primary health needs. Some medicinal plants and their products including vegetables, fruits and crops play an important role in cancer

prevention. Consumption of huge amount of fruits and vegetables can prevent the development of cancer. Doctors recommend that people who like to reduce their risk of cancer should eat several pieces of fruits and vegetables daily. Several plant-derived products exhibit potent antitumor activity against many cancer cell lines.

A good number of medicinal plants (Khare., 2007) are found mentioned in the ancient classical Ayurvedic texts 'Charaka Samhita'. 'Susruta Samhita' and 'Astanga Hrdaya Samhita'. But many of them still remain to be properly identified. During the process of urbanization the contact with plants in their natural habitat was lost, creating confusion in the correct identity of many plants.

Anticancer properties of plant derived or natural products :

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer (Cragg *et al*., 2005) lists more than 3000 plant species that have reportedly been used in the treatment of cancer. It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms.

Plant derived compounds have played an important role in the development of several clinically useful anticancer agents. Several anticancer agents including taxol, vinblastine, vincristine and topotecan are in clinical use all over the world. Vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), introduced a new era in using plant material as a medication for treatment. They were the first agents in clinical use for the treatment of cancer. Vincristine, Vinblastine are used in combination with other cancer drugs,as the treatment for various kinds of cancers, including breast and lung cancers, leukemias, lymphomas, advanced testicular cancer. A number of promising agents such as combrestatin, betulinic acid and silvesterol are in clinical or preclinical development.

Medicinal plants (Kaur *et al*., 2011) maintain the health and vitality of individual and also cure various diseases including cancer without toxicity. Natural products which are discovered from medicinal plants have played an important role in treatment of cancer. These plants possess good antioxidant and immunomodulatry properties leading to anticancer activity.

Since chemotherapy and radiation cause severe toxicity, herbal plants are becoming popular throughout the world nowadays, and are also used as a therapy for tumors or cancer. The antitumour (antineoplastic) activities of several medicinal plants (Sharma *et al*., 2009) have been reported by various authors. Many of these plants include: *Abrus precatorious* , *Aglaia roxburghiana, Cassia fistula* , *Catharanthus roseus*, *Crocus sativus* , *Ervatamia heyneana* , *Hygrophila spinosa* , *Hippocratea murcantha* , *Indigofera mysorensis* , *Ocimum sanctum* , *Olea polygama* , *Plumbago rosea, Podophyllum hexandrum* , *Semecarpus anacardium* , *Solanum dulcamara, S. indicum* , *S. khasianum, S. surattense* , *Terminalia arjuna, Trigonella foenumgraecum* , *Vanda parviflora, Wedelia calendulacea* , *Withania somnifera* and *Zingiber capitatum* .

Antioxidative properties of plant derived or natural products :

Several herbs and spices (Farrukh *et al.,* 2006) have been reported to exhibit antioxidant activity, including rosemary, turmeric, thyme, nutmeg, white pepper, ginger chili, pepper and several other chinese medicinal plant extracts. The majority of the active antioxidant compounds are flavonoids, flavones, isoflavones, coumarins, lignans, anthocyanins, catechins, and isocatechins. In addition to the above compounds found in natural foods, α-tocopherol, β-carotene and vitamins C and E are known to possess antioxidant potential. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported. Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease.

Many Indian medicinal plants are considered as potential sources of antioxidant compounds. In some cases, their active constituents are known. *Terminalia chebula, T. bellerica, T. muelleri,* and *Phyllanthus emblica,* all of which have antioxidant activity, showed high content of phenolics like gallic acid. Conversely, the antioxidant activity of *Withania somnifera, Hemidesmus indicus, Ocimum sanctum, Cichorium intybus, Mangifera indica and Punica granatum* as determined by several methods have been poorly documented.

In the present study, *Uvaria narum* seeds are used. It is a medicinal plant, fam. annonaceae (Hisham *et al*., 1991) widely distributed in foot hills of Western Ghats and is popularly used in ethno medicine for the treatment of eczema and pityriasis. The plant showed the presence of

phenols, tannins, antioxidants. The presence of these phytoconstituents in the plant has been attributed to its various medicinal properties. The whole root extract of *Uvaria narum* could be medicinally employed as a possible free radical scavenger. The plant may also be considered against ageing and other disease caused by free radicals. Hence intention of the study is to evaluate the antitumor and antioxidant activities in oil extract of their seeds.

REVIEW OF LITERATURE

Cancer remains to be one of the leading causes of death in the United States and around the world. The advent of drug targeted therapies has undeniably improved cancer patients cares. However, advanced metastasized cancer remains untreatable. Hence, continued searching for a safer and more effective chemoprevention and treatment is clearly needed for the improvement of the efficiency and to lower treatment cost for cancer care. Cancer chemoprevention with natural phytochemical compounds is an emerging strategy to prevent, impede, delay, or cure cancer (Wang *et al*., 2012).

Chemoprevention refers to the use of agents to inhibit, reverse or retard tumor genesis. Numerous phytochemicals derived from edible plants have been reported to interfere with a specific stage of the carcinogenic process. Many mechanisms have been shown to account for the anticarcinogenic actions of dietary constituents, but the attention has recently been focused on intracellular-signaling cascades as common molecular targets for various chemo preventive phytochemicals (Surh., 2003).

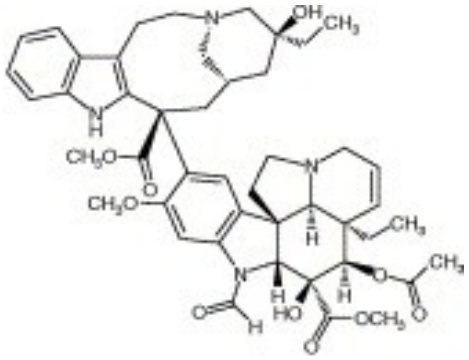
Phytochemicals are compounds made by plant-based foods that consist of nutrients such as vitamins, minerals and antioxidants that impart a great effect in the body. The majority of the phytochemicals are known as antioxidants, carotenoids,beta carotene, folic acid, vitamin C, and vitamin E. Phytochemicals could provide health benefits as: (1) substrates for biochemical reactions; (2) cofactors of enzymatic reactions; (3) inhibitors of enzymatic reactions; (4) absorbents/sequestrants that bind to and eliminate undesirable constituents in the intestine; (5) ligands that agonize or antagonize cell surface or intracellular receptors; (6) scavengers of reactive or toxic chemicals; (7) compounds that enhance the absorption and or stability of essential nutrients; (8) selective growth factors for beneficial gastrointestinal bacteria; (9) fermentation substrates for beneficial oral, gastric or intestinal bacteria; and (10) selective inhibitors of deleterious intestinal bacteria which include terpenoids, phenolics, alkaloids and fiber (Dillard et al., 2000).

There are at least 250,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties. In a study, Taxol and Podophyllotoxin were found to be beneficial in treatment of refractory ovarian, breast and other cancers. Synthetic modification of Podophyllotoxin led to the development of Etoposide, which was known to be effective for small cell cancers of lungs and testes. Camptothecin, isolated from

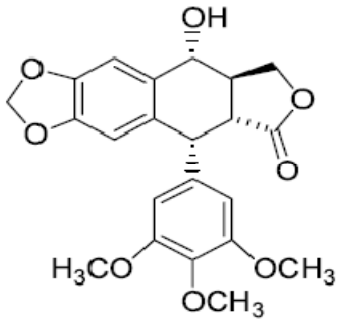
Camptotheca acuminata also have been extensively studied. Other important compounds include Vincristine, Vinblastine, Colchicine, Ellipticine and Lepachol along with Flavopiridol (Mukherjee et al., 2001).

MAJOR PLANT DERIVED CHEMOTHERAPEUTIC DRUGS:

Since 1961, nine plant-derived compounds have been approved for use as anticancer drugs in the US: vinblastine (Velban), vincristine (Oncovin), etoposide (VP-16, 1), teniposide (VM-26, 2), Taxol (paclitaxel), navelbine (Vinorelbine), taxotere (Docetaxel), topotecan (Hycamtin), and irinotecan (Camptosar). The last three drugs were approved by the Food and Drug Administration in 1996, (Dholwani et al., 2008)



Vincristine



Podophyllotoxin

Sl.no:	Compound	Source	Mechanism	Cancer Use
1	Vincristine	Catharanthus roseus	mitotic block	Lymphoma, Leukemia, breast, lung cancers
2	Vinblastine	Catharanthus roseus	mitotic block	Breast, lymphoma
3	Paclitaxel	Taxus brevifolia Nutt, Taxus baccata	Anti-mitotic	Ovary, lung, breast, bladder
4	Docetaxel	Taxus brevifolia Nutt, Taxus baccata	Anti-mitotic	Breast and lung cancer
5	Topotecan	Camptotheca acuminate	DNA topoisomerase I inhibition	Ovarian, lung and pediatric cancer
6	Irinotecan	Camptotheca acuminate	DNA topoisomerase I inhibition	Colorectal and lung cancer
7	Flavopiridol	Amoora rohituka & Dysoxylum binectariferum	Inhibits cell cycle progression at G1 or G2 phase	colorectal, lung and renal cell carcinoma, non-Hodgkin's lymphoma, leukemia, solid tumors
8	Ellipticine	Ochrosia borbonica, Excavatia coccinea, Ochrosia elliptica	DNA intercalation and inhibition of topoisomerase II	Various cancer cell types
9	Etoposide and Teniposide	Podophyllum peltatum and Podophyllum emodi	not known	Lymphomas, bronchial and testicular cancers
10	Colchicine	Colchicum autumnale and Gloriosa superba L.	Anti-mitotic	Leukemia, solid tumors

Table.3: Plant-derived anticancer agents

MAJOR SETBACK OF EXISTING CHEMOTHERAPEUTIC DRUGS:

Chemotherapeutic techniques have a range of side-effects that depend on the type of medications used. Most medications affect mainly the fast-dividing cells of the body, such as blood cells and cells lining the mouth, stomach, and intestines, (Keidan et al.,1989). Common side-effects include:

- Depression of the immune system, which results in potentially fatal infections such as typhlitis.
- Fatigue. The treatment can be physically exhausting the patient, who might be already very tired from cancer-related fatigue. It may also produce mild to severe anemia.
- Tendency to bleed easily. Medications that kill rapidly dividing cells or blood cells are likely to reduce the number of platelets in the blood, which can result in bleeding.
- Gastrointestinal distress. Nausea and vomiting are the common side effects of chemotherapeutic medications. This also produces diarrhea or constipation. Malnutrition and dehydration may result when the patient did not eat or drink enough, or when the patient vomits frequently, because of the gastrointestinal damage.
- Hair loss
- Myelosuppression and Immunosuppression
- Typhlitis (life-threatening gastrointestinal complication of chemotherapy)
- Secondary neoplasm
- Infertility
- Teratogenicity
- Neurological adverse effects

Damage to some specific organs is also possible:

- Cardiotoxicity (heart damage)
- Hepatotoxicity (liver damage)
- Nephrotoxicity (kidney damage)

- Ototoxicity, producing vertigo
- Encephalopathy (brain dysfunction)

OTHER STUDIES OF PHYTOCHEMICALS RELATED TO CANCER DRUG DEVELOPMENT:

A clinical and preclinical study was conducted in six adult patients with non resectable liver cancer as well as in mature New Zealand white rabbits with implanted VX2 carcinoma in the liver. An oily anticancer drug injected into the hepatoproximal lumen of the ligated hepatic artery can intensify the anticancer effects of a ligation of the hepatic artery for liver cancer was concluded from that study, (Kenichiro *et al*., 2006). Another antitumor study with MDV3100 was conducted in patients with castration-resistant prostate cancer by noting antitumor effects at all doses, (Howard *et al*., 2010).

The antitumor activity of saffron (*Crocus sativus*) extract against intraperitoneally transplanted sarcoma-180 (S-180), Ehrlich ascites Carcinoma (EAC) and Dalton's lymphoma ascites (DLA) tumors in mice was studied, (Nair *et al*.,1991). In a study Curcumin (diferuloylmethane), polyphenol derived from the plant *Curcuma longa* was studied for its anticancer potential to suppress proliferation of a wide variety of tumor cells, down-regulate transcription factors and down-regulate the expression of COX2, LOX, NOS, chemokines, TNF, cell surface adhesion molecules and cyclin D1. It also down-regulate growth factor receptors (such as EGFR and HER2); and inhibit the activity of protein tyrosine kinases, N-terminal kinase and protein serine/ threonine kinases (Bharat *et al.,* 2003).

In yet another antitumor study, foccused on flavanoids, polyphenolic compounds (Lazaro., 2003). The role of dietary flavanoids in cancer prevention was widely discussed. Comparing data from laboratory studies, human clinical trials and epidemiological investigations, indicated that flavanoids have important effects on cancer chemoprevention and chemotherapy. Many different mechanisms of action was identified, including carcinogen inactivation, cell cycle arrest, anti proliferation, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multi drug resistance or a combination of these mechanisms (Ren *et al*., 2003).

RELEVANT STUDIES OF ANNONACEAE FAMILY AND *UVARIA* SPECIES RELATED TO CHEMOTHERAPEUTIC DRUG DEVELOPMENT:

Another work on evaluation of anti-tumor constituent from Annonaceae plant family were done by isolating 50 new Annonaceous acetogenins, 12 new styrylpyrones and 25 new polyoxygenated cyclohexenes from 5 *Uvaria* species. Preliminary bioassay tests showed most of the new isolates exhibit significant anti-tumor activities (Yu., 1999).

In addition to the cytotoxic, antitumor, and antimicrobial activities of plant extracts from the Annonaceae family, other root bark extracts are reported in the literature as having medicinal purposes. One compound, 15-desacetylundulatone, a quassinoid, was found active against P-388 mouse lymphocytic leukemia cells and Colon-38 adenocarcmoma (Lumonadio *et al*., 1991).

By activity-directed fractionation, goniothalamicin and annonacin, two acetogenins, was isolated from the ethanolic extracts of the stem bark of Goniothalamus giganteus Hook. of Annonaceae family and studied for cytotoxic and insecticidal activity and was found to be weakly active against 3PS murine leukemia (Alkofahi.,1988). Similarly, three bioactive annonaceous acetogenins, annomocherin, annonacin and annomontacin, had been isolated from the fractionated ethanolic extracts of the seeds of Annona cherimolia, supported by brine shrimp lethality test (BST) and their cytotoxicities against the breast carcinoma (MCF-7) and kidney carcinoma (A-498) cell lines was studied (Kim *et al.,* 2001).

Similar anticancer studies was conducted by isolating three annonaceous acetogenins from seeds of Annona cherimolia (Woo *et al*., 1999); another by isolating flavone from the stems and leaves of *Dasymaschalon trichophorum* Merr. of annonaceae family (Liu *et al*., 1992) and yet another by isolating Annonaceous acetogenins from the ethanolic extract of the bark of Annona bullata of annonaceae family (Hui *et al.,* 1992).

The genus *Uvaria*, of annonaceae have been found to contain compounds such as acetogenins useful as pesticides, and recently as anticarcinogenic agents. The compounds taken from the roots of *Uvaria chamai* has also been implicated as a causative agent for liver cancer. Two separate studies out of sub-Saharan Africa have shown that the active compound in *Uvaria chamaii*, chamuvaritin, has shown that it has mutagenic effects. One of the studies also showed that sub-Saharan Africa is a high incidence area for hepatocellular carcinoma. Although the

disease is multi-factorial in etiology, including a combination of causative agents such as Hepatitis B Virus, and chemical carcinogens, the compounds contained in the medicinal plants were implicated as potential sources of causative agents for liver cancer (Uwaifo *et al*., 1984 and Uwaifo *et al*., 1972).

The phytochemical analysis and cytotoxic activity of leaves of *Uvaria chamae* against L_{929} transformed cells was studied and found that at a concentration corresponding to their IC50 against L_{929} cells, they were nontoxic against mouse thymocytes (Philipov *et al*., 2000).

In a Ethnobotanical survey of the plants used in the management of cancer, carried out in Ogun State, Nigeria, seventy-three different species of Angiosperms were found to be used for the management of cancer. Out of this bark of *Uvaria chamae* and roots of *Uvaria afzelii*, annonaceae family was found to be effective as anticancer drug (Mike *et al*., 2010). Another anticancer study was studied in the roots of the *Uvaria acuminata* by isolating Uvaricin, a potent anticancer compound from the plant (Jolad *et al*., 1982).

RELEVANT STUDIES OF ANNONACEAE FAMILY AND *UVARIA* SPECIES RELATED TO ANTIOXIDANT THERAPY:

The antioxidant and antibacterial activities of *Uvaria narum (Dunal)* Wall was studied in the essential oil and different plant extracts (petroleum ether, ethyl acetate, chloroform and methanol) which showed that the leaf methanol extract and leaf essential oil of *Uvaria narum* are good sources of natural antioxidants and antimicrobial compounds and can be incorporated into the drug formulations (Joji *et al*., 2012).

Other reported literature has shown that the compound, Uvaricin, taken from the purified root extracts of *Uvaria acuminata*, another Annonaceous plant, demonstrated an in vitro activity against P-388 lymphocytic leukemia in mice (Jolad *et al.,* 1982). Another extract, Uvaratin, also from *Uvaria acuminata*, was also shown to have anti-tumor activity (Cole *et al*., 1976).

OTHER STUDIES OF PYTOCHEMICALS RELATED TO ANTIOXIDANT THERAPY:

The *in vitro* antioxidant activity of melatonin was studied using thiocyanate method. Also, the reducing power, superoxide anion scavenging activity and free radical scavenging activity of melatonin were determined and were found that melatonin exhibited potent antioxidant activity in a linoleic acid emulsion system (Gulcin *et al*., 2002). Another antioxidant activity was studied on extract of *Mangifera indica* L. (Vimang) by using commonly accepted assays

and the extract showed a significant inhibitory effect on the peroxidation of rat-brain phospholipid and inhibited DNA damage by bleomycin or copper-phenanthroline systems, (Gregorio *et al*., 2000).

In a study it was concluded that tea possesses a strong antioxidant activity (*in vitro)* which was believed to be exerted by its poly phenols moiety and also tea had a potent *in vivo* activity in man. The inhibition of this effect by milk was thought to be due to the complexation of tea polyphenols by milk proteins. Thus it clarified the putative role of dietary poly- phenols in modulating oxidative stress *in vivo* (Serafini *et al*., 1996).

Similarly antioxidant activities of the methanolic extract of *Cinnamomum verum* bark was studied and evaluated with reference to antioxidant compounds like butylated hydroxyl anisole, trolox and ascorbic acid (Mathew *et al*., 2006**).** A Polyherbal Formulation (PHF), Rumalaya Forte that has a combination of medicinal herbs such as the powders of *Boswellia serrata, Alpinia galanga, Commiphora wightii* and *Glycyrrhiza glabra* and the extracts of *Tribulus terrestris* and *Tinospora cordifolia* was tested for its antioxidant and free radical scavenging activity in vitro and demonstrated a significant NO free radical scavenging activity **(**Subash *et al.*, 2012).

Another *in vitro* and *in vivo* antioxidant study was conducted in alcoholic and aqueous extracts of the roots of *Toddalia asiatica* and it was concluded that the extracts exhibited potential scavenging effects on hydrogen peroxide, DPPH and nitric oxide free radicals. The in vivo antioxidant activity was also investigated using Wistar albino rats **(**Madhavan *et al*., 2010). In a study the *in vitro* antioxidant effect of the ethanolic extract of *Mimosa pudica* (Mimosaceae) was evaluated against free radical damage by different standard methods such as DPPH, Nitric Oxide, ABTS and Hydrogen peroxide free radical model. Out of these four free radicals the extract showed potent activity on DPPH and Nitric oxide, which is compared to that of ascorbic acid and rutin taken as standards (Muthukumaran *et al*., 2011).

The alcoholic extract of *Medicago sativa* was studied for its *in vitro* antioxidant activity using different models namely DPPH and ABTS radical scavenging, iron chelates activity, lipid peroxidation assay, alkaline DMSO assay and nitric oxide scavenging assay. The results were analyzed statistically by the regression method and indicate that *Medicago sativa* was effective against free radical mediated disease (Rana *et al*., 2010). Similarly, the antioxidant activity of punicalagin isolated from pomegranate juice was also studied (Cerda *et al*., 2004).

In a study the total phenolic, flavonoid contents and in vitro antioxidant activity of methanol extract of *Dioscorea esculenta* (Lour). Burkill was evaluated. Total phenolic content was estimated using the Folin Ciocalteu method, flavonoid content using aluminium chloride and In vitro antioxidant activities and reducing power capacity were determined using standard methods (Manickam *et al*., 2012).

OTHER RELEVANT STUDIES OF ANNONACEAE FAMILY AND *UVARIA* SPECIES:

In a study the phytochemical analysis of the root barks essential oil of *Uvaria narum* Wall. (Annonaceae) were done by GC/MS and about 52 components were detected, out of which 22 components were identified. Bornyl acetate (15.2%) and patchoulenone (8.1%), a tricyclic sesquiterpene ketone were found to be the major constituents of the oil (Abdul *et al*., 1992).

The antiproliferative activity of Madagascan plant *Uvaria* sp. against the A_{2780} ovarian cancer cell line was done by isolating two new acetogenins from the plant and it was observed that the two acetogenins display weak antiproliferative activity against the A_{2780} ovarian cancer, the A_{2058} melanoma, and the H_{522} lung cancer cell lines (Dai *et al*.,2012). The antibacterial, antifungal and anthelmintic activities for the hexane and ethyl acetate extracts of the root barks of *Uvaria narum* Wall. and *Uvaria hookeri* King was evaluated in one of the study (Padmaja *et al*., 1993). Similarly, the antibacterial and antioxidant potential of the root of *Uvaria narum* (Dunal) Wall was carried out by using various extracts in another study (Padyana *et al*., 2011).

In fact, the literature contains numerous reports that plant extracts from the Annonaceae family have been investigated for their medicinal and toxicological effects. In one pharmacological screening, substantial antibacterial, antifungal, and anthelminthic activities were observed using extracts of the root barks of *Uvaria narum* wall, and *Uvaria hookeri* king (Padmaja *et al*., 1993).

The hepatoprotective potential of leaves of *Uvaria narum* (Annonaceae) by noting elevation in hepatic biomarkers like SGPT, SGOT, ALP, bilirubin & other biochemical parameters like Cholesterol, triglycerides, urea & tissue LPO, and decrease in total protein, albumin, glucose & tissue GSH, CAT, SOD in CCl_4 induced liver toxicity (Jagir *et al*., 2013). Yet another species, *Uvaria chamae*, has also been reported to have anti-microbial activities. In that study, cytotoxic C-benzylated flavonoids exhibited anti-microbial inhibition which compared

favorably to that of streptomycin sulfate, an inhibitor of protein synthesis (Parmer *et al*., 1978).

In a study, venom neutralizing properties of *Uvaria chamae* was evaluated by *in vivo* neutralization of *Naja nigricollis* venom in rats. For this *Uvaria chamae* leaves were extracted in methanol and the results indicated the presence of potent snake venom neutralizing capacity in the plant extract (James *et al*., 2013).

Other reported literature has shown that the compound, Uvaricin, taken from the purified root extracts of *Uvaria acuminata*, another Annonaceous plant, demonstrated an *in vitro* activity against P-388 lymphocytic leukemia in mice (Jolad *et al.,* 1982). Another extract, Uvaratin, also from *Uvaria acuminata*, was also shown to have anti-tumor activity (Cole *et al*., 1976).

In another study, species from *Uvaria* originating from Tanzanian plants were tested for their *in vitro* activity against the multi-drug resistant K I strain of *Plasmodium falciparum*, the causative agent for the disease malaria. In this study, root extracts from the stem and root barks of *Uvaria iucida* and *Uvaria* sp. (pande), were reported as having anti-malarial activity. Among the compounds isolated in this study were Uvaretin, Diuveretin, and 8',9'-dihydroxy-3-faraesylindole as the most active compounds The particular *Uvaria* species consisted of nine varieties from Tanzania and included*: U dependens, U.faulknerae, U. kirkii, U. leptocaldon, U. Iucida, Uvaria sp. (Pande), U. scheffleri, and U. tanzaniae* (Nkunya *et al*., 1991).

A *in vitro* study was done on the acaricidal activity of three *Uvaria* species, namely *U. klaineana, U. mocoli* and *U. versicolor* against *Dermatophagoides pteronyssinus*, the European house dust mite (Akendengue *et al*.,2003). In another study the methanolic extracts of the stem, root and leaves of *Uvaria chamae* were evaluated for their antibacterial activity against Methicillin-Resistant *S. aureus, S. aureus, E. coli, Klebsiella spp, Proteus spp,* and *P. aeruginosa* using standard agar diffusion method. Out of this methanoilc leaf extract was found to be significant (Oluremi *et al*., 2010). The antiplasmodial activity of *Pleiocarpa mutica, Cleistopholis patens* and *Uvaria chamae* were studied by screening its activity against *P. falciparum* (Jonathan *et al*., 2001 and Nkunya *et al*.,1991).

Another study focussed on the antioxidant and antibacterial activities of hexane, chloroform, ethanol extracts of leaves and barks of *Uvaria grandiflora* Roxb (Annonaceae) and found that flavonoids can be responsible for the strong antibacterial activity existed in the bark ethanol

extract (Noushin *et al*., 2011). The antifungal activity of dichloromethane, petroleum ether and ethanolic extracts of the stem bark and leaves of *Uvaria scheffleri* Diels (Annonaceae) was studied against *Aspergillus niger* , *Aspergillus fumigatus* and *Penicillium* species (Moshi *et al*., 2004).

OBJECTIVES

- Preparation of oil extract and its fractions of *Uvaria narum* seed.
- Screening of Phytochemical constituents of seed oil extract using standard assays as well as purification by Thin Layer Chromatography.
- Analysis of antioxidant activity of seed oil extract and its selected fractions using various *In vitro* assays.
- Analysis of *In vivo* antitumor activity of oil extract of seeds of *Uvaria narum* in mice using DLA induced solid tumor model.

PLAN OF THE WORK

The plan of work for the study of *Uvaria narum* was carried out as follows:-

A. Collection & authentication of raw materials

B. Extraction & separation of seed oil extract

C. Preliminary phytochemical analysis of seed oil extract

D. Separation of seed oil extract using HPTLC and TLC methods and Qualitative phytochemical studies

E. Pharmacological studies

- *In vitro* cytotoxic assay using DLA,EAC cell lines and Primary Spleen cells of rats
- *In vitro* antioxidant activity of seed oil extract and its fraction by:

a) Superoxide radical scavenging activity
b) DPPH radical reducing activity
c) AAPH induced human erythrocyte lysis activity
d) Ferric reducing activity
e) Hydroxyl radical scavenging activity

- *In vivo* antitumor activity by DLA induced solid tumor model to assess :

a) Body weight of tumor bearing mice
b) Total WBC count of tumor bearing mice
c) Hemoglobin count of tumor bearing mice
d) Tumor volume of treated groups

PLANT PROFILE

GENERAL DETAILS

Binomial Name	**:**	*Uvaria narum* (Dunal) Wall
Family	**:**	Annonaceae
Common Name	**:**	Narumpanal, Pulikkan
Habit	**:**	Shrub or Woody climber
Flowering Class	**:**	Dicot
Part Used	**:**	Seeds

TAXONOMICAL CLASSIFICATION

Kingdom	**:**	Plantae
Phylum	:	Magnoliophyta
Class	**:**	Magnoliatae
Order	**:**	Magnoliales
Family	**:**	Annonaceae
Genus	**:**	Uvaria

VERNACULAR NAMES

Malayalam	**:**	Narumpanal, Kureel
Tamil	**:**	Puliccan, Pulikkan
Sanskrit	**:**	Neelavalli, Valeeshakhota
Kannada	**:**	Bugadee balli, Bugadee hoo, Guavaara
Marathi	**:**	Kala-apkara

DISTRIBUTION:

Global Distribution : South India and Sri Lanka

Indian distribution:

Uvaria narum (Hishama *et al.,* 1990) is mainly seen in the dense forest of Western Ghats. It occasionally distributed in the Southern dry mixed deciduous forests. The plant is also common in the forests associated with temples or 'kavu'.

The plant is specifically seen in :

Maharashtra : Sindhudurg, Raigad, Kolhapur, Satara

Karnataka : Coorg, Chikmagalur, Mysore, N and S. Kanara

Kerala : All districts

Tamil Nadu : Madurai, Dharmapuri, Nilgiri, Salem, Namakkal, Kanniyakumari, Tiruvannamalai, Vellore, Viluppuram, Tirunelveli

PLANT DESCRIPTION:

Uvaria is a genus of flowering plants in the soursop family (Padmaja *et al.*,1993), Annonaceae. The generic name *Uvaria* is derived from the Latin *uva* meaning grape, because the edible fruits of some species in this genus resemble grapes. Scandent shrubs, branchlets sparsely hairy. It is a large climbing shrub with woody stems. *Uvaria* is a large woody stellately pubescent straggling shrub with dark bluish green leaves.

Leaves - 13-16 x 4-5 cm, elliptic, lanceolate, apex acuminate, base rounded,oblong pointed or long-pointed, stalks short less than 6 mm and leaves hairless on both sides. Crushed leaves smell like cinnamon.

Flowers - reddish, terminal or axillary, solitary, leaf opposed; tomentose, pedicels to 1 cm; sepals 8 x 5 mm, broadly ovate, basally connate; petals fleshy with outer 2 x 1.5 cm and inner 2 x 1 cm, obovate, apex incurved, golden-brown; Stamens have anthers concealed by the overlapping connectives; carpel's numerous, 5 mm, oblong, scarlet-red , tomentose.

Seeds - ovoid or compressed, chestnut brown, sub orbicular, in a single row, usually 4-5,those at the ends of carpels plano-convex, the intermediate ones compressed, nearly flat.

SOIL REQUIREMENTS:

Sandy loam soil with loose structure, good drainage.

CHEMICAL CONSTITUENTS:

Generally plant (Padyana *et al.,* 2011) contains Acetogenins, including stereoisomer's, Glutinone, Glutinol, Taraxerol, Beta sitosterol, Benzyl benzoate15.2%, & Patchoulenone. Benzoic acid ester, 2-E-(2-oxo-cyclopent-3-en-1-ylidene)ethyl benzoate, together with tetratriacontanol, tritriacontane and β-sitosterol isolate from leaves, Squamocin-28-one, Panalicin, Uvariamicin-I, II & III, novel Acetogenins isolated from bark.

The leaf oils (Ahmad *et al.,* 1998) of some genus of Annonaceae were analyzed by capillary gas chromatography (GC) and GC coupled with mass spectrometry (GC/MS). The major compounds identified were α-gurjunene (21.9%), α-phellandrene (20.1%) and bicycloelemene (9.6%). The oil contained β-selinene (12.3%), bicyclogermacrene (10.9%), caryophyllene oxide (10.4%) and bicycloelemene (5.6%) in abundance. bicycloelemene (18.3%), germacrene D (30.2%), bicyclogermacrene (26.4%) and β-bisabolene (7.7%) as the major compounds of *Uvaria*, while γ-elemene (54.0%) was the most singly abundant constituent of *Uvaria dac* oil. All the other compounds were identified in amount less than 5% with the most prominent ones being myrcene (3.8%) and limonene (3.8%).

The root bark (Schultes., 1960) essential oil were analyzed by GC/MS and about 52 components were detected, out of which 22 components were identified. Bornyl acetate

(15.2%) and patchoulenone (8.1%), a tricyclic sesquiterpene ketone, were found to be the major individual constituents of the oil.

MEDICINAL PROPERTIES:

Uvaria narum (Tran *et al.,* 2013) is mainly used in eczema, itching, varicose vein, haemorrhoids, jaundice, inflammation and fever. Leaves are recommended in rheumatic swellings, jaundice, biliousness and fevers. Root is used in the treatment of jaundice, fever, biliousness and typhoid. A decoction of the root bark is given to women to control fits at the time of delivery. It is also used in rheumatism, bowel complaints of Children, for eczema and used in skin diseases.. Oil Extracted from the root reduces burning sensation of the Liver. The whole root extract of *Uvaria narum* could be medicinally employed as a possible free radical scavenger. The plant may also be considered against ageing and other disease caused by free radicals. The aerial parts of *Uvaria* are used in stomach disorder.

USEFUL PARTS:

Aerial parts, Roots, Leaves and Seeds.

KNOWN SPECIES:

- *Uvaria calamistrata*
- *Uvaria chamae* – Finger-root
- *Uvaria grandiflora*
- *Uvaria kweichowensis*
- *Uvaria rufa* (Dunal)
- *Uvaria purpurea*

MATERIALS & METHODS

MATERIALS

I. COLLECTION OF PLANT MATERIAL :

Uvaria narum were collected locally from Thrissur, Kerala and identified using the herbarium sheets at Kerala Forest Research Institute, Peechi by Dr. Sujanapal. P (Taxonomist, KFRI, Peechi). A voucher specimen was kept (Voucher Number. KFRI PS 19033; Kannimara, Parambikulam Tiger Reserve).

II. ANIMALS :

Male Balb/C mice (average body weight 25-35g), were purchased from the Small Animal Breeding Station (SABS), Agricultural University, Mannuthy, Kerala, India. The animals were maintained under standardized environmental conditions (22-28^{o}C , 60-70% relative humidity, 12 hr dark/light cycle) in animal house of Amala Cancer Research Centre. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (No:-149/ 1999/ CPCSEA).

III. CELL LINES :

Cancer cell lines:

Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells maintained in the peritoneal cavity of mice (Swiss albino), obtained from Amala Cancer Research Centre were used in the study.

Normal cells (spleenocytes):

Male Sprague-Dawley rats weighing 250-300g were anaesthetized with chloroform and dissected. The spleen was removed and collected in a sterile nylon sieve over a petridish half filled with PBS. The spleen was gently pressed through the sieve using a plunger; PBS was then added to keep the cells moist. The disaggregated spleenocytes were transferred to a test tube and centrifuged at 1000 rpm for 5 minutes. Repeated the centrifugation process until the

suspension become free of debris. The cell pellet was re-suspended in PBS and counted using a haemocytometer.

IV. REAGENTS AND CHEMICALS :

2,2' azobis 2 amidino propane hydrochloride (AAPH), Nitro blue tetrazolium (NBT) and riboflavin were purchased from SIGMA chemical Company Inc., St. Louis, MO, USA. Ethylenediaminotetraacetic acid (EDTA) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbituric acid (TBA) was purchased from Hi-media Laboratories, Mumbai, India. Ascorbic acid and Deoxyribose from MERCK chemical company. Drabkin's reagent from Agappe Laboratories Ltd, Mumbai. All other chemicals and reagents used in this study were of analytical grade and procured from reputed Indian manufacturers.

METHODS

I. PREPARATION OF EXTRACT FROM SEEDS :

Uvaria narum seeds were dried in an oven at 40^0C, powdered using grinder and subjected for extraction using n-hexane and 95% ethanol. Approximately 20g of seeds was accurately weighed and powdered. The seeds were then defatted using 95% ethanol in 5% (w/v) suspension by mixing with a magnetic stirrer for 60 minutes. Supernatant was collected by centrifugation (3000 rpm, 45min) and kept for evaporation at room temperature. The residue thus obtained was again defatted using n-hexane (150ml) with the aid of a magnetic stirrer for overnight. The supernatant was collected by centrifugation (3000 rpm, 45min). Each time the supernatants obtained by centrifugation were saved. Finally the supernatants of each extraction were pooled, concentrated and evaporated at room temperature.

Two portions were obtained from the extract- oil portion and the water portion. Oil portion is redissolved in ethanol and used for further studies. The extracts were stored separately in air-tight bottles and kept below 4^0C. For phytochemical screening, oil extract is dissolved in ethanol, for *in vitro* antioxidant studies extract was dissolved in DMSO and for animal studies, dissolved in propylene glycol.

II. CYTOTOXICITY ANALYSIS OF SEED OIL EXTRACT:

(Short term in vitro cytotoxicity in normal and cancer cells)

METHOD: Trypan blue dye exclusion method (*Talwar.,* 1974)

PRINCIPLE:

In the normal healthy cells where the plasma membrane is intact, Trypan blue cannot enter while in cells where the cell integrity is lost, the dye enters and stain blue. Since live cells exclude the dye this method is a described as Dye- Exclusion method and used to confirm the actual cell death in a population.

PROCEDURE:

Cytotoxicity of the *U.narum* seed oil extract towards cancer and non- cancerous cells were assessed using Trypan blue method. Approximately $1x10^6$ DLA, EAC or Spleen cells were diluted to 1 ml (adding 0.9ml) with PBS, pH 7.4. From this stock cells (100µl) was added to different test tubes containing 800 µl PBS. . Different concentration of seed oil extract and individual fractions (0-100 µg/ ml) were then added to make up volume in each test tube to 1ml. Incubation was carried out for 3 hr at 37^0C. At the end of the experiments, 100µl tryphan blue was added to each tube and waited for 3minute. The cell suspension (10 µl) were applied on to a haemocytometer and observed under microscope. Live cells (non stained cells) and dead cells (blue stained cells) were separately counted and percentage cell death was determined. The percentage values were plotted on a graph against concentrations and concentration needed to induce 50% cell death (IC_{50}) was determined.

CALCULATION:

$$\textbf{\% Cell death} = \frac{\boldsymbol{number\ of\ dead\ cells}}{\boldsymbol{Total\ number\ of\ cells}} \times \boldsymbol{100}$$

III. PHYTOCHEMICAL ANALYSIS OF SEED OIL EXTRACT :

The seed oil extract obtained with n-hexane as eluent and dissolved in ethanol was used to test for the presence of various phytochemicals according to standard procedure (Rajan S *et al*., 2011). Phytochemicals such as tannins, terpenoids, flavanoids, steroids, coumarins, emodins, sterols, alkaloids and leucoanthocyanins are tested using standard methods and compared with ethanol as control.

PROCEDURE:

1) **TEST FOR TANNINS :**

Lead acetate Method

- To 2ml of the extract, added few drops of 1% lead acetate. A yellowish precipitate indicate the presence of tannins.

Ferric Chloride method

- Firstly the extract is mixed with water, heated on water bath and filtered. 4ml of 5% ferric chloride was added to 2ml of extract. Formation of dark green precipitate indicates the presence of tannins.

Potassium hydroxide method

- About 4ml of freshly prepared 10% potassium hydroxide solution was added to 4ml of concentrated extract. Formation of dirty white precipitate indicates presence of tannins.

2) **TEST FOR TERPENOIDS :**

Salkowski test:

- Acetic anhydride and concentrated sulphuric acid was added to 2ml of the extract. Formation of blue green ring indicates the presence of terpenoids.

Sulphuric acid test

- About 5 drops of concentrated sulphuric acid was added to 1ml of oil extract. Development of yellow color at lower layer suggests triterpeniods.

3) **TEST FOR STEROIDS :**

Liebermann Buchard test:

- About 1ml of acetic anhydride was added to 1ml of chloroform and cooled to 0^0C. Then one drop of concentrated sulphuric acid was added to the cooled mixture followed by the extract. The solution was observed for blue, green, red or orange colour that changes with time.

Salkowski test:

- Acetic anhydride and concentrated sulphuric acid was added to 2ml of the extract. Formation of red color indicates the presence of steroids.

4) **TEST FOR GLYCOSIDES :**

Extract was hydrolyzed with dilute hydrochloric acid for few hours on water bath and the hydrolysate was subjected to the following tests:

Legal's test:

- To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside was added and then made alkaline with sodium hydroxide solution. Appearance of pink to red color indicates the presence of glycosides.

Borntrager's test:

- To the hydrolysate chloroform was added and the chloroform layer separated. To this equal volume of dilute ammonia was added. Ammoniacal layer acquires pink color indicating the presence of glycosides.

5) TEST FOR FLAVONOIDS :

Sodium hydroxide test:

- About 2ml of extract was dissolved in 10% aqueous sodium hydroxide solution and filtered to give yellow color. A change in color from yellow to colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids.

Ferric chloride test:

- Extract was boiled with water and filtered to 2ml of the filtrate. Two drops of freshly prepared ferric chloride solution was added. Green, blue or violet colorations indicate the presence of flavonoids.

Sulphuric acid test:

- The extract was dissolved in concentrated sulphuric acid and the color change was observed. Yellow color indicates the presence of flavonoids.

6) TEST FOR COUMARINS :

About 3ml of 10% sodium hydroxide was added to 2ml of aqueous extract. Formation of yellow color indicates the presence of coumarins.

7) TEST FOR EMODINS :

About 2ml of ammonium hydroxide and 3ml of benzene was added to the extract. Appearance of red color indicates the presence of emodins.

8) TEST FOR SAPONINS :

About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

9) TEST FOR LEUCOANTHOCYANINS :

About 5ml of aqueous extract was added to 5ml of isoamyl alcohol. Upper layer appears red in color indicate the presence of leucoanthocyanins.

10) TEST FOR ALKALOIDS :

About 0.5g of extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3ml of the filtered was divided into three portions:

a) To the first portion, a few drops of freshly prepared Dragondorff's reagent was added and observed for formation of orange to brownish precipitate.

b) To the second portion, one drop of Mayer's reagent was added and observed for the formation of white to yellowish or cream color precipitate.

c) To the third portion, one drop of Wagner's reagent was added to give a brown or reddish brown precipitate.

11) TEST FOR CARBOHYDRATES :

Molisch's test:

- To the extract 2-3 drops of 1% alcoholic α- naphthol is added, then 2ml of concentrated sulphuric acid is added along the sides of the test tube. Appearance of brown ring at the junction of two liquids indicates the presence of carbohydrates.

Benedict's test:

- To the extract Benedict's reagent is added. Appearance of blue colour indicates the presence of carbohydrates.

Fehling's test:

- To the extract Fehling's solution A and B is added and heated on water bath. Appearance of red precipitate indicates the presence of carbohydrates.

12) TEST FOR PROTEINS AND FREE AMINO ACIDS :

Millon's test:

- To the extract millon's reagent is added. Appearance of red color indicates the presence of proteins and amino acids.

Biuret test:

- To the extract equal volume of 5% sodium hydroxide and 1% copper sulphate is added. Appearance of violet color indicates the presence of proteins and amino acids.

Ninhydrin test:

- To the extract ninhydrin reagent is added. Appearance of purple color indicates the presence of proteins and amino acids.

Warming test:

- About 2ml of the extract was heated in boiling water bath. Proteins get coagulated due to heating.

13) TEST FOR ANTHRAQUINONES :

To about 2ml of the extract 10ml of benzene is added, shaken and filtered. To this 5ml of 10% ammonia solution is added. Mixture was well shaken, presence of pink color in the ammoniacal (lower) phase indicate the presence of anthraquinones.

14) TEST FOR RESINS :

0.5g of the extract was diluted to 10ml with water and shaken for 5min. Formation of turbidity indicate the presence of resins.

15) TEST FOR PHENOLS :

Ferric chloride test:

- Crude extract is dissolved in water and equal amount of ferric chloride was added. Deep bluish color indicates the presence of phenols.

Ammonium hydroxide test

- To the extract 1% solution of gelatin containing 10% sodium hydroxide. White precipitate indicates the presence of phenols.

Lead acetate Test

- To the extract 10% lead acetate solution is added, white precipitate indicates the presence of phenols.

16) TEST FOR PHLOBATANNINS :

The extract was dissolved in distilled water and filtered. The filtrate was then boiled with 2% hydrochloride solution. Red precipitate shows the presence of Phlobatannins.

IV. THIN LAYER CHROMATOGRAPHY OF SEED OIL EXTRACT :

PRINCIPLE:

TLC is based on the principle of separation on the basis of relative affinity of the compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to the stationary phase travels slowly while others travel faster. Thus separation of compounds in the mixture can be thus achieved. Once the separation

occurs, individual compounds were visualized as spots at respective level of travel on the plate. Their nature or characters were identified by means of various detection techniques.

PROCEDURE:

The seed oil extract (10µl) were applied to the TLC plate 1cm above one ridge. When the spot was completely dried, the lid from the saturated chromatography chamber containing solvent system n-hexane: diethyl ether: glacial acetic acid in the ratio 30ml: 20ml: 750µl (V/ V/ V) was removed and TLC plate was inserted into the chamber. Care was taken to make sure that the solvent was well below the spot. The solvent system was run until the solvent front reached within 1cm of the top of the TLC plate. The plate was then allowed to dry. The spots were visualized under UV transilluminator (254nm), and using Iodine vapor.

SOLVENT SYSTEM:

n-hexane: diethyl ether: glacial acetic acid in the ratio 30ml: 20ml : 750µl (V/ V/ V)

CALCULATION:

$$\mathbf{R_f\ value} = \frac{\textbf{Distance travelled by the sample}}{\textbf{Distance travelled by the solvent front}}$$

V. IDENTIFICATION OF COMPOUNDS USING DIFFERENT SPRAY REAGENTS :

1. **Dragondorff's reagent (for Alkaloids) :**

   The reagent was made of two portions: Reagent 1 - 0.85g of Bismuth subnitrate was dissolved in a solution of 10 ml acetic acid and 40 ml of water. Reagent 2 - 8g of Potassium iodide was dissolved in 20 ml of water (Stock solution- mixture of equal parts of solution 1 and 2). The spray reagent was prepared by mixing 1ml of stock solution with 2 ml of fresh acetic acid and 10 ml of water. Detection of alkaloids and other nitrogen compounds is by the appearance of orange-brown spots on yellow background.

2. **Ferric ferrocyanide reagent (for Phenolics) :**

   10% Ferric chloride was mixed with Iron cyanide [$FeCN_6$ (1g/100ml)]. 0.1g of Ferric chloride and 0.1g of Potassium ferricyanide ($K_3F\ _3CN_6$) was freshly prepared by dissolving in 10 ml of distilled water. Equal portions of these two solutions was mixed , sprayed to plates and heated at 110 ^{o} C. Change of color to blue (instant) indicates the presence of Phenolic compounds.

3. **Vanillin reagent (for Terpenoids)** :

   Vanillin (10% in water) was mixed with Ethanoic acid- concentrated sulphuric acid in the ratio 2:1, and sprayed on to the plates. They were then put in the oven for 15 min. Presence of terpenoids was indicated by the appearance of brown, dark green or purple color.

4. **Ammonia reagent (for Flavonoids) :**

   TLC plates were exposed to ammonia. The presence of flavonoids was indicated by yellow, pink, grey or brown spots depending on different types.

5. **Methanol- Potassium hydroxide reagent (for Anthraquinones)** :

   TLC plate was sprayed with a 10% solution (in methanol) of Potassium hydroxide. Change of original yellowish-brown color to purple color shows a positive test for anthraquinones.

6. **20% Aqueous Sulphuric acid reagent (for Terpenoids) :**

   Aqueous sulphuric acid (20%) was prepared. The sprayed plate was heated to 110^{o}C until spots were visible. With 20% sulphuric acid, the terpenoids develop brown, pink, purple or yellow color.

7. **Anisaldehyde - Sulphuric acid reagent (AS reagent - for triterpenoids) :**

   Anisaldehyde (0.5ml) was mixed with glacial acetic acid (10ml) and diluted with methanol (85ml) and then conc. sulphuric acid (5ml) was added to it and mixed. The TLC plates were sprayed with AS reagent, heated at 100^{o}C for 5-10 min until maximal visualization of the spots was obtained. With AS reagent, triterpenoids are detected as blue, red- violet, orange or red spots.

8. **Liebermann Buchard reagent (LB reagent - for terpenoids)**

   1ml of acetic anhydride was added to 1ml of chloroform and cooled to 0^{0}C. Then one drop of concentrated sulphuric acid was added to the cooled mixture. The TLC plates was sprayed with LB reagent The terpenoids was observed as blue, green, red or orange spots that changes with time.

VI. TLC OF SEED OIL EXTRACT FOR COLLECTION OF DIFFERENT FRACTIONS:

PROCEDURE:

Seed oil extract (stock- 10mg/ml) sample (10 µl) were applied to the TLC plates and allowed to dry. Remove the lid from the saturated chromatography chamber containing solvent system n-hexane: diethyl ether: glacial acetic acid in the ratio 30ml: 20ml: 750µl (V/ V/ V)**.** Insert the

TLC plate into the chamber. Care was taken to make sure that the below the spot. The solvent system was run until the solvent front reached within 1cm of the top of the TLC plate. The plate was removed and allowed to dry. The separated bands were visualized under UV transilluminator (254nm) and the band regions were marked. The marked silica regions were scrapped out separately and collected in separate test tubes. It was then well mixed using the same solvent system and centrifuged. Each time the supernatants obtained by centrifugation were separately saved. Finally the four supernatants were separately kept for evaporation at room temperature in pre-weighed beakers. The weights of each fraction obtained were noted.

VII. HPTLC OF SEED OIL EXTRACT AND IT'S TWO FRACTIONS :

PRINCIPLE:

High performance Thin Layer Chromatography (HPTLC) is the sophisticated technique of TLC in which chromatography technique accompanied with different software systems. Similar to TLC it also works on the principle of separation on the basis of relative affinity of the compounds towards stationary and mobile phase. The compounds under the influence of mobile phase travel over the surface of stationary phase and get separated.

PROCEDURE:

CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used in the present study. All the solvents used for HPTLC analysis were obtained from MERCK. The 10 mg of the seed oil extract and two fractions, (Fraction-I, Fraction-IV) was dissolved in 1ml of ethanol and the solution was well mixed and used for HPTLC analysis as test solution. The samples (5µl) were spotted in the form of bands of width 5mm with a Camag micro litre syringe on precoated silica gel glass plate 60F-254 (20cm × 10cm) with 250 µm thickness using a Camag Linomat IV. The plates were prewashed with methanol and activated at 60^0C for 5min prior to chromatography. The sample loaded plate was kept in TLC twin through developing chamber after saturated with solvent vapour with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm. The n-hexane: diethyl ether: glacial acetic acid in the ratio 30ml: 20ml: 750µl (V/ V/ V) was employed as mobile phase. Linear ascending development was carried out in (20cm × 10cm) twin trough glass chamber saturated with the mobile phase and the chromatoplate was developed twice with the same mobile phase to get good resolution of phytochemical contents. The optimised chamber

saturation time for mobile phase was 30min at room temperature (25 ± 2^0C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under white light, UV light at 254 and 366nm. The developed plate was sprayed with reagents and dried at 100^0C in hot air oven for 3 min. The plate was photo-documented at UV 366nm and daylight using photo-documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366nm.

VIII. IN VITRO ANTIOXIDANT ACTIVITIES :

A. SUPEROXIDE RADICAL SCAVENGING ACTIVITY :

PRINCIPLE:

The superoxide anion radical scavenging activity was determined by nitro blue tetrazolium (NBT) reduction method of Mc Cord and Fridovich (1969). The assay is based on the ability of drug to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of riboflavin within the system. The superoxide radical thus generated reduce the NBT to a blue colored complex.

REAGENTS:

- Nitro blue tetrazolium (NBT) - 1.5nm (12.3mg/10ml)
- Riboflavin - 0.12µm (4.5mg/100ml)
- NaCN/EDTA - 0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

PROCEDURE:

The reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, Riboflavin (0.12mM), NBT (1.5 n moles), Phosphate buffer (67mM, pH 7.8) and various concentrations of the seed oil extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of seed oil extract and fraction-IV. Vitamin C was used as

positive control. The concentration of fraction-IV required to scavenge 50% superoxide anion (IC_{50} value) was then calculated.

CALCULATION:

$$\textbf{\% inhibition} = \frac{\boldsymbol{OD\ of\ control - OD\ of\ sample}}{\boldsymbol{OD\ of\ control}} \times \mathbf{100}$$

B. AAPH (2,2'-azobis-2-amidino propane hydrochloride) INDUCED ERYTHROCYTE LYSIS ACTIVITY :

PRINCIPLE:

AAPH generate alkoxy radicals which attack RBC membrane proteins and lipids causing hemolysis.

REAGENTS:

- AAPH (0.271g in 2ml PBS)
- Phosphate Buffered Saline (PBS)
- 20% RBC (20µl RBC + 80µl PBS)

PROCEDURE:

Packed red blood cells (RBC) obtained from human blood bank was isolated by centrifugation at 1500 rpm for 10 minutes. Following a 3 times wash with PBS, the erythrocytes were re-suspended using same buffer to a hemocrit level of 20%. The seed oil extract and fraction-IV at different concentrations (0,25,50,75,100,150,200µg/ml) was added to erythrocyte suspension and then incubated at 15 minutes at 37^0C before the addition of 200 mM AAPH for 3hr at 37^0C in order to induce free radical chain oxidation in RBC. Erythrocyte suspension incubated without any additives was the control. OD reading at 540 nm after making up the solution to 2 ml with PBS and centrifugation at 1500rpm for 10 minutes. Concentration of fraction-IV required for inhibiting the generation of 50% radicals in the assay system (IC_{50} value) was calculated and compared with seed oil extract and positive control.

CALCULATION:

$$\textbf{\% inhibition} = \frac{\boldsymbol{OD\ of\ control - OD\ of\ sample}}{\boldsymbol{OD\ of\ control}} \times \mathbf{100}$$

C. DPPH RADICAL REDUCING ACTIVITY :

PRINCIPLE:

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radical, DPPH (2, 2- Diphenyl - 1- picryl hydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stochiometric with respect to the number of electrons captured.

REAGENTS:

- DPPH - 3mg in 25ml methanol (stored in dark bottle)
- Methanol

PROCEDURE:

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of seed oil extract and fraction-IV. The volume was made up to 1ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

CALCULATION:

$$\textbf{\% inhibition} = \frac{\boldsymbol{OD\ of\ control - OD\ of\ sample}}{\boldsymbol{OD\ of\ control}} \times \mathbf{100}$$

D. FRAP ACTIVITY (Ferric reducing antioxidant power activity) :

PRINCIPLE:

FRAP measures the reducing capability of test compounds based on the ferric ion. The FRAP assay was conducted at acidic pH 3.6 to maintain iron solubility. Reaction at low pH decreases the ionization potential that drives electron transfer and increases the redox potential, causing a shift in the dominant reaction mechanism. The FRAP mechanism is totally electron transfer.

REAGENTS:

- Acetate buffer - 0.3M (pH 3.6)
- TpTz (10mM) - 31.23mg in 40mM HCl
- Ferric chloride - 32.44mg/10ml water (20mM)

PROCEDURE:

Frap reagent was freshly prepared by dissolving 20 mM Ferric chloride (2.5ml) and 10 mM TpTz in HCl (2.5ml) in 0.3M Acetate buffer (25ml). About 900µl of FRAP reagent was ataken in test tubes. The seed oil extract and the fraction-IV was added in different concentrations (0, 25, 50, 75,100,150,200µg/ml). The volume was made up to 1ml with acetate buffer. Incubated at 37^0C for 15 minutes and read against distilled water at 595nm. Ferric chloride was taken as standard. The OD values of fraction-IV were plotted in the standard graph and compared with crude oil extract.

E. HYDROXYL RADICAL SCAVENGING ACTIVITY (TBARs METHOD):

PRINCIPLE:

Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals generated from Fe^{3+}/ ascorbate / EDTA / H_2O_2 systems. The hydroxyl radicals attack deoxyribose which eventually results in TBARs.

REAGENTS:

- $KH_2 PO_4$ - KOH buffer - 20mM (pH 7.4)
- Hydrogen peroxide - 1mM (113µl in 100ml - freshly prepared)
- EDTA - 0.1mM (3.72mg/10ml)
- Ferric chloride - 0.1mM (16.22mg/100ml - freshly prepared)
- Ascorbic acid - 0.1mM (17.61mg/100ml - freshly prepared)
- Deoxyribose - 2.5mM (9.38mg/2.5ml buffer)
- Acetic acid - 20% (pH 3.5)
- TBA - 400mg/50ml (freshly prepared)
- SDS buffer

PROCEDURE:

About 100µl each of Deoxyribose, Ferric chloride, EDTA, Ascorbic acid and Hydrogen peroxide were added to test tubes. Then the seed oil extract and the fraction-IV was added in different concentrations (0, 25, 50, 75,100,150,200µg/ml). The volume was then made up to 1ml with KH_2 PO_4 - KOH buffer pH 7.4. Incubation was carried out at 37^0C for 1hr. Approximately 400µl of the reaction mixture was added to fresh tubes to which added SDS (200µl), acetic acid (1.5ml) and TBA (1.5ml). The volume was made up to 4ml with distilled water and mixed thoroughly. Incubation was carried out at 100^0C for 1hr. Cooled and added 1ml of distilled water. The mixture was then centrifuged for 15min at 3000rpm and the OD of supernatant was read at 532nm. The concentration of the fraction-IV to scavenge 50% hydroxyl radical (IC_{50} value) was calculated from the graph plotted using % inhibition Vs concentration.

CALCULATION:

$$\textbf{\% inhibition} = \frac{\boldsymbol{OD\ of\ control - OD\ of\ sample}}{\boldsymbol{OD\ of\ control}} \times \mathbf{100}$$

IX. ANTI-TUMOUR STUDIES USING DLA INDUCED SOLID TUMOUR MICE MODEL SYSTEM:

A. ASSESSMENT OF TUMOR VOLUME:

PRINCIPLE:

Tumor was induced in the hind limb of the mice by injecting DLA cell. The volume of tumor is assessed and compared fraction-1 with the standard drug (cyclophosphamide).

MATERIALS REQUIRED:

- Male Balb/C mice
- DLA cell lines
- Cyclophosphamide
- Trypan blue dye

PROCEDURE:

Male Balb/C mice were divided into five groups :

High dose - Fraction-1 (10mg/kg) dissolved in propylene glycol

Low dose - Fraction-1 (5mg/kg) dissolved in propylene glycol

Standard - Cyclophosphamide dissolved in propylene glycol

Vehicle - Propylene glycol

Control - no treatment

Viable DLA cells (1×10^6) in 0.1ml PBS (aspirated from 15 day old DLA ascites tumor in mice) were transplanted by injection on to the right hind limb of mice. This was taken as day zero. From the first day onwards Fraction-I was administered orally (5 & 10mg/kg body weight) and continued for 10 consecutive days. The group that received only the DLA cell line served as the control and the standard group was treated with cyclophosphamide (25mg/kg body weight). The tumor development on animals in each group was administered by measuring the diameter of tumor growth in two perpendicular planes using vernier caliper twice every week for 4 weeks. The tumor volume was calculated using the formula and a graph was plotted with tumor volume in X-axis and number of days in Y-axis.

$$V = \frac{4}{3}\pi r_1^2 r_2$$

V - Tumor volume

r_1 - minor radius

r_2 - major radius

At the end of the experiment, the % Inhibition of solid tumor induced tumor volume in mice by fraction-I treatment was assessed. It was calculated by:

$$\textbf{\% Inhibition} = \frac{T.vol\ of\ control\ on\ 30th\ day - T.vol\ of\ treated\ on\ 30th\ day}{T.vol\ of\ control\ on\ 30th\ day} \times 100$$

B. ASSESSMENT OF BODY WEIGHT :

The body weight of all the animals in 5 groups were assessed in alternate days up to 21 days. The values were tabulated and compared among the five groups.

C. HEMATOLOGICAL PARAMETERS:

1. DETERMINATION OF TOTAL WBC COUNT :

PRINCIPLE:

The cells were diluted in Turk's fluid which contains a weak acid (acetic acid) to lyse RBC and a stain for WBC (crystal violet).

REAGENTS:

- Diluting fluid (by dissolving 1.5ml of acetic acid and 1ml of 1% crystal violet in 98ml water)
- Haemocytometer

PROCEDURE:

The total WBC count was measured from freely flowing tail vein blood. Collect the blood in EDTA coated tubes (Heparin coated tubes). 20µl of blood and 380µl of diluting fluid were added and kept for 2-3min. It was loaded on haemocytometer and the number of cells in 4 large squares of haemocytometer was counted under 10x objective of a compound microscope.

CALCULATION:

$$\textbf{Total WBC count} = N \times 50 \text{ cells}$$

N - Number of cells in 4 large squares of haemocytometer

2. DETERMINATION OF HAEMOGLOBIN :

PRINCIPLE:

The haemoglobin is converted to cyanmethemoglobin in the presence of potassium ferricyanide and potassium cyanide. The absorbance of cyanmethemoglobin is proportional to the haemoglobin concentration (Drabkin and Austin., 1932).

PROCEDURE:

The reagents used were procured from Agappe diagnostic kit. The hemoglobin count was measured from freely flowing tail vein blood. For this, 20µl of fresh blood was mixed with 5ml of Drabkin's reagent and incubated for 5 minutes at room temperature and followed by the measurement of absorbance at 546 nm against reagent blank. The concentration of hemoglobin was calculated.

CALCULATION:

$$\textbf{Hb (mg/dl)} = \frac{OD\ of\ test}{OD\ of\ standard} \times 15$$

X. STATISTICAL ANALYSIS:

Each *in vitro* assays were performed at least thrice and the data were expressed as Mean value ± SD. Animal experiments had six animals per group and data are mean ± SD. The data were analyzed by the one-way ANOVA followed by the Dennett's test.

RESULTS

I. EXTRACTION OF OIL COMPONENTS FROM UVARIA NARUM SEEDS :

Dried and powdered seeds were serially extracted with 95% ethanol and n-hexane to obtain water and oil extracts of the seeds respectively. Out of this **oil extract** is used in this study. Considerable yield of pale yellow colored oil was obtained from the seeds. The yield was approximately 8.36% (Table.4).

TYPE OF EXTRACT	COLOUR	CONSISTENCY	PERCENTAGE YIELD
Oil extract	Yellow	Oily nature	8.36 %

Table.4: Yield and characteristics of *U. narum* seed oil extract. Value is the average of two extractions.

II. IN VITRO CYTOTOXICITY ANALYSIS OF OIL EXTRACT ON DLA, EAC AND NORMAL SPLEEN CELLS :

In vitro cytotoxicity analysis of the seed oil extract and its separated fractions (Fraction-I, Fraction-II, Fraction-III, Fraction-IV) on the DLA cells, EAC cells and the normal spleenocytes were studied.

EFFECT OF SEED OIL EXTRACT AND ITS SEPARATED FRACTIONS ON DLA CELL LINES:

The seed oil extract showed an increase in cytotoxicity with increase in dose. Maximum cytotoxicity was observed at 100µg/ml (Fig.4A). The IC_{50} value obtained was 77 ± 5.37µg/ml (Tab.5). Similar dose dependent cytotoxiciy were also observed with the isolated fractions on this cell line. The IC_{50} value of the four separated fractions were found to be 78, 95µg/ml for Fraction-I (Fig.4B, Tab.5), Fraction-II (Fig.4C, Tab.5), respectively and greater than 100µg/ml for Fraction-III (Fig.4D, Tab.5) and IV (Fig.4E, Tab.5) respectively.

EFFECT OF SEED OIL EXTRACT AND ITS SEPARATED FRACTIONS ON EAC CELL LINES:

In the EAC cell population, the seed oil extract and its isolated fractions showed significant cytotoxicity. The seed oil extract had an IC_{50} value of 100 ± 1.41µg/ml (Fig.5A, Tab.5). Similarly the IC_{50} values for the four separated fractions were found to be 79ug/ml for Fraction-I (Fig.5B, Tab.5), 70ug/ml for Fraction-II (Fig.5C, Tab.5) and above 100ug/ml for the other two fractions, Fraction-III (Fig.5D, Tab.5) and Fraction-IV (Fig.5E, Tab.5).

EFFECT OF CRUDE OIL EXTRACT AND ITS SEPARATED FRACTIONS ON NORMAL SPLEEN CELLS:

Figure.6 shows the cytotoxicity of *U.narum* seed oil extract and their purified fraction on primary spleenocyte of rats. The cytotoxicity was minimal in these groups. However in the seed oil extract and fractions, the IC_{50} values were above 100ug/ml concentrations. The seed oil extract showed an IC_{50} value of 100 ± 0.96µg/ml (Fig.6A, Tab.5). Similarly the IC_{50} value of all the four separated fractions were found to be >100µg/ml (Fig.6B, C, D&E, Tab.5).

Sl no:	Test Compounds	IC_{50} values (µg/ml)		
		DLA	EAC	SPLEEN
1	Seed oil extract	77 ± 5.37	100 ± 1.14	100 ± 0.96
2	Fraction - I	78	79	Above 100
3	Fraction - II	95	70	Above 100
4	Fraction - III	Above 100	Above 100	Above 100
5	Fraction - IV	Above 100	Above 100	Above 100

Table.5: IC_{50} values of *U. narum* seed oil extract and its separated fractions. Values are mean ± SD of three independent experiments.

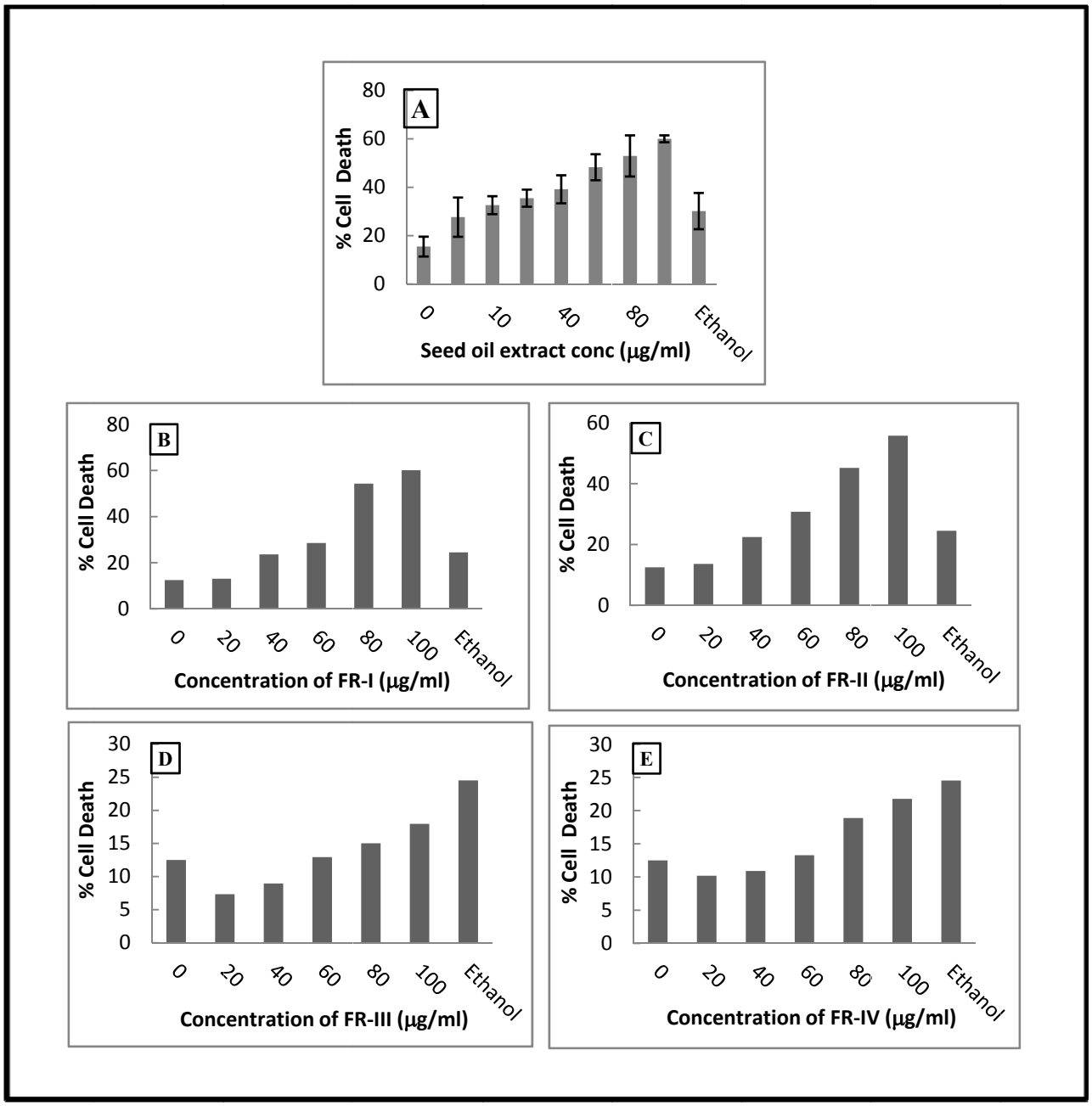


Fig.4: *In vitro* cytotoxicity of *U. narum* seed oil extract (A) and its separated fractions, (Fraction- I (B), II (C), III (D), and Fraction-IV (E)) on **DLA** cells . Cells ($1\times10^{6)}$ were suspended in PBS and different concentrations of drug added. The cytotoxicity was determined by trypan blue assay following 3hr incubation and the % inhibition was calculated. Values are ± SD of at least three consecutive experiments.

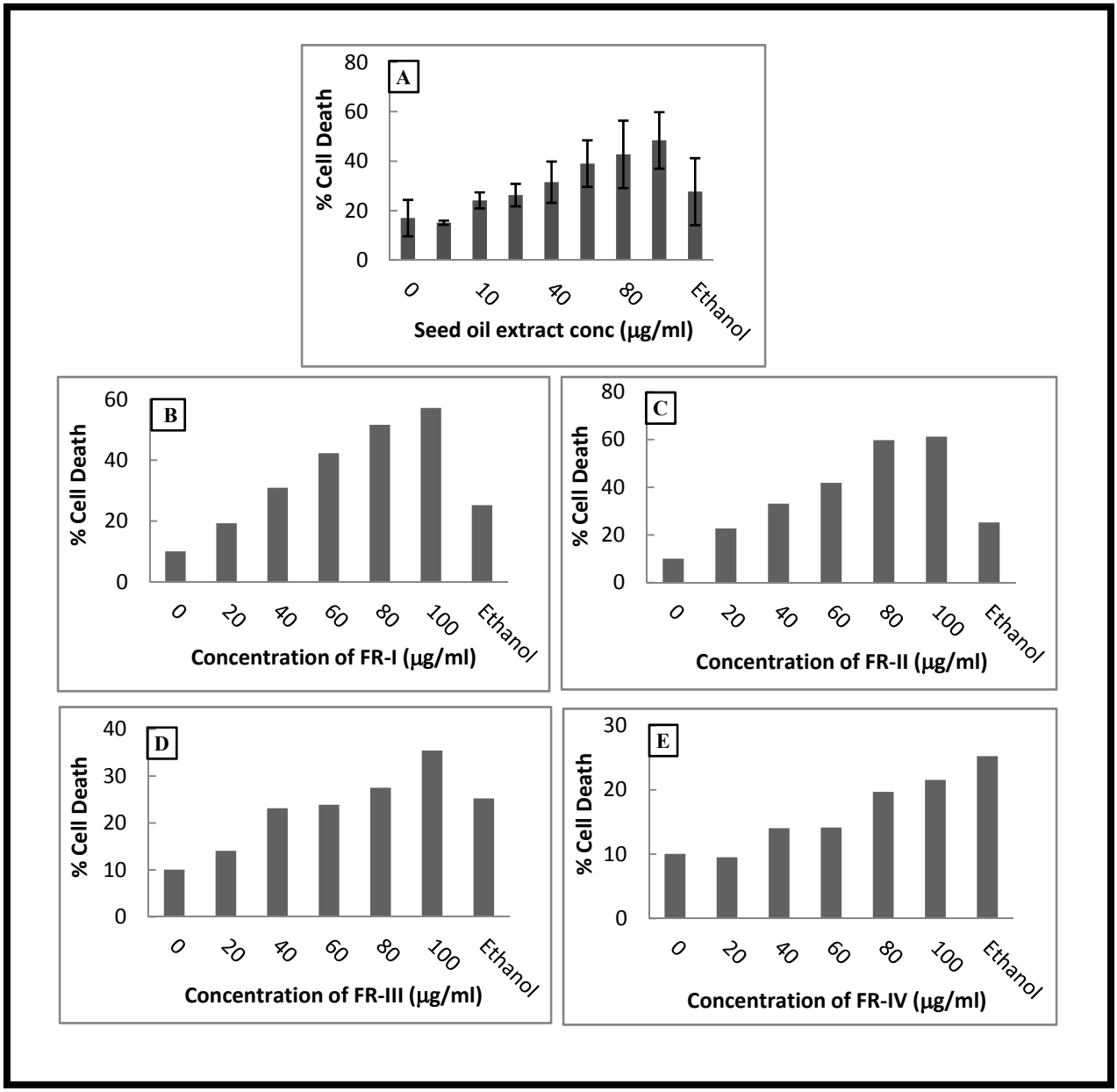


Fig.5: *In vitro* cytotoxicity of *U. narum* seed oil extract (A) and its separated fractions, (Fraction- I (B), II (C), III (D), and Fraction-IV (E)) on **EAC** cells. Cells ($1\times10^{6)}$ were suspended in PBS and different concentrations of drug added. The cytotoxicity was determined by trypan blue assay following 3hr incubation and the % inhibition was calculated. Values are ± SD of at least three consecutive experiments.

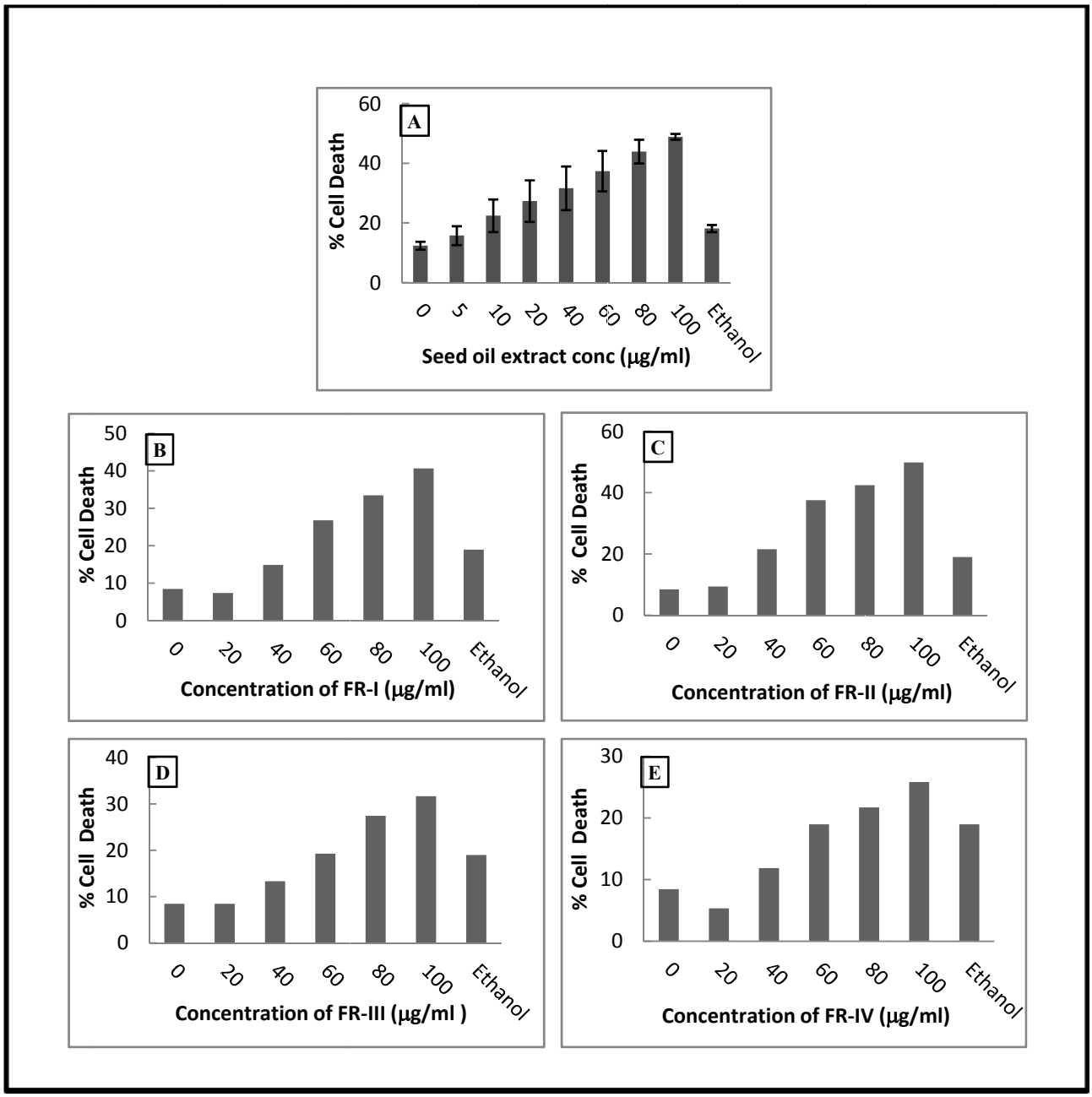


Fig.6: *In vitro* cytotoxicity of *U. narum* seed oil extract (A) and its separated fractions, (Fraction- I (B), II (C), III (D), and Fraction-IV (E)) on **normal spleen** cells. Cells ($1\times10^{6)}$ were suspended in PBS and different concentrations of drug added. Cytotoxicity was determined after 3hr incubation and the % inhibition was calculated. Values are ± SD of at least three consecutive experiments.

III. PHYTOCHEMICAL ANALYSIS OF U. NARUM SEED OIL EXTRACT:

Phytochemical evaluation was performed with *U. narum* seed oil extract. The extract showed positive result for dragondorff's reagent, mayer's reagent (indication of alkaloids); lead acetate test, ferric chloride test (indication of tannins); salkowski test, conc. sulphuric acid test (indication of terpenoids); benzene-ammonia test (indication of anthraquinones) and lead acetate test, ferric chloride test and 10% sodium hydroxide test (indication of phenolics) (Table.6).

IV. THIN LAYER CHROMATOGRAPHY OF SEED OIL EXTRACT :

Thin layer chromatography of the seed oil extract was studied. The seed oil extract showed four band regions in both UV transilluminator (254nm) and Iodine vapor (Table.7). The R_f value range of the four bands in UV was found to be 0.01 (Fraction-I), 0.21 (Fraction-II), 0.48 (Fraction-III), 0.77 (Fraction-IV) and in Iodine vapor was that of 0.01 (Fraction-I) , 0.27 (Fraction-II), 0.47 (Fraction-III) and 0.73 (Fraction-IV).

V. IDENTIFICATION OF COMPOUNDS USING DIFFERENT SPRAY REAGENTS :

Various reagents were sprayed on the developed TLC plates for the identification of the compounds. The Dragondorff's reagent (A) showed orange spot on yellow background at R_f value 0.01 which indicate the presence of alkaloid in the first band. The AS Reagent (B) and 20% aq. sulphuric acid reagent (D) showed a dark brown spot at R_f value 0.48 which indicate the presence of terpenoids in the third band. The Ferric ferrocyanide reagent (C) showed a blue spot on green background at R_f value 0.77 which indicate the presence of phenolics in the fourth band. Finally, the Methanol- potassium hydroxide reagent showed a yellow spot at R_f value 0.21 which indicate the presence of anthraquinones in the second band. The results obtained were depicted in Table.8 & Figure.8.

VI. COLLECTION OF DIFFERENT FRACTIONS BY TLC OF THE SEED OIL EXTRACT :

The seed oil extract were developed by TLC using the mobile phase, n-hexane: diethyl ether**:** glacial acetic acid in the ratio 30ml: 20ml: 750µl. Four bands were obtained with R_f values in the range of 0.01 (Fraction-I), 0.21 (Fraction-II), 0.48 (Fraction-III), and 0.77 (Fraction-IV) respectively. The characterstics of the four fractions were described in the Table.9.

SL. NO:	PHYTOCONSTITUENTS	INFERENCE
1	Carbohydrates	-
2	Proteins	-
3	Tannins	+
4	Terpenoids	+
5	Flavanoids	-
6	Steroids	-
7	Alkaloids	+
8	Leucoanthocyanins	-
9	Glycosides	-
10	Coumarins	-
11	Emodins	-
12	Saponins	-
13	Anthraquinones	+
14	Resins	-
15	Phenolics	+
16	Phlobatannins	-

Table.6: Phytochemical analysis of *U. narum* seed oil extract (+ present, - absent).

Sl.no:	Bands	R_f values	
		UV (254nm)	Iodine vapor
1	Band – I	0.01	0.01
2	Band – II	0.21	0.27
3	Band – III	0.48	0.47
4	Band – IV	0.77	0.73

Table.7: Rf values of fractions obtained in TLC analysis of *U. narum* seed oil extract by viewing it under UV transilluminator (254nm) and iodine vapor.

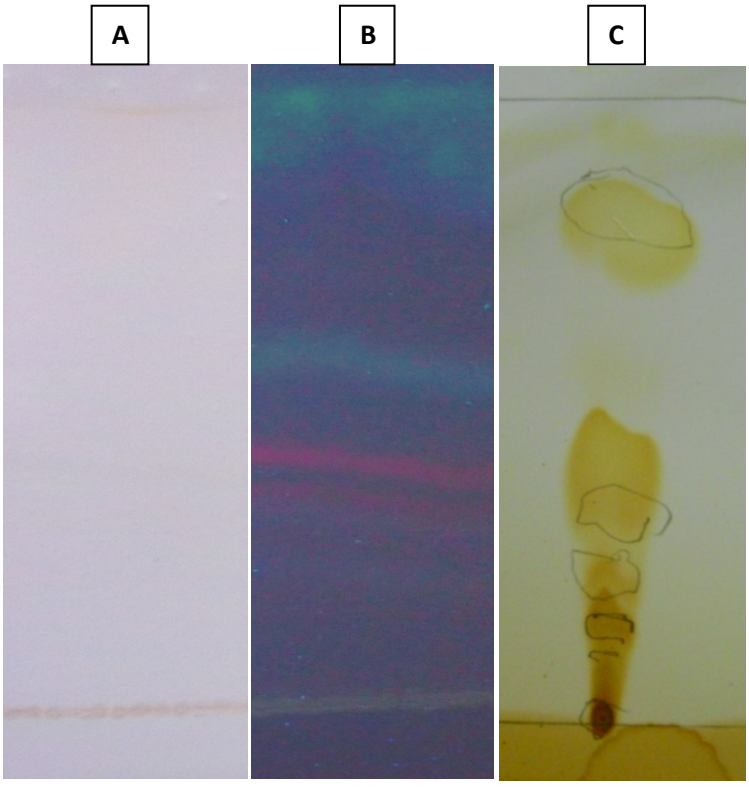


Fig.7: TLC analysis of the *U. narum* seed oil extract under (A)-white light, (B)-UV 254 nm and (C)-Iodine vapor

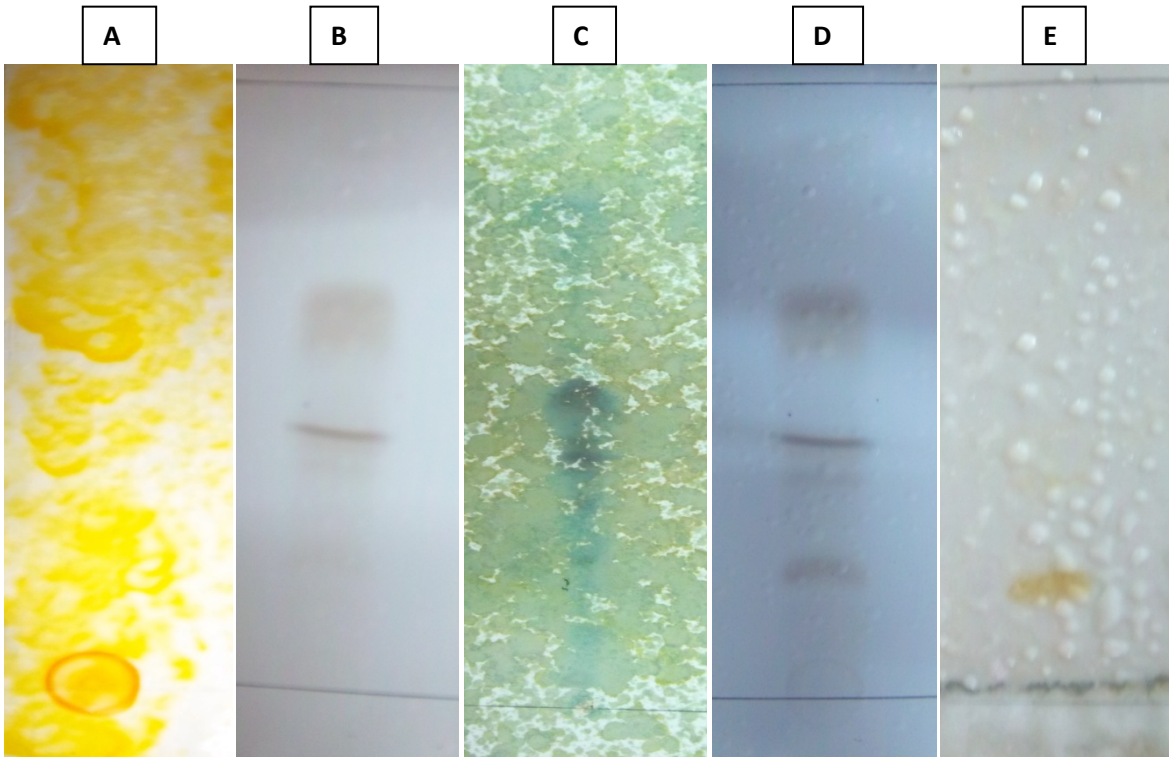


Fig.8: Phytochemical detection of *U.narum* seed oil extract on TLC plates spraying with (A)-Dragondorff's reagent,(B)- AS reagent,(C)- Ferric ferrocyanide reagent,(D)- 20% aq. sulphuric acid reagent and (E)- Methanol- potassium hydroxide reagent

Sl.no:	Reagents Used	Assigned compound	Inference
1	Dragondorff's reagent	Alkaloids	++
2	Ferric ferrocyanide reagent	Phenolics	++
3	Vanillin reagent	Terpenoids	+
4	Liebermann- Buchard reagent	Terpenoids/ Steroids	-
5	20% aq sulphuric acid reagent	Terpenoids	++
6	AS reagent	Terpenoids	++
7	Ammonia reagent	Flavanoids	-
8	Methanol-Potassium hydroxide reag	Anthraquinones	++
9	5% Ferric chloride reagent	Tannins	++

Table.8: Phytochemicals analysis of the fractionated seed oil on TLC by various spray reagents (++ present, + moderately present, - absent).

PROPERTIES	FRACTION- I	FRACTION - II	FRACTION - III	FRACTION- IV
Percentage yield	61.66 %	53.33 %	55 %	43.33 %
R_f value	0.01	0.21	0.48	0.77
Phytochemicals				
Alkaloids	+	-	-	-
Anthraquinones	-	+	-	-
Terpenoids	-	-	+	-
Tannins / Phenolics	-	-	-	+

Table.9: Characteristics of the four fractions obtained from *U. narum* seed oil extract by TLC separation (+ present, - absent).

VII. HPTLC OF SEED OIL EXTRACT AND TWO FRACTIONS USED IN ANTIOXIDANT AND ANTITUMOR STUDIES :

Seed oil extract and the two fractions (Fraction -I and Fraction -IV) were examined by HPTLC. The presence of similar bands were identified at R_f value, 0.08 of seed oil extract and 0.08 of Fraction-I (Table.10,11, Fig.9B). Similarly comparable bands were identified at R_f value, 0.81 of seed oil extract and 0.84 of Fraction-IV (Table.11, 12, Fig.9B). Other bands were also seen in fraction-I and fraction-IV due to impurity of fractions. At 254 nm remission a prominent band was seen in seed oil extract (Fig.9A). At 366 nm remission, two similar bands were seen in seed oil extract- fraction-I and seed oil extract- fraction-IV (Fig.9B). However in case of white remission, there were no visible bands (Fig.9C). HPTLC of the seed oil extract showed different bands. Out of these bands, HPTLC and TLC of Fraction-I showed only a single band. But in case of Fraction-IV, there were 3 bands in HPTLC and 2 bands in TLC.

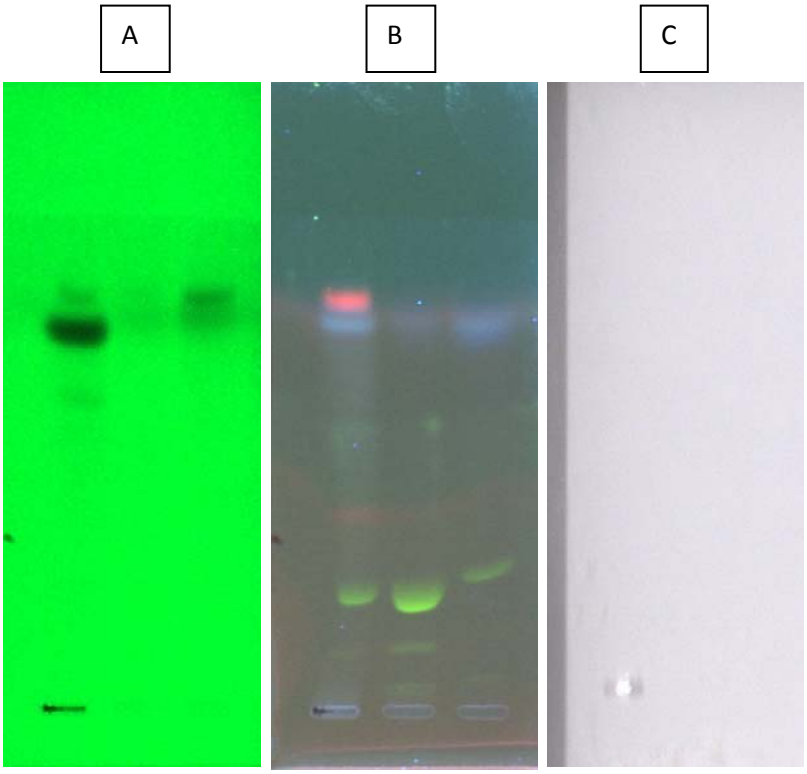


Fig.9: HPTLC analysis of the *U.narum* seed oil extract, fraction -I and IV under (A)254 nm , (B) 366 nm and(C) white remission.

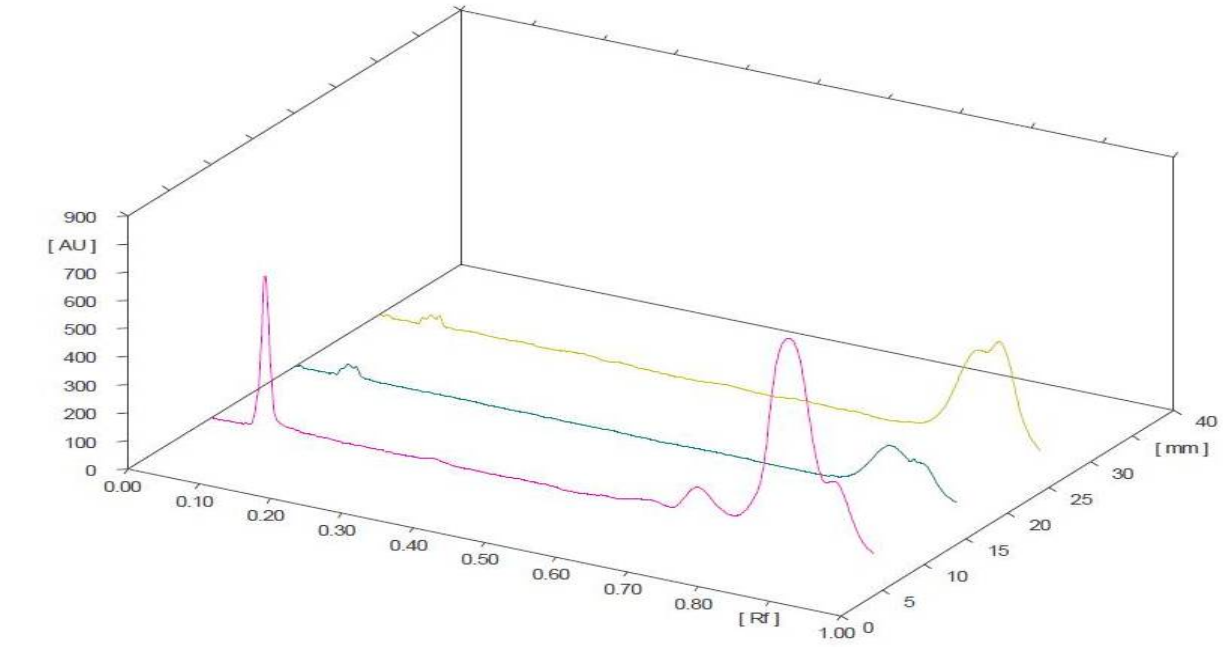


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Fig.10: 3D image of HPTLC chromatogram of *U.narum* seed oil extract, fraction-I and IV measured at wavelength 254nm using D2 & W lamps.

SEED OIL EXTRACT:

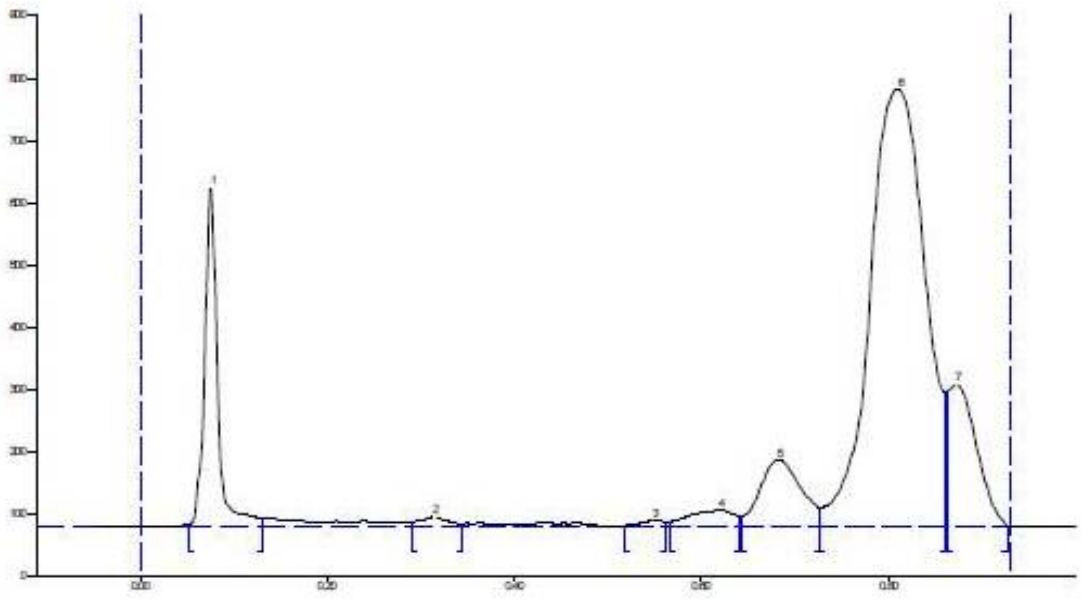

Fig.11: Integrated graph showing individual peak of *U.narum* seed oil extract in HPTLC analysis at wavelength 254nm shows 7 peaks. Out of this peak 6 (R_f value-0.81) and peak 1 (R_f value-0.08) are the prominent ones.

Peak	Max R_f	Max Height	Area %
1	0.08	545.0	12.12
2	0.31	17.6	0.89
3	0.55	11.9	0.48
4	0.62	29.2	2.21
5	0.68	105.7	7.60
6	0.81	703.2	65.40
7	0.87	228.6	11.30

Table.10: Characteristics of the various compounds obtained by HPTLC of *U. narum* seed oil extract.

FRACTION -I :

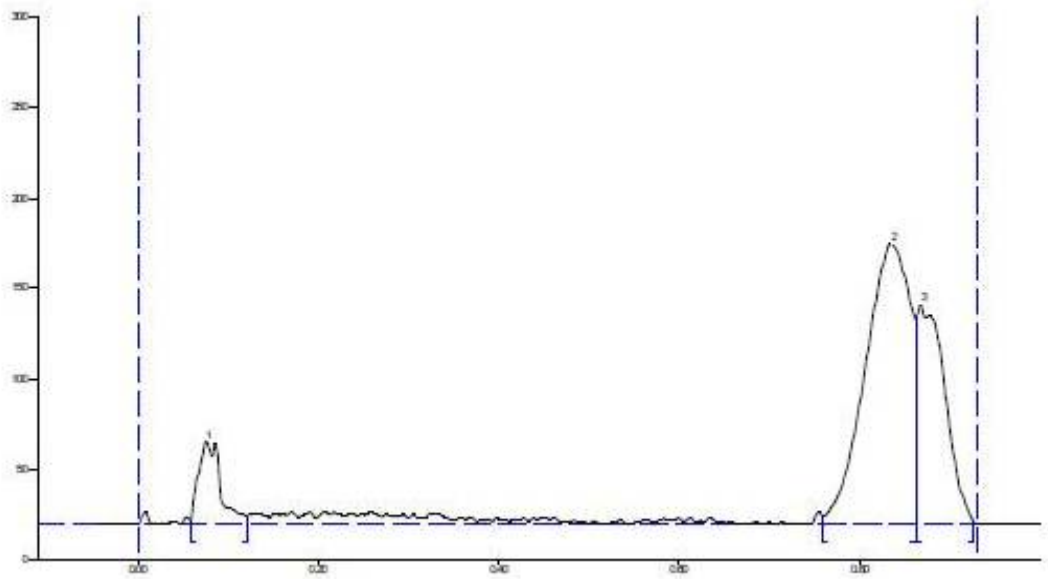

Fig.12: Integrated graph of showing peaks of fraction-I in HPTLC analysis measured at wavelength 254nm shows 3 peaks. Out of this peak 2 (R_f value-0.84) is the prominent one.

Peak	Max R_f	Max Height	Area %
1	0.08	45.9	8.98
2	0.84	155.0	61.53
3	0.87	122.1	29.49

Table.11: Characteristics of the compounds obtained by HPTLC of fraction - I

FRACTION -IV :

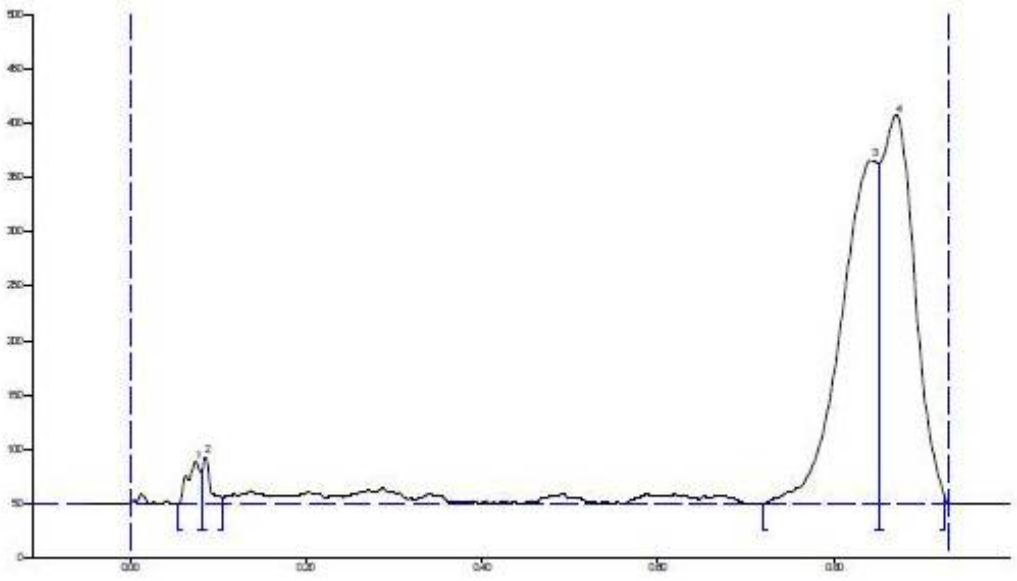

Fig.13: Integrated graph of showing peaks of fraction-IV in HPTLC analysis measured at wavelength 254nm shows 4 peaks. Out of this peak 4 (R_f value-0.87) is the prominent one.

Peak	Max R_f	Max Height	Area %
1	0.08	38.7	2.04
2	0.09	43.2	1.34
3	0.84	316.0	48.20
4	0.87	357.9	48.42

Table.12: Characteristics of the compounds obtained by HPTLC of fraction - IV

VIII. IN VITRO ANTIOXIDANT ACTIVITIES :

A. EFFECT OF SEED OIL EXTRACT AND FRACTION-IV ON SUPEROXIDE RADICAL SCAVENGING ACTIVITY:

Superoxide generated in the photo reduction of riboflavin was effectively inhibited by the addition of varying concentrations (0-200 μg/ml) of Seed oil extract and Fraction-IV (Fig.14). The concentration of the seed oil extract and fraction-IV needed to scavenge 50% superoxide anion (IC_{50}) was found to be 95 and 82.5 μg/ml (Table.13) respectively. Vitamin C which was used as a positive control had an IC_{50} value of 50 μg/ml.

B. EFFECT OF SEED OIL EXTRACT AND FRACTION- IV ON AAPH INDUCED ERYTHROCYTE HAEMOLYSIS ACTIVITY :

AAPH generate alkoxy radicals which attack RBC membrane proteins and lipids causing hemolysis (Fig.15).The IC_{50} value of the seed oil extract and fraction- IV was found to be 98.5 and 70 μg/ml respectively (Table.13) . Vitamin C which was used as a positive control had an IC_{50} value of 11.5 μg/ml.

C. EFFECT OF SEED OIL EXTRACT AND FRACTION-IV ON DPPH RADICAL REDUCING ACTIVITY :

The DPPH radical was effectively scavenged by seed oil extract and Fraction- IV. A dose dependent reduction of was observed within the range of concentrations (0-200μg/ml) of seed oil extract and fraction- IV added to the reaction system (Fig.16). The IC_{50} value of seed oil extract and fraction-IV was found to be 101 and 149 μg/ml (Table.13) respectively. Vitamin C which was used as the positive control exhibited an IC_{50} value of 2.6 μg/ml.

D. EFFECT OF SEED OIL EXTRACT AND FRACTION- IV ON FERRIC REDUCING POWER :

FRAP assay showed positive correlation between reducing power and phenolic content in seed oil extract and fraction -IV. The ferric reducing power of 50μg of seed oil extract of *Uvaria narum* is equal to the reducing power of 0.255 m mole/ml $FeSO_4.7H_2O$ and that of the 50 μg of Fraction-IV is equal to the reducing power of 0.318 m mole/ml $FeSO_4.7H_2O$ (Table 14). Linearity of FRAP (dose–response line) for standard solution (ascorbic acid) is shown in Figure 18.

E. EFFECT OF SEED OIL EXTRACT AND FRACTION-IV ON HYDROXYL RADICAL SCAVENGING ACTIVITY :

The seed oil extract and fraction- IV shows inhibition of hydroxyl radicals generated by Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fig.17). The IC_{50} value of seed oil extract was found to be 87.5 µg/ml where as that of Fraction-IV was found to be 82.5 µg/ml (Table.13). Vitamin C, used as positive control has IC_{50} value of 90 µg/ml.

Compounds	**IC_{50} values (µg/ml)**			
	Superoxide radical scavenging assay	AAPH induced erythrocyte lysis	DPPH radical reduction assay	Hydroxyl radical generation assay
Seed oil extract	95	98.5	101	87.5
Fraction - IV	82.5	70	149	82.5
Standard (Vitamin C)	50	11.5	2.6	90

Table.13: IC_{50} values for *U. narum* seed oil extract, fraction IV and standard, vitamin C in various *in vitro* anti oxidant assay systems

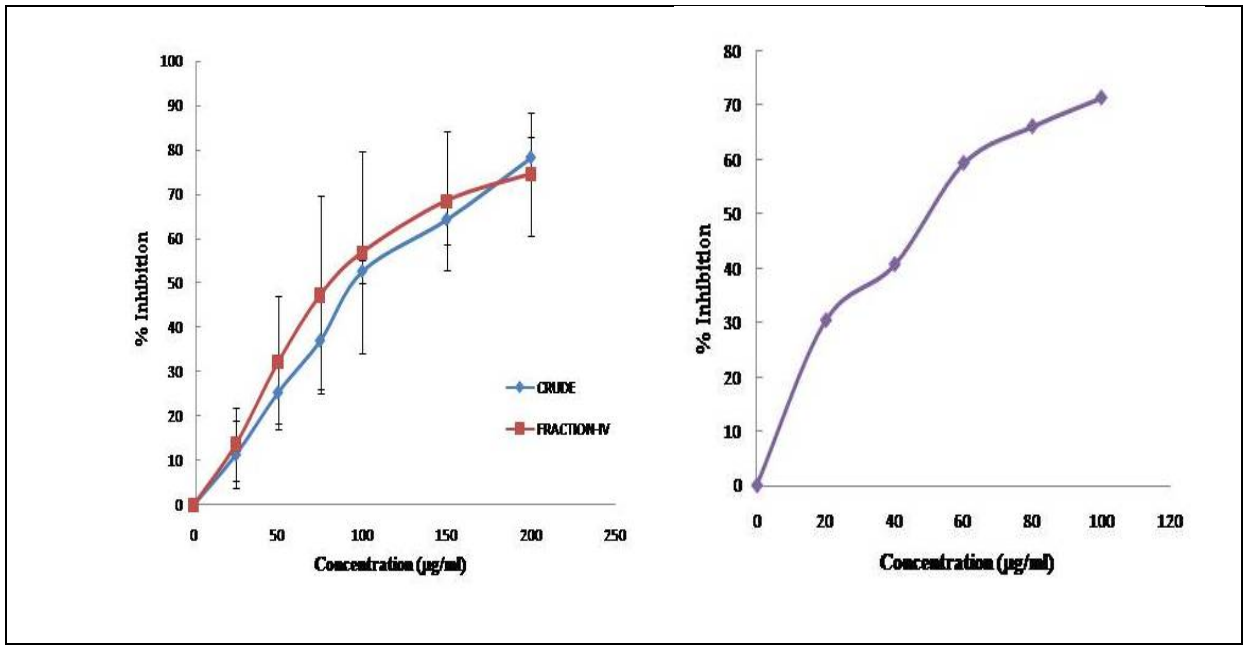

Fig.14: Effects of *U.narum* seed oil extract, fraction-IV and the vitamin C on superoxide radical scavenging activity. All data expressed as mean ± SD of three individual experiments.

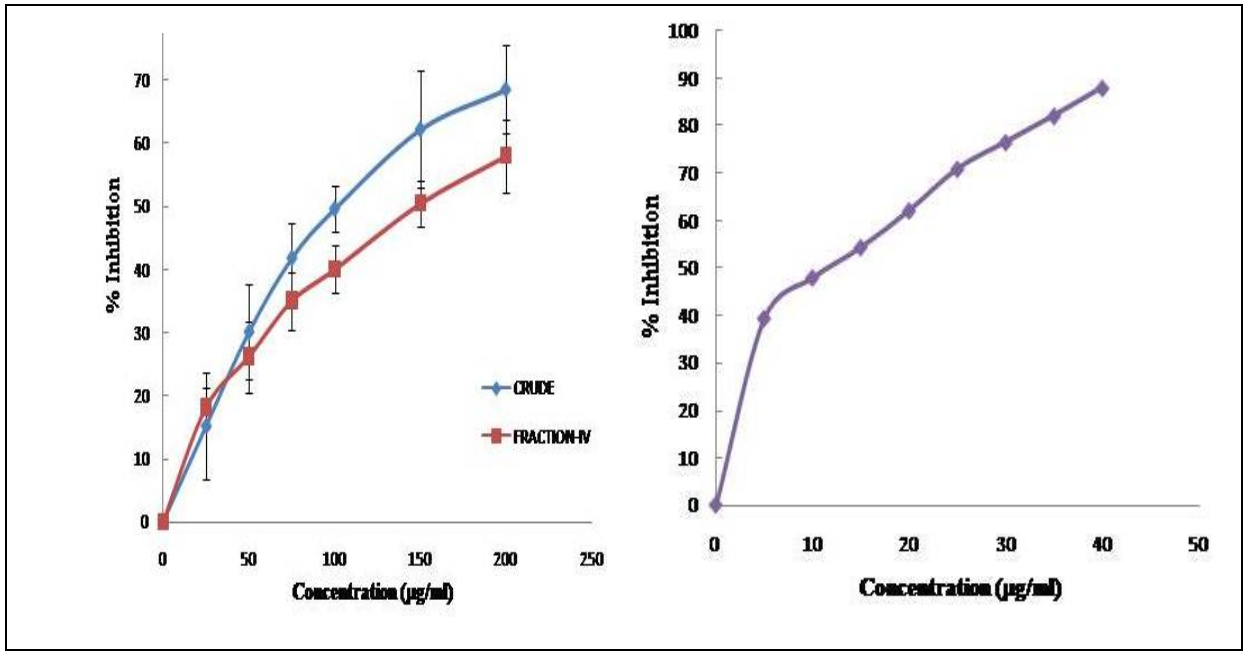

Fig.15: Effects of *U.narum* seed oil extract, fraction-IV and vitamin C on AAPH-induced lysis of human erythrocyte. All data expressed as mean ± SD of three individual experiments.

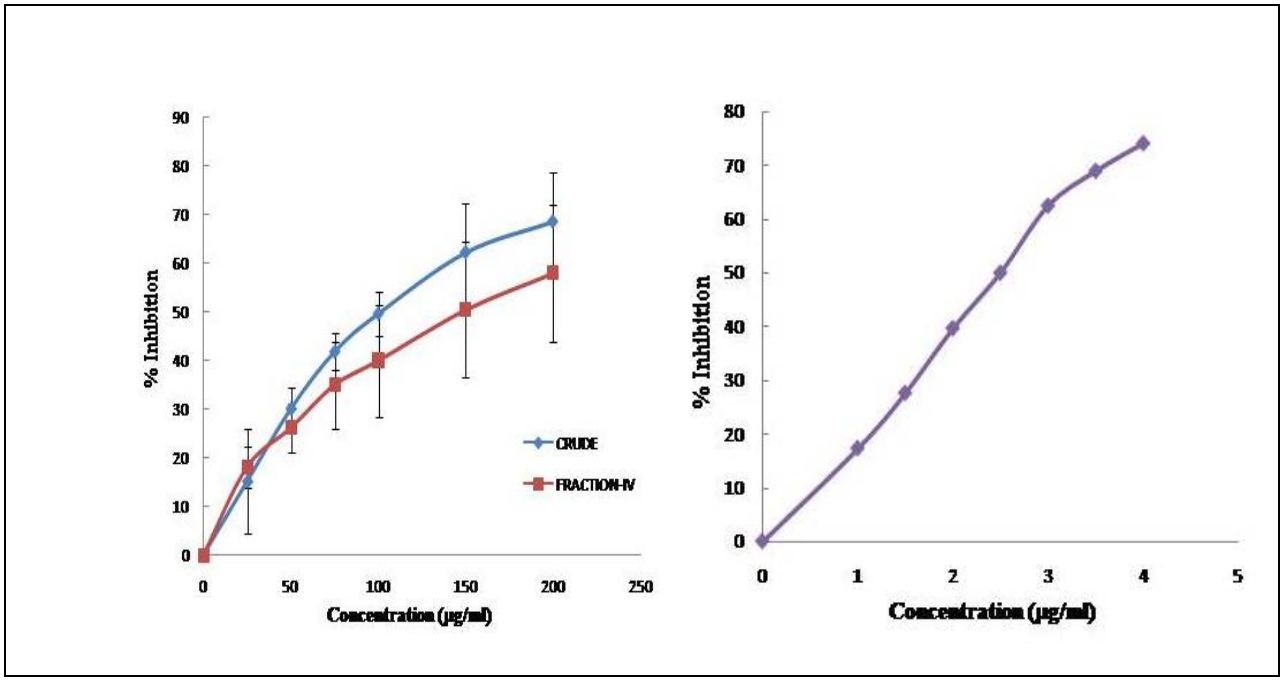

Fig.16: DPPH radical reducing activity of *U.narum* seed oil extract, fraction-IV and vitamin C. Results are mean ± SD of three individual experiments.

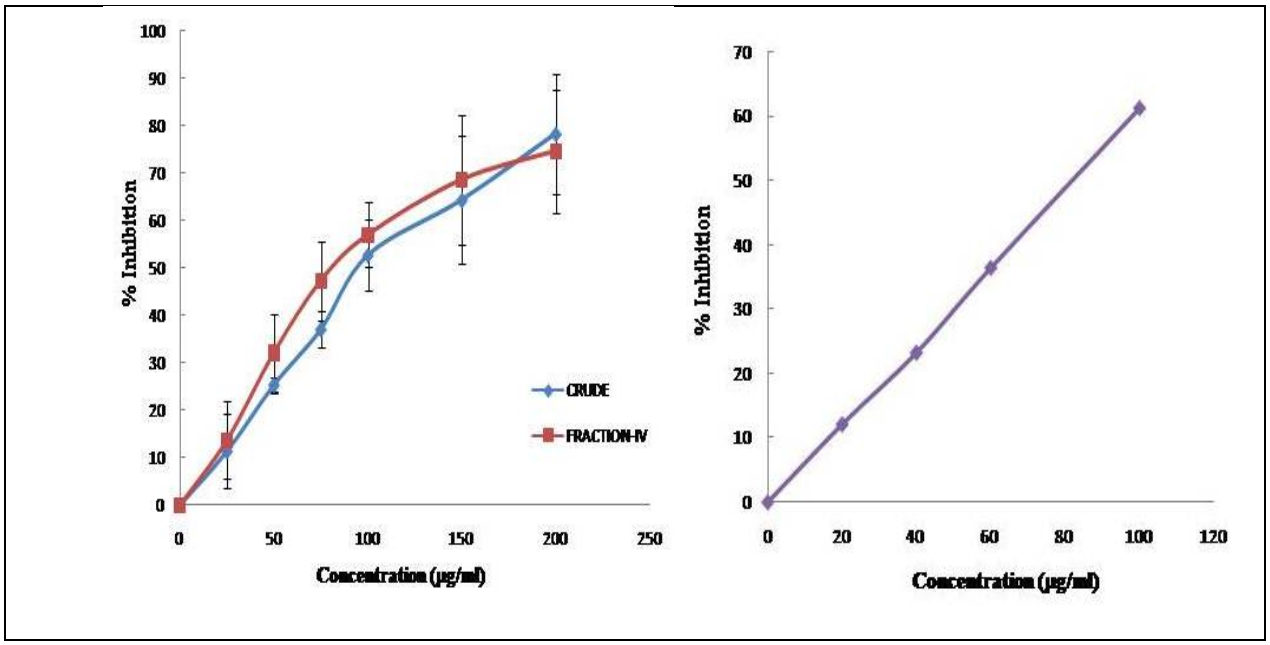

Fig.17: Hydroxyl radical scavenging activity of *U.narum* seed oil extract, its fraction-IV and the standard, vitamin C. All data expressed as mean ± SD of three individual experiments.

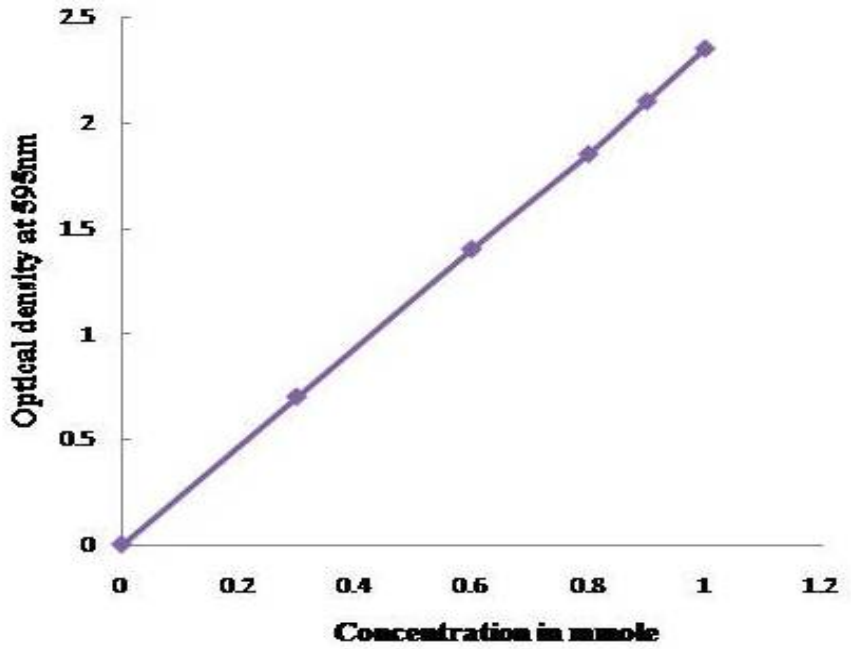

Fig.18: Ferric-reducing antioxidant power of ascorbic acid. Ascorbic acid reduced ferric to ferrous which has the optical absorbance at 595 nm; therefore, the increase in absorbance indicated the ferric reducing activity.

Concentration (µg/ml)	Ferric reducing power (m mole/ml of $FeSO_4$)	
	Seed oil extract	Fraction -IV
0	0.165	0.125
25	0.209	0.231
50	0.255	0.318
75	0.338	0.434
100	0.396	0.52
150	0.589	0.60
200	0.660	0.685

Table.14: Ferric reducing power of *U. narum* seed oil extract and its fraction- IV.

IX. IN VIVO ANTITUMOR STUDIES BY SOLID TUMOR METHOD :

A. EFFECT OF FRACTION- I ON TUMOR VOLUME :

Fraction-I of seed oil extract of *Uvaria narum* showed significant antitumor activity against solid tumor induced by DLA cell lines (Fig.19). Fraction-I of oil extract when administrated at concentration, 5 and 10mg/Kg body weight after implantation of tumor cell decreased 34.94 % and 61.43% tumor volume (Tab.15,19) after five weeks when compared to control and vehicle groups.

B. EFFECT OF FRACTION- I ON BODY WEIGHT :

The weight of tumor in the treated group (high dose- 10mg/kg b.wt and Low dose- 5mg/kg b.wt) at the end of fifth week was significantly lesser than the control and vehicle groups. Fraction-I doesn't showed much decrease in body weight (Tab.16) as compared to standard reference drug, cyclophosphamide (25 mg/kg b.wt).

C. EFFECT OF FRACTION- I ON TOTAL WBC COUNT :

From the study it was revealed that there was no much difference in total WBC count in the treated group (high dose- 10mg/kg b.wt and Low dose- 5mg/kg b.wt) when compared to the normal and control groups (Tab.17). However in case of standard group, (cyclophosphamide) the total WBC count decreased significantly.

D. EFFECT OF FRACTION- I ON HEMOGLOBIN COUNT :

From the study it was revealed that the hemoglobin count of the treated group (high dose- 10mg/kg b.wt and Low dose- 5mg/kg b.wt) was comparable with the control and vehicle groups (Table.18). But in case of standard group, hemoglobin count decreased to a greater extend.

Fig.19: Inhibition of DLA induced solid tumor by fraction-I of *U. narum* seed oil extract in Swiss albino mice.

Control

Vehicle

Fraction- I (5mg/kg)

Fraction-I (10mg/kg)

Cyclophosphamide (25mg/kg)

TREATED GROUPS	DAYS										
	Initial	3	6	9	12	15	18	21	24	27	30
CONTROL	0.675± 0.18	1.18± 0.17	2.29±1.61	5.64±4.01	11.65± 8.37	11.82± 8.51	15.08± 18.82	1.41± 0.0	2.23± 0.0	4.42±0.0	7.06±0.0
VEHICLE	0.556± 0.144	1.09± 0.47	2.01±0.75	3.99± 0.107	5.33± 1.43	5.53± 1.65	6.23± 3.08	7.55± 4.32	8.88± 5.31	11.48±4.82	18.23±7.03
HIGH DOSE	0.467± 0.06	0.692± 0.15	1.0±0.44	3.54±1.04	6.19± 3.35	7.09± 4.04	7.26± 3.47	6.95± 4.28	7.28± 4.74	10.51±6.08	10.95±5.78
LOW DOSE	* 0.464± 0.097	0.694± 0.16	1.31±0.46	3.58±1.63	5.06± 2.72	6.42± 4.48	7.64± 4.49	11.86± 8.4	13.74± 9.65	13.52±7.08	18.47±5.44
STANDARD	0.566± 0.18	0.954± 0.25	0.936± 0.16	1.41±0.48	1.78± 0.82	1.495± 1.11	1.33± 0.84	1.626± 1.0	1.87± 1.29	2.14±2.48	3.73±4.01

Table 15: Effect of fraction-I of *U. narum* seed oil extract on tumor volume (in cm^3) of mice. The values are mean ± SD of 5 animals in each group. P value : *P<0.05 when compared to the control group.

TREATED GROUPS	DAYS														
	Initial	3	5	7	9	12	14	16	18	20	22	24	26	28	30
CONTROL	28.4±3.17	28.56±3.19	29.6±2.95	30.7±2.19	30±1.71	30.6±03	28.56±1.25	30.46±0.41	32.76±0.92	31.9±1.21	32.76±1.38	33.33±1.28	33.86±1.22	34.36±1.20	34.7±1.1
VEHICLE	27.2±2.06	27.7±1.96	28.2±2.29	26.7±3.84	24.67±4.58	23.27±5.61	20.42±6.11	21.42±5.31	23.12±5.85	23.35±5.85	23.35±5.78	23.42±5.86	24.07±6.04	24.27±5.95	24.72±7.26
LOW DOSE	28.34±1.93	28.58±1.83	28.66±1.98	27.34±2.11	27.58±0.66	25.66±1.32	26.02±1.19	24±0.71	25.56±1.58	27.26±1.67	26.6±1.35	26.62±1.81	26.18±2.43	25.38±2.98	25.66±2.94
HIGH DOSE	32.68±0.70	33.12±0.91	31.2±1.49	30.8±1.96	32.2±2.44	30.32±2.67	31.84±2.99	29.86±2.78	32.24±3.03	32.66±3.09	29.44±3.35	30.44±4.06	30.9±4.38	31.16±4.82	32.5±3.96
STANDARD	28.1±1.66	28.58±1.81	26.4±3.08	25.76±3.69	26.38±3.58	24.86±3.99	25.85±3.54	24.86±4.36	25.7±5.96	26.93±7.08	26.36±8.24	26.73±8.95	26.8±9.13	26.9±9.29	30.55±4.31

Table.16: Effect of Fraction-I of *Uvaria narum* seed oil extract on body weight (in gms) of tumor bearing mice. The values are mean ± SD of 5 animals in each group. P values : *P<0.05, **P<0.01 when compared to the control group.

TREATED GROUPS	DAYS						
	4	8	12	16	20	24	28
CONTROL	18350±229	20300±400	28450±260	54417±681	57950±322	59733±313	60500±255
VEHICLE	15263±630	18413±104	22725±942	26750±367	51663±724	57325±781	58088±465
HIGH DOSE	** 8560±118	** 10690±129	* 19230±261	* 24540±439	** 47110±292	* 54790±435	56560±474
LOW DOSE	** 8460±538	* 12900±219	22020±570	* 17420±159	44520±501	51190±997	* 52480±423
STANDARD	** 5530±256	* 6170±325	4820±564	6100±557	11810±107	9980±922	9320±863

Table.17: Effect of Fraction-I of *U.narum* seed oil extract on total WBC count of tumor bearing mice. The values are mean ± SD of 5 animals in each group. P values : *P<0.05, **P<0.01 when compared to the control group.

TREATED GROUPS	DAYS						
	4	8	12	16	20	24	28
CONTROL	18.09 ±0.93	17.94 ±1.28	17.26 ±1.68	15.33 ±0.45	10.66 ±1.21	20.12 ±1.48	16.79 ±1.44
VEHICLE	17.95 ±0.91	16.24 ±1.7	16.10 ±2.0	14.27 ±2.23	14.41 ±3.12	17.0 ±1.09	16.01 ±0.75
HIGH DOSE	14.97 ±3.46	15.88 ±2.6	16.53 ±2.0	14.09 ±1.74	15.22 ±1.61	21.82 ±2.59	* 14.66 ±0.5
LOW DOSE	16.18 ±1.8	15.52 ±1.34	17.92 ±1.14	15.53 ±1.08	** 14.81 ±1.56	20.48 ±3.02	15.23 ±3.09
STANDARD	* 11.31 ±1.6	12.22 ±1.44	9.2 ±2.4	* 6.19 ±2.27	10.73 ±1.51	13.5 ±1.0	* 7.14 ±0.56

Table.18: Effect of Fraction-I of *U.narum* seed oil extract on hemoglobin count (in mg/dl) of tumor bearing mice. The values are mean ± SD of 5 animals in each group. P values : *P<0.05, **P<0.01 when compared to the control group.

Sl no:	Groups	Doses	Tumor volume in cm3 (30 days after tumor inoculation)	Percentage of Inhibition of tumor volume after 30 days
1	Control	-	28.39 ± 18.82	-
2	Vehicle	Propylene glycol	18.23 ± 7.03	35.78
3	Standard	Cyclophosphamide	3.73 ± 4.01	86.86
4	High dose	10 mg/Kg body wt	10.95 ± 5.78	61.43
5	Low dose	5 mg/Kg body wt	18.47 ± 5.44	34.94

Table.19: Inhibition of DLA induced solid tumor in mice by fraction-I of *U. narum* seed oil extract.

DISCUSSION

Plants are important source of biologically active secondary metabolites. For instance triterpenes found to have hypotensive and cardio depressant properties (Olaleye., 2007), anthraquinones posses astringent, purgative, anti-inflammatory, moderate antitumor and bactericidal effects (Muzychkina.,1998), Alkaloids having antimicrobial activity (Bonjean *et al*.,1998), Tannins reduce the risk of coronary heart diseases (Janaky Ranjithkumar *et al*., 2010) and phenolic compounds are potential antioxidants and free radical scavengers (Rice-Evans *et al*.,1995). Alkaloids and terpenoids are the class of compounds, predominant in the currently using plant derived chemotherapeutic compounds.

The plant *Uvaria narum* (Dunal) Wall is widely distributed in India and Srilanka particularly in dense forest of Western Ghats (Hishama *et al.,* 1990). The leaves of the plant have been studied for its antibacterial and antioxidant activities (Joji *et al*., 2012) but the seeds have never been studied for pharmacological activities. Hence the objective of the study is to determine the potential of *Uvaria narum* seeds by extracting oil from the seeds.

In this study, the oil extract of *Uvaria narum* seeds showed considerable cytotoxicity towards neoplastic cells (DLA and EAC). However towards the primary spleen cells, cytotoxicity was minimal. This suggests the specificity of the oil extract towards cancerous cells without affecting the normal cells. As it does not affect the normal spleenocytes, there is strong indication that the seed oil would not have immunosuppressive effect *in vivo.*

The preliminary phytochemical screening of the oil extract indicates the presence of various bioactive secondary metabolites constituents such as alkaloids, anthraquinones, terpenoids, tannins and phenolics which may accounts for its cytotoxic potential. In the TLC analysis oil extract separates into four fractions when illuminated under UV (254nm) and exposed to iodine vapour.

Out of the four fractions obtained in the TLC analysis, Fraction-I which is positive to alkaloid and Fraction-II, an anthraquinone positive show significant cytotoxicity towards DLA and EAC cell lines with comparatively less cytotoxicity towards Spleen cells. Fraction-III (positive to terpenoids) and Fraction-IV (positive to tannins/phenolics) have shown less cytotoxic towards normal cells and cancer cells. The anthraquionone fraction (Fraction-II) possesses cytotoxicity but comparatively lesser than the alkaloid fraction (Fraction-I).

Further, oral administration of alkaloid fraction (Fraction-I) at 5 and 10 mg/kg body weight for 30 days in mice considerably reduce DLA induced solid tumour volume by 34.94 % and 61.43% respectively. The body weight, total WBC count and hemoglobin level in the alkaloid fraction treated animals are similar to that of normal untreated mice. However, oral administration of the standard drug, Cyclophosphamide show significant reduction in body weight, total WBC count, haemoglobin and. This result is very encouraging, as today majority of the tumour reducing drugs specifically show drug induced myelosuppression and other complications. In the present study, the alkaloid fraction obtained from *Uvaria narum* seed oil extract does not produce such secondary lethal effects. Eventhogh the tumor reducing potential of alkaloid fraction is lower than cyclophosphamide, it requires only low dose to produce a tumor reduction. This seems to be advantageous over other chemotherapeutic drugs which require higher dose to achieve tumor reduction..

It is thus clear that Seed oil of *Uvaria narum* is a good source of promising cytotoxic alkaloids and antraquinones. Tumoricidal property of alkaloid fraction has been revealed in the present study. At present the tumoricidal property of anthraqunone fraction has not been assessed. Since it possess moderate toxicity towards cancer cell lines, further investigation are needed in this line. Several studies have shown that plant derived anthraquinones and alkaloids posses tumoricidal property (David *et al*., 2010). The well known chemotherapeutic vincristine and vinblastin are alkaloid compounds obtained from roots of *Catharanthus roseus*. These molecules achieve its anticancer property by inhibiting mitotic spindle formation. In the present study the mechanism of *Uvaria narum* seed alkaloid fraction is not yet identified. Considering the myelosuppression and other side effects of vinca alkaloids, the non myelosuppressive nature of the *Uvaria narum* seed alkaloid fraction seems to be very promising and suggested to have different mode of action.

In HPTLC analysis of seed oil extract, two major bands observed with R_f values 0.84 and 0.87 corresponds to alkaloid fraction (Fraction-I) and tannin/phenolic fraction (Fraction-IV) respectively. This is confirmed from results of individual HPTLC analysis of these two fractions. HPTLC results also revealed the partial purification of these two fractions from the seed oil extract. However in a different solvent system these individual fraction may get

separated. In such scenario, further purification active component has to be performed to obtain the active molecule.

Since fraction-IV, a tannins/phenolics positive fraction, shows minimum cytotoxicity it is used in the antioxidant studies. However in the antioxidant studies it showed reducing power to DPPH radicals. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Shirwaikar *et al.*, 2006). When considering the IC_{50} value for DPPH radical reduction of crude oil extract (101µg/ml), Fraction-IV (149 µg/ml) and standard Vit.C (2.6 µg/ml), it is likely that Fraction-IV may not be responsible for the entire antioxidant potential of the seed oil but other compounds do exist which together provide higher antioxidant potential to the oil. But the efficacy seems to be far below to vitamin C.

The ferric reducing power of Fraction-IV however is higher than that of the seed oil extract. Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents, e.g., oxygen radicals, hydroxyl radicals (Halliwell et al., 1999). In the present study the crude seed oil extract and Fraction-IV show almost same radical scavenging effect which is more or less similar to vitamin-C. In the Hydroxyl radical generation assay crude seed oil extract, Fraction-IV and vitamin C have been found to possess similar efficacy which is highly significant. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins (Spencer et al., 1984), especially membrane peroxidation. By inhibiting hydroxyl radical mediated damage, Fraction-IV find promising role in various degenerative conditions where lipid peroxides and carbonyl are involved.

Further in an *in vitro* assay system where erythrocyte haemolysis activity was evaluated, the crude seed oil extract and Fraction-IV show significant protective effect. The haemolysis reaction induced by alkoxy radical generated from AAPH molecules and is an indication of *in vivo* for drug toxicity. Thus it is clear that radical mediated membrane lipid peroxidation can be effectively inhibited by crude seed oil and Fraction-IV. These preliminary antioxidant activities suggest that the tannin/phenolics fraction of seed oil extract may be responsible for its major anti oxidant potential.

A number of antioxidants have been isolated from the plant sources. However they require in higher amounts in many of the *in vitro* assays mentioned above. Exceptions are curcumin, ellagic acid, ferulic acid etc. None of these molecules however found effective in clinical trials. Curcumin, which is currently in various stages of clinical trial, face set back due to its lower bioavailability. Fraction-IV in the present study is less cytotoxic but possesses significant radical reducing; scavenging and lipid peroxidation inhibiting activities require less than 100 µg/ml in all the *in vitro* assays. Since it is only a partially purified fraction, further level of purification may yield better non toxic antioxidants. It is expected that the actual antioxidant molecule may require only in nanogram quantities to produce its effect. Further, due to its lipophilic nature, (as it derived from seed oil) the molecule may possess good bioavailability and hence it can be pharmacologically active

CONCLUSION

The seed oil fraction of *Uvaria narum* possesses significant cytotoxic effect towards malignat cells than normal cells. In the present study two cytotoxic fractions one positive to alkaloid stains and the other positive to anthraquinone in TLC could be purified. Both these fractions exhibited siginificant cytotoxicity towards tumor cells however they are less toxic to normal cells. The alkaloid fraction reduce DLA induced tumor progression in mice but does not show weight loss, decrease in WBC count and haemoglobin level in the tumor bearing animals suggesting a promising antitumor property. The other two fractions, Fraction-III (positive to terpenoids) and Fraction-IV (positive to tannins and phenolics) are also obtained from this seed oil extract of *Uvaria narum* of which Fraction-IV exhibits significant antioxidant potential. Overall partial purification of promising antitumor alkaloids and antioxidant tannin/phenolic has been achieved from *Uvaria narum* seeds. It is expected that further level of purification of these fractions may yield pharmacologically important phytochemicals.

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