Chapter -1

Review of Literature

1 Introduction

Plants are constantly been used as medicinal agents since centuries. Even today, herbal products derived from plants are being employed worldwide in a variety of health care systems and also as nutraceuticals. Growing interest in the utilization of medicinal plants/derived materials has prompted need for development of elaborative study on their screening. This also has generated a serious concern regarding quality, safety and efficacy as many of these still lack sound scientific database.

1.1 Free radicals and Reactive oxygen species

Free radical are defined as chemical species, an atom or a molecule that has one or more unpaired electrons in its valance shell and is capable of existing independently. These free radicals contain an odd number of electrons which makes them unstable, short lived and highly reactive, therefore a free radical react quickly with other available chemical moieties in order to capture the needed electron to gain stability.

Generally, free radical attacks the nearest stable molecule, "stealing" its electron. When the attacked molecule looses electron, it becomes a free radical in itself, thus beginning a chain reaction cascade, resulting in disruption of a living cell physiology (Mitchell RN, 2003).

Most common radical derivatives of oxygen like superoxide free radical anion ($O_2^{\cdot-}$), hydroxyl free radical ($OH^{\cdot}$), lipid peroxyl ($LO^{\cdot}$), lipid alkoxyl ($LOO^{\cdot}$) and lipid peroxide (LOOH) as well as non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are collectively known as reactive oxygen species (ROS). These free radicals/reactive oxygen species are produced mainly from two important sources in the biological system ie cellular metabolism and environmental sources.

1.2 Sources of Free Radicals

1.2.1 Cellular Metabolism

Mitochondrial electron transport, endoplasmic reticulum oxidation, enzymatic activity, prostaglandin synthesis, autooxidation of (adrenaline, Thiol, Ascorbic

acid), nitric oxide sythatase, stimulated neutrophils, activated phagocytic cells, reperfusion injury and cytochrome P_{450} are some sources of generation of free radicals.

1.2.2 Environmental

Drugs, CCl4, pesticides, transition metals (Cu, Fe), tobacco smoke, alcohol, radiations, X-ray by water radiolysis, light by photo ionization and high temperature also generate free radicals.

Normally, there is an equilibrium between a free radical/reactive oxygen species formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, it can produce oxidative stress (Murray RK, 2000; Sen CK, 1995). This state of oxidative stress can result in injury to all the important cellular components like proteins, DNA and membrane lipids which can cause cell death.

In recent years experimental and clinical data have provided evidences for the involvement of free radicals in large number of pathophysiological disorders (Mitchell RN, 2003). This has led to increasing curiosity and interest among the scientists and researchers to evaluate potential benefits from antioxidant therapy. There are many evidences which showed that low levels of antioxidants are associated with increased risk for a pathological state. (Boaz M, 2000; Holick CN, 2002).

1.3 Free Radicals and Human Diseases

Oxidative damage to the cells occurs whenever the level of the cellular antioxidant systems goes down or when the ROS reach abnormally high levels leading to several pathological conditions.

Several disorders, like cardiovascular diseases, hemorrhagic shock, metabolic disorders, rheumatoid arthritis, cystic fibrosis, neurodegenerative diseases, and AIDS have been reported as the ROS-mediated disorders. Some specific examples of the ROS-mediated diseases are Alzheimer's disease, Parkinson's disease, oxidative modification of low-density lipoprotein in atherosclerosis and cancer (Bandyopadhyay, 1999).

1.4 Free radical and cardiac disorders

Cardiac tissue injury after myocardial infarction is due to free radicals generated at the site of damage (Tappel, 1973). Detoxification of ROS by antioxidants therefore affords protection against such diseases. Although antioxidant administration has been shown to reduce the severity of the myocardial injury, yet some properties of antioxidants such as cytotoxicity (Visweswaran, 1997), pro-oxidant activity (Edge, 1997) or high molecular weight in case of SOD, limited their therapeutic application.

Use of plant extracts, food supplement and even drugs which augment major cellular endogenous antioxidants following chronic administration have been identified as a promising therapeutic approach to combat oxidative stress associated with cardiac disorders.

Hyperlipidemia and excess of free radicals formed are the important risk factors in the initiation and progression of atherosclerosis. As a result there is increased awareness for the need to lower the elevated plasma lipoproteins and the oxidative stress due to excess of free radicals. Many plant products are increasingly recognized as having protective role in coronary artery diseases through several mechanisms including antioxidant and hypercholesterolemic properties. Cholesterol and other dietary fats become harmful after they are damaged by oxygen and oxygen radicals. The resulting oxidized and peroxidized lipids are not only toxic, but are also precursors for further chain reactions of free radical propagation.

1.5 Antioxidants

Antioxidants are the compounds of exogenous or endogenous nature which either prevent the generation of toxic oxidants or intercept or inactivate those which are generated and thereby block the chain propagation reaction produced by these free radicals (Rangan U, 1993).

Some antioxidants found in biological systems

1.5.1 Endogenous Antioxidants

1.5.1.1 Intracellular

The major intracellular antioxidants in the human body are probably the enzymes superoxide dismutase, catalase and glutathione peroxidase. In

addition, membranes contain the chain-breaking antioxidant α-tocopherol, a lipid soluble molecule that is located in the interior of biological membranes. α-tocopherol is the most important chain-breaking antioxidant present in human membranes. (Halliwell, 1991)

1.5.1.2 Extracellular

In contrast with the intracellular environment, antioxidant defence enzymes are less prominent in extracellular fluids. Blood plasma, tissue fluid, cerebrospinal fluid, synovial fluid and seminal plasma contain little or no catalase activity, and only low activities of superoxide dismutase and selenium-containing glutathione peroxidase can be measured. There is also very little reduced glutathione (GSH) present in most extracellular fluids (Halliwell, 1991).
Ascorbic acid, present in plasma has multiple antioxidant properties, probably including the ability to regenerate α-tocopherol by reducing α-tocopheryl radicals at the surface of lipoproteins and membranes. High concentrations of ascorbic acid are also present intracellularly, especially in brain and lung cells.
Albumin can also bind copper ions and it usually inhibits copper ion-dependent lipid peroxidation and $^{\bullet}OH$ radical formation. Binding to albumin might also help to stop copper ions from accelerating the peroxidation of low-density lipoproteins and promoting atherosclerosis. The copper ion-albumin complex might be a safe 'transit form' of copper that can be removed from the circulation by the liver. (Halliwell, 1991).

Uric acid can act as an antioxidant, both by binding iron and copper ions in the forms that do not accelerate free radical reactions, and by directly scavenging oxidizing species such as singlet O_2, $HOCl^{\cdot}$ and peroxyl radicals. Uric acid does react with some oxidants *in vivo*; however, reaction of uric acid with certain oxidizing species, such as $^{\bullet}OH$ or peroxyl radicals, can generate uric acid radicals that are themselves capable of doing biological damage, such as by inactivating certain enzymes. But, these uric acid-derived radicals can be reduced by ascorbic acid. (Halliwell, 1990).
Thus endogenous antioxidants are also categorized as

1.5.1.3 Enzymatic origin

Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase, (GPX), Glutathione Reductase (GRD), Glucose–6 Phosphate dehydrogenase (G6PD), Cytochrome oxidase system and Peroxidase (Rangan U, 1993; Mitchell RN, 2003; Stephens NG, 1996).

1.5.1.4 Non-enzymatic origin

Nutrient: Carotenoid, α-tocopherol and ascorbic acid

Metabolic: Glutathione, uric acid, albumin, ferritin, hepatoglobin, cysteine and bilurubin (Rangan U, 1993).

1.5.2 Exogenous Antioxidants

Endogenous antioxidant defence systems, though scavenge and minimize the formation of oxygen free radicals, are not 100% effective, especially so, in pathological conditions demanding the use of exogenous antioxidants. Hence large number of drugs and natural compounds has been studied as antioxidants.

The antioxidant property of glutathione has led to the study of several sulfhydryl-containing compounds for their activity. Lipoic acid and N-acetylcysteine act by replenishing the reduced glutathione level (Moldeus, 1994). Lipoic acid also acts as a singlet oxygen scavenger. Dihydrolipoic acid inhibits hepatic microsomal lipid peroxidation. N-acetylcysteine protects against free radical mediated cellular damage (due to its ability to act as a precursor for the cysteine portion of the tripeptide glutathione) and is a powerful scavenger of hypochlorous acid. (Egan, 1988)

Flavonoids have exhibited prominent antioxidant activity. These are reported to chelate metal ions preventing free radical damage. Many of the flavonoids are reported to be scavengers of superoxide anion, hydroxyl radicals, singlet oxygen and inhibitors of iron stimulated lipid peroxidation (Halliwell, 1990). Curcumin, the major colouring component of 'turmeric', which is credited with several pharmacological properties including anti-cancer and anti-inflammatory activities, is a scavenger of oxygen free radical and a potent inhibitor of lipid peroxidation.

Potentiation of the protection against free radicals has also been attempted by administration of the endogenous enzymes superoxide dismutase and catalase. Chemically modified SOD or catalase is being developed in order to extend their half-lives, target them and reduce their antigenecity (Ogino, 1988).

Thus exogenous antioxidants are also categorized as

1.5.2.1 Antioxidants of natural origin

Flavonoids, coumarins, phenolic acid, linolenic fatty acid, α-tocopherol, β-carotene and ascorbic acid etc. (Czinner E, 2001; Frank BHU, 2002)

1.5.2.2 Antioxidants of synthetic origin

Probucol, Xanthine oxidase inhibitor (allopurinol, folic acid), Superoxide dismutase, Catalases, NADPH inhibitors (adenosine, calcium channel blockers), Antioxidants raising endogenous glutathione peroxidase activity (glutathione, acetylcysteine) (Rangan U, 1993)

1.6 Antioxidants in disease prevention

There is evidence concerning the participation of reactive oxygen species in the pathophysiology of various human diseases and disorders, such as cancer, atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, neurodegenerative and hepatic disorders. (Repetto, 2002). Antioxidants function by offering easy electron targets for free radicals. In absorbing a free radical, antioxidants "trap" the lone free-radical electron and make it stable enough to be transported to an enzyme, which combines the two stabilized free radicals together to neutralize both. Antioxidants can act at different levels in an oxidative sequence. Antioxidants could act against lipid peroxidation by

- Decreasing localized O_2 concentrations.
- Preventing initiation of peroxidation.
- Quenching or scavenging singlet O_2 that can react directly with membrane lipids to produce peroxides.
- Binding metal ions in forms that will not generate reactive species.
- Removing peroxides by converting them into non-radical products, such as alcohols.

- Chain breaking, such as reacting with chain-propagating radicals (peroxyl and possibly alkoxyl), so preventing continued hydrogen abstraction from fatty acid side chains. Example phenols or aromatic amines and α-tocopherol. (Halliwell, 1990).

1.7 Herbal drug as Antioxidants

Ayurveda is recorded in ancient scripture, handed down through generations and developed over 6000 years. This time-tested holistic medicinal system maintains that good health exists when the body, mind, spirit and environment are in perfect harmony. Good health is a phenomenon rare in today's fast moving world, where people live in a stressful environment and follow an unplanned diet and unbalanced life style. It has become the need of the hour that a new vibrant medical system evolved which is devoid of any side effects and which leads resurgence of Ayurvedic traditions. Herbal and herbal-based molecules are expected to form the basis for such a development. (Dasture AV 2002)).

This global awareness for everything natural is the biggest challenge for Indian Pharmacist to come out with newer technologies. World health organization has recognized the traditional medicines as a part of the healthcare system. WHO currently encourages, recommends and promotes traditional herbal medicines in National healthcare programmes due to their case of availability, low cost, safety, and people's faith in such remedies (Divakar MC, 2002).

As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) ravage and restore the optimal balance by neutralizing the reactive species. Thus, herbal drugs containing antioxidants are gaining immense importance by virtue of their critical role in disease prevention.

Literature revealed that both *Ailanthus* and *Butea* were known traditionally for their effect in liver disorders. Both these plants constitute an important ingredient of most of the ayurvedic preparations used in different pathological conditions. Free radicals might be involved in development of these pathological disorders and both these plants may be acting by scavenging these free

radicals. In our previous studies we have already reported the hepatoprotective effect of Ailanthus leaves in chemical induced toxicity. Flowers of *Butea* were also worked out for their hepatoprotective activity. *Ailanthus excelsa* is reported to possess cardioprotective and rejuvenating property (Loizzo MR, 2007). Recently the leaves of Ailanthus were worked out for its antihypertensive activity.

Thus to know the exact mechanism involved and to explore antioxidant potential, both these plants were selected for the present study.

1.8 Plants selected for the present study

Ailanthus excelsa (Simaroubaceae)

Butea monosperma (Leguminosae)

1.8.1 Ailanthus excelsa Roxb

Ailanthus is a genus of tall, lofty trees, distributed in Indo-Malaya, China, Japan and Australia (Kirtikar KR, 1995). The genus is noted for its antidiarrhoel and antidysenteric properties (Chopra RN, 1958). The plant is known for its high commercial and economic importance (Singh U, 1983) (Fig-1.1 & 1.2).

Common Names:

Ailanthus excelsa Roxb (Simaroubaceae) is commonly known as "Mahanimba" due to its resemblance with neem tree (*Azadirachta indica*). The term *Ailanthus* is from ailanto which means *Tree of Heaven* and is the name for one of the species in the Moluccas, while in Latin *excelsa* means tall. The plant is known by different names like, *tree of heaven* in English, *ardusi, aralavo* in Gujarati, *maruk, ghoda karanj, aakashneem, arlu* in Hindi, *peruvagai* in Tamil and *peddamanu* in Telgu (Kirtikar KR, 1995).

Distribution: It is a fast growing tree extensively cultivated in many parts of India towards the vicinity of villages. The tree is indigenous to central and southern India and is distributed in Madhya Pradesh, Gujarat, some costal districts of Andhra Pradesh, Ganjam and Puri districts of Orissa (Nadkarni KM, 1976).

Useful Parts: Root, bark, leaves, and seeds.

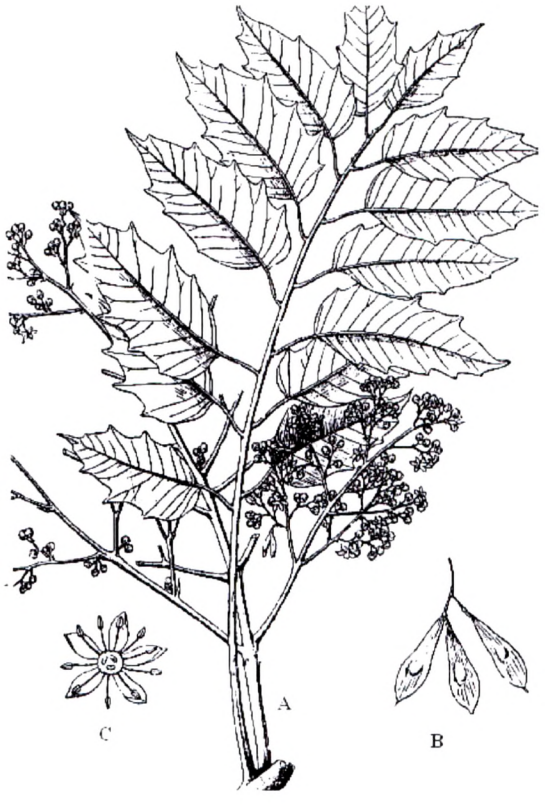


Fig-1.1 *Ailanthus excelsa* tree.
Fig-1.2 *Ailanthus excelsa* (A) A flowering twig, (B) fruits, (C) flower.
(Fig-1.2 Ref. K.R. Kirtikar, 1995)

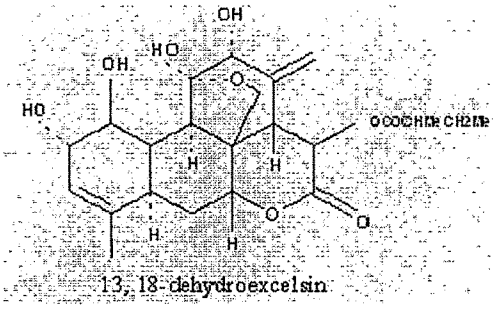
Ethnopharmacology:

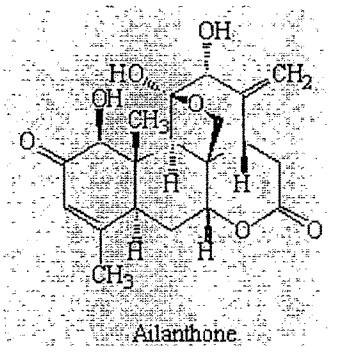
In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery, especially when there is a blood in stool (Chopra RN, 1958; Dash SK, 2006). The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma (Chevellier A, 1996). It has marked antispasmodic and cardiac depressant properties (Nadkarni KM, 1976). The root bark is used to cure epilepsy and heart troubles. In Africa the plant is used to treat cramps, gonorrhea epilepsy, tape warm infestation and high blood pressure (Anonymous, 1998; Sharma PV, 1996). Alcoholic extract of the leaf and stem bark shows anti-implantation and early abortificient activity (Dhanashekaran S, 1993). Traditionally the mattress made of leaves is used as bed for children suffering from fever. In Bombay the bark and leaves are of great repute as a tonic especially in debility after child birth. They are used in dyspepsia, bronchitis and asthma (Kirtikar KR, 1995; Nadkarni KM, 1976). In Konkan the juice of the leaves is usually administered in *khir*, or the juice of the fresh bark is given with coconut juice and treacle or with aromatics or honey to stop after pains (Kirtikar KR, 1995; Nadkarni KM , 1976; Nadkarni KM, 1976). It is also used to cure wounds and skin eruptions (K.R. Kirtikar, 1995). The plant is used as natural antifertility agent by the Irula women in Mavanahalla region of the Nilgiri district in Tamil Nadu (Abraham Z, 1981). The fresh juice of stem bark mixed with either honey or sugar is given to pregnant woman during evening for three consecutive days to induce permanent sterility (Anonymous, 1956). In Kanakpura taluka in Karnataka, the paste of stem barks along with goat milk and neem oil is used for curing the nose rope wound in ox. The bark is used as bitter, refrigerant, astringent, appetizer, anthelmintic, febrifuge, in dysentery, earache, skin disease, troubles of the rectum, fever due to *tridosha* and allay thirst (Kirtikar KR, 1995; Anonymous, 1956; Polonsky J, 1973). It is also used in gout and rheumatism (Jain SK, 1994). In Ayurveda it is used to remove the bad taste of mouth (Kirtikar KR, 1995). The bark is a good substitute for kurchi, *Holarrhena antidysenterica* (Anonymous, 1956). *A. excelsa* along with *Arjuna myrobalans* strengthen the body's natural rejuvenative processes. Fruits are used in diarrhea, polyurea, piles and fever (Yoganarashimhan SN, 2000). Leaves along with twigs are found to be suitable

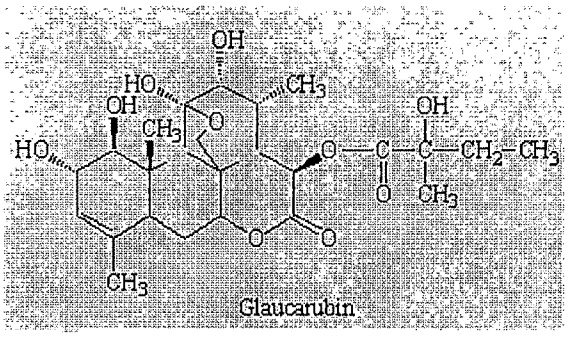
fodder for cattle, sheep and goats (Singh NP, 1977; Mandal L, 1997). The tree yields an inferior quality of bassora or hog gum. The plant serves as one of the host for silk worms. In France the tree is cultivated for its leaves, on which the caterpillar of the silk spinning *Ailanthus* moth (*Bombyx cynthia*) is fed yielding a silk of more durable and cheaper than mulberry silk. The wood is short fibered, admixture with long fiber pulp, such as bamboo pulp, used in the manufacture of paper (Anonymous, 1956). It is also used for the preparation of pencils (Pandey CN, 2000).

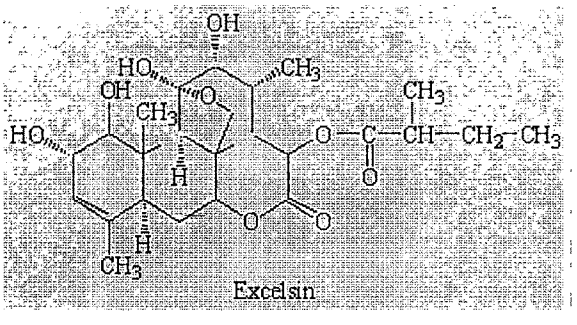
Phytochemistry and Pharmacology: A few phytoconstituents from the plant have already been isolated and also studied for pharmacological actions.

Quassinoids: Plants from Simaroubaceae are known to contain compounds with highly oxygenated triterpenens and bitter taste called as quassinoids (Khosa RL, 1985). Stem bark of *A. excelsa* contains quassinoids like excelsin, 1,4-dihydroexcelsin (Tripathi AK, 1993; Khan SA, 1990), 2,4-dihydroexcelsin, 3,4-dihydroexcelsin (N. Bhatia, 1985), 13,18-dehydroexcelsin, glaucarubin (Khan SA, 1978), glaucarubol (Khan SA, 1980), ailanthinone, 1,12-deoxy-13-formyl ailanthiol, ailanex A, ailanex B, polyandrol and glaucarubolone (Pandey B, 2004; Joshi BP, 2003) while the root bark is reported to contain ailanthinone, glaucarubinone and mixture of glaucarubin -15 - isovalerate, 13, 18-dehydroglaucarubol 15-isovalerate (Suroor AK, 1978). Ailanthone is toxic to some fungi and may therefore acts to protect plants against fungal pathogens and is associated with the observed toxicity of this species (Ogura M, 1977). A total control on *Chenopodium album* and *Amaranthus retroflexus*, the two weeds associated with soybean was observed with excelsin (Khan SA, 1980). Quassinoids from *Simarouba amara* were tested in vitro against a multi drug resistant strain of *Plasmodium falciparum* and in vivo against *Plasmodium berghei* in mice. Although the in vitro studies indicated activity in the region of 23-52 times greater than that for chloroquine, the toxicity was found to be very high (Ang HH, 1995). Quassinoids also play an important role in treating Epstein- Barr virus infection (Tamura S, 2003), HIV infection (Chang YS, 2003; Morre DJ, 1998; Geoffrey AC, 1994), and neoplasms (George RP, 1980) possibly by depolarization of mitochondrial membranes (Rosati A, 2004) (Fig- 1.3).









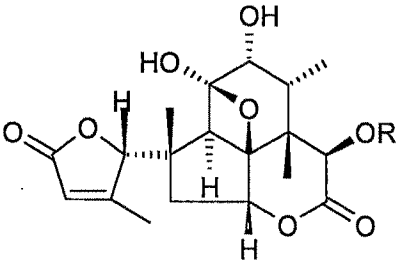


Fig-1.3 Structures of quassinoids from Ailanthus excelsa.

2, 6-dimethoxy benzoquinone and malanthin: Yellowish green viscous oil obtained from trunk bark gives colorless crystalline malanthin and 2, 6 dimethoxy benzoquinone.

Steroidal compounds: The petrol extract of stem bark contains β –sitosterol and Stigmasta-4, 22-diene-3-one (Mandal S, 1999) (Fig-1.4).

Triterpine: Root bark showed the presence of a new triterpene alcohol, 3S,

24S, 25-trihydroxytirucall-7-ene (Mahendra Kumar Jain, 1964; Khan SA0, 1980; Bhatia N, 1985) (Fig-1.4).

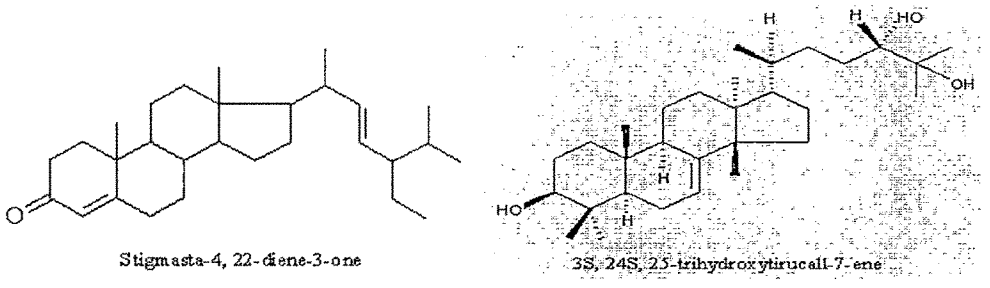
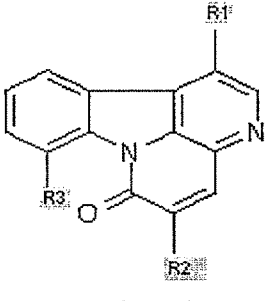


Fig-1.4 Structures of Steroid and Triterpenoid from *Ailanthus excelsa* bark.

Triacontane and Hexatriacontane: Stem Bark showed the presence of triacontane and hexatriacontane (Mehta CR, 1959).

Alkaloids: Root bark contain canthin-6-one, 1-methoxy canthin -6-one, 5-methoxy canthin -6-one and 8-hydroxy canthin-6-one alkaloids (Haynes HF, 1952; Ogura M, 1978; Ogura M, 1980). These alkaloids were studied for nasopharynx carcinoma in Eagles but none of the compounds were sufficiently active to meet the required criteria. On the other hand these alkaloids have shown significant cytotoxicity against 12-O-tetradecanoylphorbol-13-acetate induced Epstein-Barr virus early antigen (EBV-EA). Canthin-6-one and 4-methoxy canthin-6-one showed potent antiulcerogenic activity in gastric lesions induced animals, as well as significant antinociceptive activity in mice (Murakami C, 2004; Anderson LA, 1983) (Fig-1.5).



	R1	R2	R3
Canthin-6-one	H	H	H
1-methoxy canthin-6-one	OCH3	H	H
5-methoxy canthin-6-one	H	OCH3	H
8-hydroxy canthin-6-one	H	H	OH

Fig-1.5 Structure of Alkaloids from *Ailanthus excelsa* root bark.

Proteins: Leaves contain considerable amount of proteins where, cytoplasmic protein fraction can be used for human consumption; while the unfractionated and chloroplastic fractions could be utilized as a nutritious feed for ruminants and nonruminants (Nag S, 1994).

Flavonoids: The leaves were reported to contain different flavonoids like kaempferol (5, 4', 5, 7-Tetrahydroxy flavone), luteolin (3', 4', 5, 7-tetrahydroxy flavone), apigenin (4'.5, 7-trihydroxy flavone) while fruits contains quercetin (Kapoor SK, 1971; Khan MSY, 1994). These flavonoids were reported to possess many biological activities such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory properties. The flavon-C-glycosides like vitexin show antioxidant, analgesic and antithyroid activities (Gaitan E, 1995; Wagner H, 1999; Sethuraman MG, 1990) (Fig-1.6).

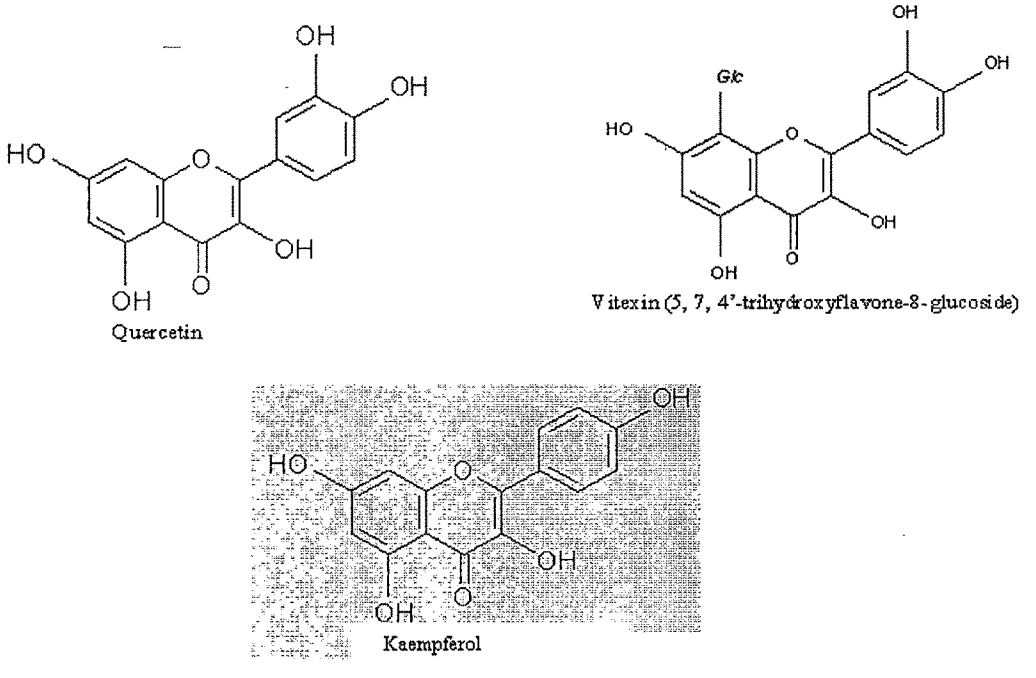


Fig-1.6 Structures of phenolics from *Ailanthus excelsa* leaves.

Ailantic acid: Bark contains wax like, reddish brown, water soluble bitter principle, known as ailantic acid. It is given as a tonic and alterative in dyspepsia and constipation (Nadkarni KM, 1976).

PHARMACOLOGICAL PROPERTIES

Antifertility activity: The alcoholic extract of the leaf and stem bark at a dose of 250mg/kg body weight exhibited a remarkable antimplantation and early abortificient activity in female albino rats (Dhanashekaran S, 1993).

Antifungal activity: Chloroform fraction of the methanol extract of stem bark showed significant fungistatic and fungicidal activity against *Aspergillus fumigatus, Penicillium frequentence, Aspergillus niger, Penicillium notatum* and *Botrytis cinerea* (Joshi BC, 2003).

Antimalarial activity: Excelsin was found to inhibit the growth of malarial parasites even at a concentration of 0.2μM (Khan SA, 1980). Glaucarubinone is much more potent than that of chloroquine and acts by inhibiting the protein synthesis in mammalian cells as well as in malaria parasites. It has been suggested that this effect also accounts for their amoebicidal activity (Kirby GC, 1989; Monjour, 1987). However, their antimalarial action is different from that of cytotoxicity, as some quassinoids have shown greater selectivity against *P. falciparum* than against KB cells (Anderson MM, 1991; Kardono LBS, 1991). The cytotoxicity of glaucarubinone against KB cells is 285 times of its activity against *P. Falciparum* (Wright CW, 1993). All quassinoids inhibits protein synthesis more rapidly than nucleic acid synthesis in the *P. falciparum* infected human erythrocytes which is mainly due to its effects upon ribosome rather than upon nucleic acid metabolism. Inhibition of nucleic acid synthesis was observed following the failure of protein synthesis. As chloroquine does not affect protein synthesis so the chance of cross-resistance of malaria between quassinoids and chloroquine is less (Kirby GC, 1989).

Antibacterial activity: Ethyl acetate fraction of dried stem bark inhibited the growth of *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* (MIC: 6mg/disc). Three active principles, excelsin, 13, 18-dihydroexcelsin and 1, 12-deoxy-13-formylailanthinol, isolated from bark are said to be responsible for this activity. The antibacterial activity of all three compounds was more pronounced than the antifungal potency (Patel RD, 1967; Bhatia N, 1989; Shrimali M, 2001).

Hypoglycemic activity: A single administration of leaves or stem bark extracts of *A. excelsa* lowered the blood glucose of normal rats in a glucose tolerance

test. Administration of each extract for 60 days produced a significant hypoglycemic effect on STZ-induced diabetic rats, with improved renal parameters which suggest of its potential use in the treatment of diabetes (Genta S, 2005).

Insect feedent-deterrent: Excelsin prevents feeding at a concentration of 1000 ppm against destructive lepidopterous pest *Spilosoma oblique* (Tripathi AK, 1993).

Antipyretic activity: Ethanol extract of *A. excelsa*, showed moderate to significant degree of antipyretic activity against yeast suspension induced hyperthermia in an experimental rat model (Suresh B, 1995).

Leishmanicidal: A genus of parasitic flagellate protozoans causes leishmania. In man it invades the cells of the lymphatic system, spleen, and bone (kala-azar). Canthin-6-one alkaloid from *Ailanthus* was found to be active against these protozoans (Thouvenel C, 2002).

Antitumor and cytotoxicity: Aqueous extracts of roots when screened by the brine shrimp lethality assay it showed significant toxicity to the brine shrimp (<60μg/ml) (Krishnaraju AV, 2006). The quassinoids like Ailanthione, glaucarubinone and a mixture of glaucarubol 15-isovalerate have shown substantial antitumor and cytotoxic activities against the P 388 lymphocytic leukemia and KB test system respectively (Asolkar LV, 1992; Anderson MM, 1991). The observed antitumor activity is by inhibiting the protein synthesis of ribosomal peptidyl transferase leading to the termination of chain elongation. (Hall H, 1983).

Hepatoprotective activity: Ethanol extract of leaves showed protective effects against CCl_4 induced liver injury as evidenced by a significant reduction in the CCl_4 induced elevated enzyme levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and serum alkaline phosphatase. The presence of phenolics might be the responsible factor for the above activity (Lavhale MS, 2003; Lavhale MS, 2003).

Toxicity: Large dosage of drug are said to lead queasiness, dizziness, headache, tingling in limbs and diarrhea, myocarditis associated with fever, chills, epigastric pain, substernal chest pressure, and shortness of breath which may likely due to exposure to quassinoids present in tree sap (Bisognano

JD, 2005).

Preparations:

Ailanthus is an important ingredient in most of the ayurvedic preparations like, *Pusyanuga churna, Brahat Gangadhara churna and Aralu putpaka,* used in the management of *atisara, krimi, arsa, sannipatajwara, brama, tvakroga, chardi, kustha, pravahika, grahani, prameha, gulma, swasa, musaka and visaja roga* (Anonymous. 2001). *Dasmularista,* a highly prized ayurvedic formulation for fatigue, is actually a mixture of ten different herbs out of which one is Shyonak/Sonapatha. It aids in cellular regeneration to hasten removal of dead or weak cells and replace them with fresh, vital ones (Anonymous, 1978). In ayurvedic literature there happens to be a controversy between the common name used for both *A. excelsa* and *Oroxylum indicum* mentioned as *Shyonak* (Bopalal Vaidya, 1982). In the Bhavprakash nighantu also *A. excelsa,* is described under the name of 'Aralu' and Sonapatha/Shyonak is mentioned as its synonym (Pandit Bhavmishra, 1963; Lala Shaligramji Vaishya, 1981). In Amarkosh *aralu, shyonak* and *tintuk* are the names given to the same plant (Baburanjitsingji Vaidya, 1965). In the title "Some controversial drugs in Indian medicine" the Nighantu writers have confounded it with *Oroxylum indicum* (Bignoniaceae) (Bopalal Vaidya, 1982). As per the Adarsha Rajniguntakar Nighantu, the description under Shyonakyugal mentioned that in case of two *Shyonakas,* one should be *aralu* and other is *tintuk,* whereas European practitioners consider both *A. excelsa* and *Oroxylum indicum* as totally different plants; however *A. excelsa* is said to be a substitute for Shyonak (Shree Bopalal G Vaidya, 1968).

Pilex, the most popularly used ointments for piles contains bark of *A. excelsa* and is indicated in hemorrhoids, anal fissures, fistulae, proctitis, venous stasis, varicose veins, thrombophlebitis, varicocele and varicosity (M.S. Mohd Shafi Misger, 1977). Lukol tablets used in leucorrhoea contains Loha bhasma, along with extracts of *Withania somnifera, Saraca indica, Woodfordia floribundi, Symplocos racemosa, A. excelsa, Leptadenia reticulata* and *Asparagus racemosus* which acts synergistically as uterine tonics, nervine sedatives and have a stimulating action on the endometrium and ovarian tissues (Bhatnagar, 1984; E. Rajyalaxmi, 1982; S.M. Dabadk, 1984; Bose C, 1996). Sports massage

oil prepared from the bark of A. *excelsa* is used to keep muscles relaxed. "Rain tree's Simarouba extract" the preparation of *Simarouba amara*, contain quassinoids like ailanthinone and glaucarubinone as the main active constituents, which are also present in *A. excelsa*, and are considered to be the main therapeutic constituents for dysentery (amoebic and bacterial) and diarrhea; intestinal worms and internal parasites; malaria; as an astringent to stop internal bleeding (stomach ulcers, hemorrhages) and externally for wounds and in viral infections (Bonte F, 1996).

1.8.2 Butea monosperma Lam.

Syn. *Butea frondosa* (Fabaceae, Papilionaceae)

Common names:

The plant is known by different names like, Kino tree in Bengali, Hindi- dhak, palas, Gujarati- *khakaro*, Sanskrit- *palasa*, Tamil- *pilasu*, Telgu- *mooduga*, *palasamu* and *palas* in Marathi (Kirtikar KK, 1975).

The tree is leafless and with flowers from January to April and its certainly one of our most gorgeous trees, with a riot of orange and vermilion blossoms covering the entire crown and looking truly a tree a flame that's why its commonly known as "Flame of the forest". The cup-shaped velvety brownish-green calyx is in perfect contrast to the vermilion-red of the petals. The curved keel recalls the curved beak of a parrot so also called as "Parrot Tree" (Kirtikar KK, 1975; Chakravarti Venkatesh, 1976; Randhawa, MS, 1965) (Fig-1.7& 1.8).

Distribution:

This herb is indigenous to India, found chiefly in the mixed or dry deciduous forests of central and western India. It is mostly grown in mountainous regions of India, Burma and Ceylon (Kirtikar KK, 1975).

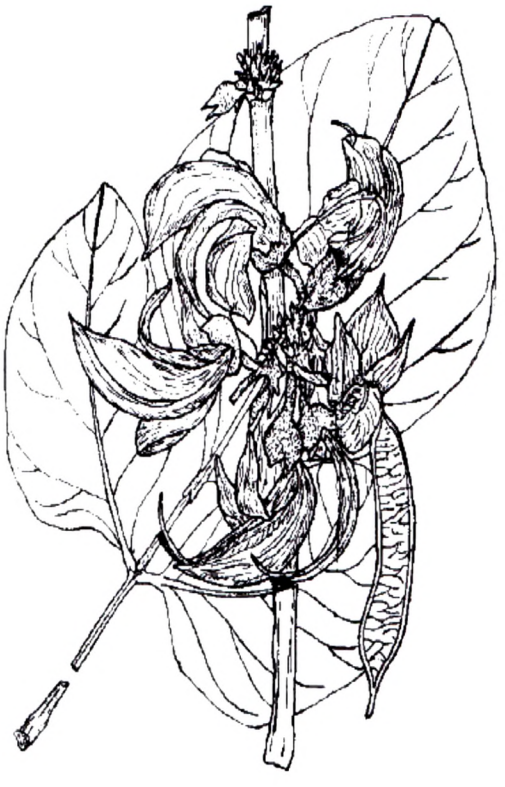


Fig- 1.7 Flowers of *Butea monosperma*

Fig-1.8 *Butea monosperma* leaves, flowers and fruit.

Parts used: Bark, flowers, leaves, gum and seeds

Ethnopharmacology:

B. monosperma possess many medicinal properties such as onic, astringent, aphrodisiac and diuretic. The flowers are widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-implantation activity (Chopra, 1956). Roots are reported to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumors. Pippali rasayana, an ayurvedic drug, from *B. monosperma* is used in the management of giardiasis (Agarwal, 1997).

In Ayurvedic system of medicine, the flowers of *Butea* are used for relieving burning sensation, in the treatment of gout, leprosy and other skin diseases. In Unani system of medicine they are used as aphrodisiac, expectorant, tonic,

emmenagogue, diuretic and in biliousness (Jayaprakash GK, 2001; Kirtikar KR, 1995; Anonymous, 1999)

According to Charaka and Sushruta, the bark and the seeds are used in snake bite (Chopra IC, 1958; Andrew Chevallier Mnimh). Gum fried in ghee is taken by women twice daily for 15 days to relieve post- delivery backache and weakness. Six gram powder of bark, flowers and gum, in equal quantity is given twice a day to patients with gonorrhea, until symptoms disappear. (Mohammad Anis1, 2000). Roots of *Kuchila* (Snakewood, *Strychnos nux-vomica*) and roots of *Surjamukhi* (Common sunflower, *Helianthus annuus*) is mixed with *Palas* (*B. monosperma*) petals and mustard oil and applied topically over the wound. (Amitendu, 2004). Seeds of palash are to be powdered and taken with water for three days during the time of fertilization. Fruit of Kshirivriksha (*Mimusops bexandra*) and flower of Shalmali are added to seeds of palash and then taken with alcohol for 15 days for producing sterility in the woman. Seed of Palasha tree is made into a paste by adding honey and then taken during the menstrual period. This will prevent both menstruation and conception in future. Seeds, when pounded with lemon juice and applied locally, act as a powerful rubefacient (Unesco, 1960). The flowers are used in the herbal antistress formulation, 'Jigrine'. (A.D.Bhatwadekar, 1999)

Phytochemistry:

Flowers contain butein, butin, butrin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, palasitrin, monospermoside (butein 3-β -D-glucoside) and isomonospermoside. Bright colour of the flowers is attributed to the presence of chalcones and aurones. Stem bark contains stigmasterol, stigmasterol-β-D-glucopyranoside, nonacosanoic acid, 3α-hydroxyeuph-25-ene and 2, 14-dihydroxy-11,12-dimethyl-8-oxo-octadec-11-enylcyclohexane (Mishra M, 2000) (Fig-1.9)

The gum from the bark contains leucocyanidin and its tertramer procyanidine, along with riboflavin and thiamine. It also contains monospermin [1-N-acetyl-2-oxo-4-methoxy-3H, 5H-imidazole] and (-)-palasonin (Husain A, 1992). The seed oil shows the presence of fatty acid like myristic, palmitic, stearic, arachidic, behenic, lignoceric, oleic, linoleic and linolenic acids. The

unsaponifiable part of the oil contains β-sitosterol, its β-D-glucoside and α-amyrin. Jalaric esters I and II and laccijalaric esters III and IV have been reported from the roots. A potential antiviral flavone glycoside 5, 20-dihydroxy-3, 6, 7-trimethoxyflavone-5-O-β-D-xylopyranosyl-O-β-D-glucopyranoside has been isolated from the seeds. (Yadava RN, 2005).

Analyses of seeds showed phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol as the major components. Small amounts of lysophosphatidylcholine and cardiolipin were detected (Prasad R, 1987). Seeds yield a yellow oil, proteolytic and lipolytic enzymes palsonin h-heneico-sanon and lactone, α-amyrin, β-sitosterol, β-D-glucoside, sugars and monospermin. (Monika V, 1998)

The red-colored gum, obtained from the tree is rich in gallic and tannic acids. The seeds contain a yellow fixed oil called moodooga oil or kino-tree oil, small quantities of a resin and large quantities of a water soluble albuminoid. Fresh seeds contain proteolytic and lipolytic enzymes.

A structurally related toxin, namely palasonin was isolated from seeds (Raj, 1967). Palasonin was found to be effective against ascarids, stomach worms of sheep, oxyurids of mice, and hook worms of dogs (Mehtar, 1966). The biochemical mechanism of the anthelmintic action of palasonin is based upon inhibition of glucose uptake affecting the energy generating mechanisms and motor activity of the parasite (Kumar, 1995).

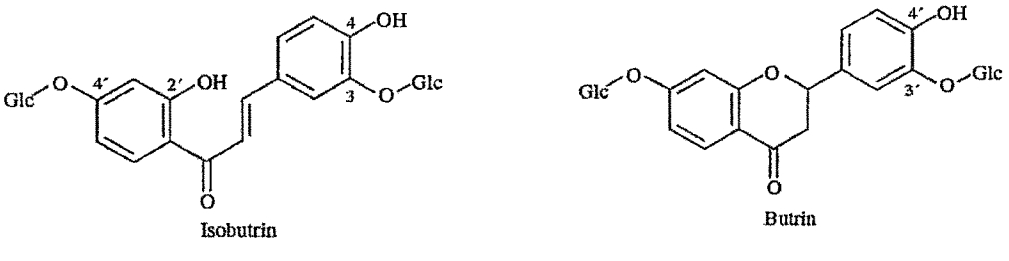


Fig-1.9 Structure of Isobutrin and butrin from *B. monosperma* flowers

Pharmacological properties

Different parts of the tree are worked out for their antifertility (Kholkute SD, 1976) antifungal (Bandara BMR, 1989), anthelmintic (Prashanth D, 2001), hepatoprotective (Sehrawat A, 2006; Wagner H, 1986), anti-inflammatory (Nazimuddin SK, 1982), antidibetic, anti-diarrhoeal (Gunakkunru, A, 2004), antipyretic, antimicrobial (Kram M, 1980), antiulcer, and anticonvulsant (Kasture VS, 2002) properties. The flowers are used in the herbal antistress formulation, "Jigrine™" which was proposed to be due to the decrease in brain 5-HT and suppression of hypothalamo-hypophyseal-adrenocortical axis. (Bhatwadekar AD, 1999)

Anticonvulsant property

Acetone soluble part of petroleum ether extract of flowers protected animals from maximum electro shock, electrical kindling and pentylenetetrazole-induced convulsions in mice. The fraction also inhibited convulsions induced by lithium-pilocarpine and electrical kindling. (Kasture VS, 2002)

Diarrhoea and dysentery

The gum is useful in the treatment of diarrhoea and dysentery. Further the ethanolic extract of stem bark potential antidiarrhoeal in castor oil induced diarrhoea and PGE (2) induced enteropooling in rats. (Gunakkunru A, 2005)

Skin disorders

The seeds are beneficial in the treatment of certain skin diseases. The seeds, ground and mixed with lemon juice, can be daubed on dhobi's itch-an eczema-type of skin disorder, characterized by itching. They can also be applied with gratifying results on ring worms. A hot poultice of the leaves can be applied to resolve boils, pimples, tumorous piles, ulcers and swellings. The crushed seeds can be used for killing maggots in wounds and sores.

Diabetes

The leaves of the tree are very useful in diabetes. They reduce blood sugar and are useful in glycosuria.

Leucorrhoea

The leaves are also beneficial in the treatment of leucorrhoea. Decoction or infusion of leaves should be used as a vaginal douche for this purpose.

Sore Throat

The leaves are useful in congested and septic throat. A decoction of the leaves obtained by boiling them in water should be used as a mouth-wash in the treatment of this disorder.

Retention of Urine

The leaves are useful in treating the difficulty of retention of urine. The pubic region should be fomented with the leaves in this disorder

Non medicinal properties

Dye obtained from the flowers is used to colour cotton fabrics, wooden articles and woolen carpets and to control white ants in the field. It is also used as gulal during the festival of holi. The gum from the trunk is used in tanning leather and more commonly for making quer bangles etc. The gum exuded from the trunk is used for tanning leather. The bark and young roots make a strong coarse fibre. The large leaflets are much used in South for making round plates, stitched together with the steams of the broomstick grass. Young leaves are fed to buffaloes. The tree is also one of the principal hosts of the Lac insect. The palas is sacred to the Hindus. The three leaflets are symbolic of Trimurti—the trinity of Brahma, Vishnu and Shiva. The flowers are offered as in place of blood in sacrifice rituals to goddess Kali (Chakravarti Venkatesh, 1976; Dutt VC, 1995; Nadkarni KM, 1976).

Toxicity

Chronic oral exposure to substantial doses of the seeds of the plant leads to nephrotoxicity and anaemia in rats, dogs and rabbits. Hepato and splenomegaly, with congestion, gross dilatation of the stomach and gastric inflammation were also seen. The seed oil showed cardiac effects with inhibition of ventricular movement, reduction in the amplitude of contraction of isolated frog heart and duodenal muscle relaxation in the rabbit.

Preparations

"Hair Loss Cream" is a polyherbal formulation recommended for the management of telogen effluvium, and contains the extracts of the flowers of *B. monosperma* and bark of *B. parviflora*. (Rawal R, 2005)

The seeds of *Butea* are one of the important constituents of *Pippali rasayana*, an immunomodulatory ayurvedic drug (Agrawal AK, 1997)

It is also an important component of some other ayurvedic formulations like *Palsabijadi churna, Krimikuthara rasa, Maha Narayana taila* and *Khakarano ark* used in the management of various disorders (Anonymous, 1999).

The flowers of *B. monosperma* were in the herbal antistress formulation, 'Jigrine'. (Bhatwadekar AD, 1999)

1.9 Objectives of the study

The role of reactive oxygen species in several diseases and the potential antioxidant effect of natural compounds on affected tissues are topics of high interest. A number of plants and plant isolates are reported to protect free-radical induced damage in humans and animals.

On the basis of the available information on these plants it was felt that these offer scope for further investigation upon their potential as antioxidant. Systematic studies were therefore proposed including isolation and characterization of active chemical constituents from the bioactive fractions of these plants. The biological standardization was also proposed to justify their role in traditional systems of medicine and hence the objectives of the present investigation were set as follows:

- To select suitable extracts from the plant material
- To fractionate and identify bioactive fraction from the selected extracts
- To evolve method of separation and characterization of bioactive molecules from active fractions
- To study isolated chemical moieties for their anti-oxidant activity using various *in vitro* and *in vivo* methods

1.10 Evaluation of antioxidant activity

1.10.1 In-vitro Methods

Assessment of Radical Scavenging activity

Radical scavenging activity of various extracts is determined by measuring their reducing power (Oyaizu M, 1986), percentage inhibition of DPPH radical (Blois MS, 1993), superoxide anion radical (Robak J, 1998; Beauchamp C, 1971), nitric oxide radical (Green LC, 1982), hydroxyl radical (Halliwell B, 1987) and erythrocyte hemolysis induced by 2, 2'- azo-bis (amidinopropane) dihydrochloride (AAPH) (Zhu QY, 2002; Ko FN, 1997). Further rapid screening

for radical scavengers in the extract done on TLC using DPPH reagent (Cuendet M, 1997).

Determination of Total Phenolic Content

As most of the phenolic compounds are reported to possess antioxidant activity, extracts are subjected for their total phenolic content (Shahidi F, 1992).

1.10.2 Acute toxicity study

As per OECD guideline 425 toxicity studies were performed for selected extracts (Ghosh MN).

1.10.3 *In vivo* Antioxidant Activity

1.10.3.1 Isoproterenol induced myocardial infarction

In vivo studies are carried out on the selected extracts using isoproterenol (ISO) induced myocardial infarction in rats. Serum levels of creatine kinase Isoenzyme (CKMB), lactate dehydrogenase (LDH) and glutamate oxaloacetate transaminase (GOT) are the diagnostic indicators of myocardial infarction. An increase in the activity of these enzymes in serum is due to their leakage from heart as a result of necrosis induced by ISO. Increase in serum uric acid could be due to excessive degradation of purine nucleotides and proteolysis (Iriama, 1987).

Protective effect of extracts are to be seen by analyzing the endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), in heart homogenate along with serum marker enzymes like GOT, CK-MB, LDH, and uric acid. The study can be supported by histopathological examination of heart. (Hearse DJ, 1979; Manjula TS, 1992).

1.10.3.2 Effect of extracts on Isoproteronol induced liver damage

Myocardial infarction produces significant abnormal liver functioning. In the present study the effect of selected extracts on liver of myocardially infarcted rats is investigated. Alterations in tissue marker enzymes of liver injury like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and Alkaline Phosphatase (ALKP) are studied (C.J. Thirupurasundari, 2005).

The protective effect of extracts can be supported with reversal of isoproterenol-induced changes in liver histopathology.

1.10.3.3 Isoproterenol Induced Hyperlipidemia

Mathew et al. reported an altered lipid metabolism in myocardial necrosis following isoproterenol administration. High level of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage. Administration of isoproterenol mainly raised LDL cholesterol and decreased HDL cholesterol level in serum. High levels of LDL cholesterol have positive relation and high levels of HDL cholesterol have negative relation with myocardial infarction. HDL inhibits the uptake of LDL by arterial wall and also facilitates the transport of cholesterol from peripheral tissue to the liver where it is catabolised and excreted out of the body. Isoproterenol administration resulted in significant increase in the free fatty acid level. Hypertriglyceridemia seen in isoproterenol treated rats, a condition observed in ischemic heart disease, is due to a decrease in the activity of lipoprotein lipase in the myocardium resulting in decreased uptake of triglycerides from circulation (Mathew S, 1981)

For evaluating isoproterenol induced hyperlipidemia, analysis of serum lipid profile was carried out in isoproterenol induced myocardial infarction. Various factors like, total cholesterol, LDL, HDL, VLDL and triglycerides were analyzed under this study.

Selected extracts from the *in vivo* studies are to be studied for their antioxidant property using rat myocardial cell lines H9c2.

1.10.4 In vitro studies using H9c2 cell lines

1.10.4.1 Analysis of cell viability

The viability of cells after treatment with test material was assayed by the reduction of 3-(4, 5-dimethylthiazole-2-yl)-2, 5- diphenyl-tetrazoliumbromide (MTT) to formazan as described previously (Plumb, 1989).

1.10.4.2 Measurement of intracellular ROS

The selected extracts and isolated compounds selected from the *in vivo* studies were further studied for their protective effect against H_2O_2 and Xanthine – xanthine (X+XO) generated reactive oxygen species (ROS) in rat myocardial cell lines (H9c2) after loading the cells with ROS specific molecular probes like, Dichlorodihydro fluorescein diacetate (DCF-DA) (Kuo, 1998), Dihydroethidium (DHE) (Cai J, 1998), Diaminofluorescein diacetate (DAF-2DA) (Kojima H, 1998; Zhong Zhang, 2004; Roychowdhury S, 2002), and dihydrorhodamine (DHR123) (Crow JP, 1997). Effect of extracts and compounds were studied on H_2O_2 affected mitochondrial membrane potential using tetramethyl rhodamine methyl ester (TMRM) (Desagher S, 1999; Narita M, 1998) and 3, 3'-dihexyloxacarbocyanine iodide ($DiOC_6$) [Rottenberg H 1998]. Nuclear staining was done with 4', 6-diamidino-2-phenylindole (DAPI),

While studying Ailanthus root bark, chloroform extract of root bark was found to affect cardiovascular system in rats, which offers further scope for its detailed study.

1.10.5 Studies on *Ailanthus excelsa* root bark

1.10.5.1 Effect of chloroform extracts *Ailanthus excelsa* root bark on mechanical properties of rat heart

Circulatory functions are measured with ultra miniature catheter pressure transducers in spontaneously breathing animals. Various haemodynamic variables, like heart rate, electrocardiogram, systolic pressure, diastolic pressure, mean pressure, systolic and diastolic duration, left ventricular contractility (dP/dt_{max}) and relaxation (dP/dt_{min}), contractility index, pressure time index can be recorded using the Power Lab system (Power Lab, AD Instruments).

1.10.5.2 Effect of chloroform extract of Ailanthus root bark and its isolates on electrophysiological characteristics of neonatal rat cardiomyocytes

Ventricular cardiomyocytes are isolated from the neonatal rats and their mechanical properties can be assessed by using a SoftEdge MyoCam system (IonOptix, Milton, MA). Following contractile indices like peak shortening (PS) amplitude, maximal velocity of shortening/relengthening ($\pm dL/dt$), time to PS (TPS), and time to 90% relengthening (TR_{90}) are measured.

1.10.5.3 Effects of chloroform extract isolates on perinuclear and nuclear calcium level in H9c2 cells (Fluo-3/AM assay)

Fluo-3 dye is used to detect effect of compounds on intracellular calcium concentration (Kao, 1989; Minta, 1989). With the help of this calcium-sensitive fluorescent dyes and video microscopic imaging detection of the changes in intracellular calcium in the cytoplasm, in the perinuclear region, and in the nucleus gets possible.

While studying chloroform extract isolate using H9c2 cells, the compound was found to affect the cell growth, which offers further scope for evaluating its antitumor activity using various *in vitro* and *in vivo* methods in cancer cell line.

1.10.6 Studies on compound isolated from root bark

1.10.6.1 In vitro antitumor activity for chloroform extract isolates

1.10.6.1.1 Cell viability

Effect on viability of prostate cancer (PC3), breast cancer (MDA-MB) and melanoma (B16) cells can be studied by using MTT assay

1.10.6.1.2 Tritiated thymidine ([3H]-TdR) incorporation assay

Tritiated thymidine incorporation assay is used to study the cell proliferation. The radioactivity measured as counts per minute (CPM) determined by using a liquid scintillation counter (Packard, USA).

1.10.6.1.3 Flow cytometry analysis of DNA content for cell cycle

Cell cycle analysis based on measurements of DNA content generates a clear pattern of distribution of G0/G1 phase, S phase and G2/M phase. DNA content can be measured using fluorescent, DNA-selective stains propidium iodide that exhibit emission signals proportional to DNA mass. Flow cytometric analysis of these stained populations is then used to produce a frequency histogram that reveals the various phases of the cell cycle.

1.10.6.1.4 Annexin V-PI staining for apoptosis detection

In this assay Annexin V is used for detection of apoptosis by flow cytometry microscopy. Since membrane permeabilization is observed in necrosis, necrotic cells will also bind Annexin V. Propidium iodide is used to distinguish between viable, early apoptotic and necrotic or late apoptotic cells. Necrotic cells will bind Annexin V and stain with propidium iodide while propidium iodide will be excluded from viable and early apoptotic cells. (Frey T, 1997)

1.10.6.2 In vivo antitumor activity

B16-F10 melanoma cells are injected subcutaneously in the flank of C57Bl/6 mice. After 15 days inoculation animals are grouped like control, treated cis-platin respectively (n=6). Intratumor injections of test material and cis-platin were given to animals, while control received only PBS for 12 days. Tumor volume is to be measured at definite interval during the study. At the termination of the experiment tumor sample are prepared for biopsy and western blotting. Histopathologies of all the organs in mice are carried out. (Raymond M., 2005).

1.10.6.2.1 Western blot analysis of tumor cells isolated from animals.

Western blot analysis of the tumor samples using SDS-polyacrylamide-gel electrophoresis gel provides information regarding the expression of proteins in cell cycle.

1.11 RESEARCH ENVISAGED

A) Studies on *Ailanthus excelsa* leaves

- Generation of phytochemical profile, TLC fingerprint of Ailanthus leaves.
- Screening the extracts for preliminary antioxidant activity.
- Selection of the active extracts and their toxicity study as per OECD guidelines.
- Studies on selected extracts using isoproterenol (ISO) induced myocardial infarction and liver damage in rats.
- Studies for active extract on H_2O_2 and xanthine- xanthine oxidase induced stress in myocardial cell lines H9c2.
- Development of methods for estimation of possible active compounds in different extracts different analytical methods.

B) Studies on *Ailanthus excelsa* root bark

- Successive extraction of Ailanthus roots and development of phytochemical profile and TLC fingerprint.
- Screening the extracts for preliminary antioxidant activity.
- Toxicity studies of selected active extracts as per OECD guidelines.
- Isolation of compounds from root bark their characterization with UV, NMR, IR, CHN and mass spectroscopy.
- Biological evaluation of isolated compounds.
- Cell line studies on isolated compounds.
- Development of methods for estimation of compounds in extracts.

C) Studies on *Butea monosperma* flowers

- Generation of phytochemical profile, TLC fingerprint of Butea flowers.
- Preliminary screening of extracts for radical scavenging activity.
- Selection of active extracts and their toxicity studies as per OECD guidelines
- Biological studies on selected active extracts.
- Isolation of compound from active extracts and their characterization with NMR, IR and mass spectroscopy.
- Cell line studies on isolated compounds
- Quantification of isolated compounds in different extracts.