1.1. Introduction

A state of enhanced oxidant production either in animal or in human cells, distinguished by the removal of free radicals and consequentally cellular degeneration is kown as oxidative stress (Beyani et al., 2009).

Neuro degenerative, cardiovascular and hepatic disorders are generally associated with oxidative stress. H_2O_2 remains inert and never accumulates in the tissues or other organs under normal circumstances.

The genesis of reactive oxygen species (ROS) is an unavoidable consequence of life in an aerobic environment; ROS are characterized by their high chemical reactivity and include both free radicals and non-radical species. ROS concentration may scavenge or reduce since there is equilibrium between ROS generation and the action of enzymatic and non-enzymatic antioxidant systems. Redox imbalance caused either increased ROS production or reduced antioxidant defense. It results oxidative stress and there is probability of living cells to react with ROS (Sanberg and Giordano, 1988).

1.1.1. Study of Medicinal Plant

The surroundings provided the complete accommodation to cure all diseases. Herbs today are being increasing used to treat all types of ailments. The medicinal plants are studied to promote the proper use of herbal medicine and to find out the possible basis for novel drugs (Kokate, 2004). The history of herbal medicines is as old as human civilization. Natural products are more acceptable for our body than synthetic substances. These synthetic drugs are out of reach of millions of people and have very much side effects. For a variety of reasons, more individuals now a day prefer to take personal control over their health with the use of herbal medicines (Akerele, 1993).

In India and China plants are directly used as medicines. The humans and the animals can be protected from plant toxicity if studied. Cultivation and preservation of medicinal protect biological diversity, for example metabolic engineering of plants (Pandey et al., 2008).

India may be designated as botanical garden of the world since it is the largest creator of medicinal herbs. In Indian medicinal systems the practitioners generally prepare and serve formulae; therefore there are requirements of appropriate documentation and research. The traditional systems of medicine serve 70% of the population in rural India. An approximately 40% of the population is taking herbs to treat diseases in western world. This may be due to increased prevalence of the adverse drug reactions and financial load of the present man-made drugs (Ali, 2008).

1.1.2. Importance of plants as a source of new drugs

The 25% of all drugs prescribed today come from plants. This estimate suggests that plant-derived drugs build up a important section of natural product–based pharmaceuticals. Out of many families of secondary metabolites, nitrogen-containing alkaloids have given the largest number of drugs to the modern pharmacopoeia, ranging in effects from anticholinergics (hyoscine) to analgesics (morphine) and from antiparasitics (quinine) to anticholinesterases (galantamine) to antineoplastics (vinblastine/vincristine). Terpenoids (as well as steroids) have also made an equally important contribution to human health with alkaloids in the modern pharmacopoeia. The Na+/K+ pump-inhibitor like cardiac glycosides obtained from *Digitalis* species, antineoplastic (paclitaxel), antimalarial (artemisinin), anti-inflammatory (triptolide) are naturally occurring materials (Joy et al., 1998). The methanolic root extract of *Hemidesmus indicus* was responsible for snake venom neutralization. There is a big need of plant-derived therapeutic antagonist against snakebite (Verma and Singh, 2008).

1.1.3. Current and Future perspectives

The interest in Nature as a source of potential chemotherapeutic agents continues. More than 50% of all the drugs in clinical use are natural occuring products globally. During the last 40 years, at least a dozen potent drugs have been derived from flowering plants. Diosgenin was obtained from *Dioscorea* species. It showed anovulatory contraceptive properties. Some alkaloids like reserpine obtained from *Rauwolfia* species acted as antihypertensive medications. Some American trees (Pilocarpus species) constituted pilocarpine which is used as an antiglaucoma drug and in sjogren syndrome. Some anti-cancer agents like vincristine and vinblastine obtained from the *Catharanthus roseus.* Some *Cassia* sp. contained laxative agents and *Digitalis* species are also used as a cardiotonic agent to treat congestive heart failure (CHF). Approximately half (125,000) of the world's flowering plant species live in the tropical forests (Ameenah, 2006).

Although some troubles are there with plant materials but no one can not be forgotten value of plants in olden days. The chances are provided to the scholars in the field of Natural Product Chemistry, Pharmacognosy, Pharmacology, Ethnobotany etc. New drugs can be derived from plants. The structure activity platform may be made if the knowledge gained by traditional system strengthened. The drug development process may be smoothly run since traditional knowledge and experiential database can provide new functional leads to reduce time, money and toxicity. The extract libraries may be developed and it will be responsible for cost effective collection of plants. The plant materials will be available which will be encoded accurately. The extracts may be preserved in large amount and screened accordingly.

Modern equipment and biological assay methods provide the possibility of developing suitable quality control criteria for herbal drugs. the new plant constituents can be determined by sophisticated spectroscopic (UV, FT-IR, NMR, Mass spectroscopy) and X-

ray crystallographic techniques. The biological profile can be obtained by high-throughput automated bioassays. The clues are existed which showed exact utilization of plants to improve the human health and it will be continued in the 21st century (Kinghorn and Balandrin, 1993).

1.1.4. Quality control and Quality Assurance of Herbal Drugs

Several problems influence the quality of herbal drugs. the herbal drugs are usually mixtures of many constituents, however the active principle(s) is (are), in most cases are unknown. Since selective analytical methods or reference compounds may not be available commercially. The plant materials are chemically and naturally variable therfore the source and quality of the raw material are varied. The methods of harvesting, drying, storage, transportation, and processing (mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect on plant materials. Hence strict guidelines have to be followed for the successful production of a quality herbal drug. The proper botanical identification, phytochemical screening, and standardization should be established for analytical purposes (Gupta, 2003).

Quality control requirement of new formulation of traditional medicines comprised recipe, literature and basis of scientific and raw data after physico-chemical studies. The batch production report which included the quality standard and explanation of medicinal material, literature and test data of initial stability for clinical research (accelerated stability study etc.), the information of quality detection and hygiene standard detection of the preparation for clinical research, property and specification of the packing material include packaging instructions of the medicament, labeling specifications and applied instructions which may vary country to country.

International scheme for quality assurance of pharmaceutics involves the standard practices like GAP: Good Agricultural Practice, GLP: Good Laboratory Practice, GMP: Good

Manufacturing Practice, GCP: Good Clinical Practice and GALP: Good Analytical/Automated Laboratory Practice (Waxler, 1998).

1.1.5. Free radical and the antioxidant defense system

To better comprehend the relation between changes in the brain membrane lipids and oxidative stress, we must look at the question of the free radical biochemistry and oxidative stress. A consequence of aerobic metabolism is the generation of potentially toxic free radicals, which are chemical species with unpaired electron (primarily the reactive oxygen species and hydroxyl radicals) they are generated in vivo during many normal biochemical reaction involving oxygen, including the mitochondrial electron chain, NADPH – dependent oxidases, and oxidation of PUFA and the and the catecholamine (Rice – envas,1994). The superoxide radical produced during these reactions are dismuted to hydrogen peroxide. Hydrogen in itself is not a free radical, but is susceptible to autoxidation to yield the hydroxyl radical, one of the most reactive species. Complex protective strategies have evolved against free radicals toxicity.

Under normal physiological conditions, free radical mediated damage is kept under control by the antioxidant defence system, comprising of series of enzymatic and non-enzymatc component. The important enzyme include superoxide mutase (SOD), catalase(CAT) and Glutathione peroxidase (GSH-px). These enzyme acts cooperatively at different sites in the metabolic cascade of free radicals.

1.1.6. Brain and liver is highly susceptible to oxidative damage

All aerobic organism are susceptible to oxidative stress simply because semi reduced oxygen species, super oxide and hydrogen peroxide, are produced by mitochondria during respiration (Chance et al., 1979). The exact amount of ROS produced is considered to be about 2% of the total oxygen consumed during respiration, but may vary depending on several parameters. Brain as consider abnormally sensitive to oxidative damage and in fact

early studies demonstrating the ease of peroxidation of brain membranes supported this notion (Floyd and Carney, 1992). Brain is enriched in the more easily peroxidizable fatty acid consumed an inordinate fraction (20%) of the total oxygen consumption for its relatively small weight (2%), and is not particularly enriched in antioxidant defenses (Zaleska and Floyd,1985). Additionally, human brain has higher level in iron (Fe) in certain region and in general has high level of ascorbate. Thus if tissue organizational disruption occur, the Fe/ ascorbate mixture is expected to be an abnormally potent pro-oxidant for brain membranes (Poli et al., 1989).

Rigorous measurement of hydrogen peroxide production from isolated brain mitochondria shows that it amounts to about 2% of the total oxygen consumed when NADH supplies the reducing equivalents (Hensley et al., 1998). In addition to mitochondria, additional sources of ROS include mixed function oxidases as well as other oxidative process. Of particular importance to brain is the hydrogen peroxide produced by oxidative deamination of catecholamine.

The brain is an organ that works as the center of the nervous system in all vertebrate and most invertebrate animals and important functions are co-ordination of boy.

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion.

1.1.7. Natural products and defense against oxidative stress induced diseases

The role of natural products as a source for remedies has been known since ancient times (Cragg and Newman, 1999). There are some examples of agents summarized below derived from natural sources and are currently used in clinical practice (Gupta and Sharma, 2006).

Table 1.1. List of natural products

SN	Plant	Common name	Parts used	Chemical constituents	Biological activities
1	*Curcuma domestica*	Turmeric	Leaf	Curcumin, β -pinene, Eugenol	For cleaning blood, in treatment of cough and dyspnea
2	*Cuscuta reflexa*	Akasabela	Leaf	Flavanoids Dulcitol Coumarines	Expectorant Carminative
3	*Daucus carota*	Carrot	Root	Carotene Carotenoids Flavanoids	Used in bronchitis Chest trouble
4	*Embilica officinalis*	Amla	Fruit	Vit C, Polyphenols	Used in inflammation Piles, Vomiting
5	*Foeniculum vulgare*	Sauf	Fruit	Fenchon, Limolene	Stimulant, Carminative
6	*Glycyrrhiza glabra*	Mulethi	Root	Glycyrrhizin Flavanoids	Diuretics, Emmenegogue
7	*Magnifera indica*	Aam	Leaf Root Fruit	Cynogetic Glycoside Polyphenol, Vit. A	Leucorrhoea, Dysentery Bronchitis
8	*Momordica charantia*	Karela	Leaf Fruit Seed Root	Triterpen glycoside Stearic acid	Laxative, Carminative
9	*Ocimum sanctum*	Tulsi	Leaf	Volatile oil, Eugenol	Expectorant, Bronchitis
10	*Psoralea coriliforia*	Babchi	Seed	Essential oil, Resin	Leucoderma, Aphrodiasic
11	*Santlum album*	Safed-chandan	Heart wood Bark	Santalol	Useful in disease of heart Antipyretic
12	*Solanum nigrum*	Makoi	Leaf	Polyphenol, Flavanoids	Hepatoprotective Diuretics
13	*Swertia chirayita*	chirayita	Whole plant	Xanthones Chirantin	Febrifuge, Antimalarial
14	*Withania somnifera*	Ashwagndha	Root Leaf Seed	Steroidal lactone, Withanolides	Aphrodiasic, Hepatoprotective

Recently it has been observed that *A. racemosus* has been extensively used for treatment of ulcer and as antioxidant activities and decrease the tumor occurrence in female rats treated with DMBA (7,12dimethyl benz (a) anthracene (Rao, 1981). Besides these scientifically supported claims; root of *A. racemosus* have also been mentioned in ayurveda for their uses as it stimulate the milk secretion during lactation in women and *E. hirta* has been extensively used in treatment of gastrointestinal disorders (diarrhea, dysentery and intestinal parasitosis), bronchial and respiratory diseases, as well as antidiabetic, immunomodulatory and nephroprotective activity (Subramanian et al., 2011).

It is likely that in future safe and effective medicines will be developed from medicinal plants to treat various degenerative diseases.

Free radical causes various diseases eg. parkinsons, hepatic and cardiac disease. If we control free radicals by different methods/enzyme like super oxide dismutase, glutathione and catalase then we are protecting our body from different diseases. For this various phytoconstitiuents (Flavanoids and polyphenols) play an important role.

Flavanoids and polyphenols scavenging of ROS generated free radicals and get few of them are reused with endogenous oxido-reductases or via intracellular reducing shuttles.

These agents are present in various herb and plants.eg: polyphenols and flavonoids. Some dietary supplements like apple, onion and carrot also kill the free radicals.

After doing extensive literature survey we have selected two plants namely *Asparagus racemosus* Willd. and *Euphorbia hirta* Linn. to see the effects of their extracts on free radicals through various methods/enzyme which have own significance and compare to known non enzymatic antioxidant quercetin and literature survey also reveals there are no scientific claim has been made on anti-stress activity on these selected plants. These observations are the main important criteria to choose these plants for good candidate to

explore scientifically. The main objective of the research work is to explore the plant *A. racemosus* and *E. hirta* for their therapeutic potential in in-vivo model of oxidative stress.

1.1. Hypothesis

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants.

Phenolic and flavonoid compounds are belief to alter the stress induced changes due to its antistress properties. During the literature survey we came to know that *Asparagus racemosus* and *Euphorbia hirta* contains these compounds, so we hypothesized that *Asparagus racemosus* and *Euphorbia hirta* may have antistress property due to which it prevents the stress induced changes in behaviour and oxidative biomarkers. To test this hypothesis behavioural assessment, cytokines estimations, level of oxidative biomarkers and liver function test was observed, by using lipopolysaccharide model in (Sprague Dawley) rats.

1.2. Aim and Objectives

It is believed that herbal drugs provide a safer and natural way to human body in both pharmaceutical and nutraceutical aspects. According to Food and Drug Administration (FDA) herbal drugs can be developed more rapidly and less costly than conventional single entity pharmaceuticals.The aim of the study was to evaluate and determine anti-stress potential of *Asparagus racemosus* and *Euphorbia hirta* against lipopolysaccharide (LPS) induced –oxidative stress in rats.

Following objectives were carried out.

- Collection, Identification and Quality parameters for authentication of the crude drugs.
- Preparation, Optimization and Chemical analysis of various extracts.
- To determine the total phenolic and flavonoidal contents in Plants extracts

- To established the safety profile studies of the optimized and standardized methanolic extracts.
- To investigate the anti-stress activity of the methanolic extract of *Asparagus racemosus* and of *Euphorbia hirta.*

1.4. Significance

Pharmacognostical characters of both plants provide useful information with regards to its correct identity and to evaluate the quality and purity of the plants material. The preliminary phytochemical screening of both the plants indicates the presence of primary and secondary metabolities, which are reported to play an essential role in protective activity against LPS (lipopolysaccharide) induced oxidative stress in rats.

The present study scientifically supports the traditional uses, religious believers and uses of these drugs in the formulations of Unani/Ayurveda as anti-stress enhancers. Therefore, it is suggested that clinical and pharmacological studies should be carried out to investigate other unexploited potential of these drugs and further investigations are also required to isolate and purify novel pharmacologically active compound of industrial importance and to see its effects at molecular level.

1.3. Review of Literature

1.5.1. Plant profile of *Asparagus racemosus Willd.*

A. racemosus is an important medicinal plant belong to family Asparagaceae. One such important medicinal plant which is regarded as a ‘rasayana’ (plant drugs promoting general well-being by increasing cellular vitality and resistance) in the Ayurvedic system of medicine commonly known as shatavari (Hussain et al., 2011).

1.5.1.1. Synonyms

Asparagus rigidulus

Protasparagus racemosus

1.5.1.2. Vernacular names

Hindi	:	Satavar, stavari, satmuli
English	:	Wild asparagus
Gujarati	:	Satavari
Telugu	:	Pilli-gaddalu
Tamil	:	Tannirvittan, Kadumulla
Marathi	:	Asvel
Kashmiri	:	Sejnana

1.5.1.3. Botanical classification

Kingdom	:	Plantae
Clade	:	Angiosperms
Order	:	Asparagales
Family	:	Asparagaceae
Subfamily	:	Asparagoideae
Genus :	:	*Asparagus*
Species	:	*racemosus*

1.5.1.4. Geographical sources

The plant grows throughout the tropical and subtropical parts of India and quite commonly met within most states from sea level to about 1,400 m elevation.

1.5.1.5. General Description

The plant is a spinous under-shrub; the genus *Asparagus* includes about 300 species around the world. The genus is considered to be medicinally important because of the presence of steroidal saponins and sapogenins in various parts of the plant. Out of the 22 species of *Asparagus* recorded in India; *Asparagus racemosus* is the one most commonly used in traditional medicine (Goyal et al, 2003).

Flower- Flowers white, in simple or branched racemes.

Leaves- The leaves are like pine needles, small and uniform.

Root- numerous succulent tuberous roots (30–100 cm long and 1–2 cm thick) that are silvery white or ash coloured externally and white inter-nally.

Stem- is woody, climbing,whitish grey or brown coloured with small spines.

Fig 1.1. Roots of *A. racemosus*

1.5.1.6. Phytoconstitiuents

Steroidal saponins, known as shatvarins. Shatvarin I to VI are present. Shatvarin I is the major glycoside with 3-glucose and rhamnose moieties attached to sarsapogenin (Bopana and Saxena, 2007). Oligospirostanoside referred to as Immunoside. Polycyclic alkaloid-Aspargamine A, a cage type pyrrolizidine alkaloid (Krtikar and Basu, 1975). Isoflavones-8-methoxy-5,4,6-tri hydroxyl isoflavone-7-o-beta-D-glucopyranoside (Gautam et al.,2009). Flavanoids-Glycosides of quercitin, rutin and hyperoside are present in flower and fruits. Sterols-Roots also contain sitosterol, 4,6-dihydryxy-2-O (-2-hydroxy isobutyl) benzaldehyde and undecanyl (Narayanan et al.,2011). Trace minerals are found in roots-zinc, manganese, copper, cobalt along with calcium, magnesium, potassium zinc and selenium. Kaepfrol-Kaepfrol along with Sarsapogenin from woody portions of tuberous roots could be isolated. Miscellaneous Essential fatty acids-Gamma linoleinic acids, vitamin A, diosgenin and quercetin 3-glucourbnides (Hannan et al., 2011).

1.5.1.7. Traditional medicinal uses

Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic and as tonic. Beneficial effects of the root of *A. racemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentry, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases.

1.5.1.8. Pharmacological literature review

1.5.1.8.1. Immunomodulatory activity

Aqueous extract of *A. racemosus* was fractionated and screened for the polysaccharide fraction. The characterization was done by enzymatic, Size Exclusion, gas chromatography with flame ionization detector (GC-FID), high pressure anion exchange chromatography (HPAEC) and thin layer chromatographic analyses. Phytochemical evaluation confirmed

the presence of 26.7% of 2→1 linked fructo-oligosaccharides (FOS). They have a degree of polymerization (DP) of nearly 9-10. Cytotoxicity evaluation on P388 cell lines was consistent with low cytotoxicity of the extracts. In vitro Natural Killer (NK) cell activity was evaluated using human peripheral blood mononuclear cells (PBMC) isolated from whole blood on a ficoll-hypaque density gradient. K562 a myeloid leukemia cell line, were used as target cells. ARC, tested over the range 0.2-50μg/ml, showed a dose-related stimulation of NK cell activity with a peak increase of 16.9±4.4% at 5.6μg/ml. However, ARP demonstrated a higher stimulatory activity of 51.8±1.2% at 25μg/ml. The results indicate that the FOS from A. racemosus potentiates the NK cell activity and this could be an important mechanism underpinning the 'Rasayana' properties of this plant (Gautam et al., 2009).

1.5.1.8.2. Antibacterial activity

Methanolic extract of *A. racemosus* shows antibacterial activity and it was tested by agar disc diffusion method in order to analyse the inhibitory activity of plant extract on the organisms (Narayanan et al., 2011).

1.5.1.8.3. Hypolipidemic Activity

Aqueous extract of roots of *A. racemosus* shown hypolipidemic activity by increase the level of catalase, SOD and ascorbic acid in hypercholesterolemic rats (Visavadiya and Narasimhacharya, 2011).

1.5.1.8.4. Antidiabetic activity

Ethanolic extracts of *A. racemosus* roots have been shown to enhance insulin secretion in perfused pancreas and isolated islets. The extract significantly suppressed postprandial hyperglycaemia after sucrose ingestion and reversibly increased unabsorbed sucrose content throughout the gut. The extract also significantly inhibited the absorption of glucose during in situ gut perfusion with glucose. Furthermore, the extract enhanced glucose transport and

insulin action in 3T3-L1 adipocytes. Daily administration of A. racemosus to type 2 diabetic rats for 28 d decreased serum glucose, increased pancreatic insulin, plasma insulin, liver glycogen and total oxidant status. These findings indicate that antihyperglycaemic activity of *A. racemosus* is partly mediated by inhibition of carbohydrate digestion and absorption, together with enhancement of insulin secretion and action in the peripheral tissue (Hannan et al., 2011).

1.5.1.8.5. Enzymes inhibitory activity

Methanolic extract of *A. racemosus* significantly inhibited cholinesterase and MAO activities as compared to hexane and chloroform extracts of *A. racemosus* as evident from the IC(50) values (Meena et al., 2011).

1.5.1.8.6. Hepatoprotective activity

A. racemosus extract (50 mg/kg) orally shows beneficial effect on isoniazid-induced hepatotoxicity in male albino rats. by Evaluated body weight, serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, G-glutamyl transferase, total protein, albumin, hepatic malondialdehyde content, superoxide dismutase, catalase, and glutathione level (Palanisamy and Manian, 2011).

1.5.1.8.7. Fertility activity

Lyophilised aqueous extracts of *A. racemosus* were orally administered at 100 mg/kg body weight to Wistar strain male albino rats shows beneficial effect. Penile erection index and sperm count were determined by visual observation, the seminal fructose concentration was measured spectrophotometrically using resorcinol reagent; and NO release was assessed in a mouse macrophage cell line (RAW264) spectrophotometrically using a commercial Griess reagent kit. Penile erection index, sperm count, seminal fructose concentration and in vitro NO release were the parameters measured. A significant effect on the sperm count, seminal

fructose content and penile erection index was observed upon treatment with the extracts (Thakur et al., 2011).

1.5.1.8.8. Anti-HIV activity

Various extracts were prepared from *A. racemosus* plant. Anti-HIV activity was measured in a human CD4+ T-cell line, CEM-GFP cells infected with HIV-1NL4.3. AR reduced viral production in CEM-GFP cells infected with HIV-1NL4.3. *Asparagus racemosus* demonstrated promising anti-HIV potential and were investigated for their active principles (Sabde et al., 2011).

1.5.1.8.9. Diuretic activity

Aqueous extract of the roots of *A. racemosus* utilizing three doses 800 mg/kg, 1600 mg/kg and 3200 mg/kg was shows diuretic activity in comparison with standard drug furosemide (Kumar et al., 2010).

1.5.1.8.10. Antiamnesic activity

Methanolic extract of *A. racemosus* was rats pre-treated with MAR (50, 100 and 200mg/kg, p.o) for 7 days showed significant decrease in escape latency in the MWM test indicating nootropic activity. MAR also significantly reversed scopolamine and sodium nitrite-induced increase in transfer latency on EPM indicating anti-amnesic activity (Ojha et al., 2010).

1.5.1.8.11. Anticandidal Activity

The in vitro anticandidal activity of *A. racemosus* roots and tubers extract was investigated against Candida albicans, Candida tropicalis, Candida krusei, Candida guillermondii, Candida parapsilosis and Candida stellatoida, which are isolated from vaginal thrush patients. The extract of *A. racemosus* showed high degree of activity against all the Candida strains. The inhibitory effect of the extract against all the Candida tested was found comparable with that of standard antibiotics used (Uma et al., 2009).

1.5.1.8.12. Anti-parasitic activity and cytotoxicity

Aqueous extracts of *A. racemosus* shows antiparasitic and cytoxicity against 2 laboratory-adapted Plasmodium falciparum isolates (D6, CQ-sensitive and W2, CQ-resistant) (Kigondu et al., 2009).

1.5.1.8.13. Antidepressant activity

Methanolic extract of roots of *A. racemosus* shows antidepressant effect. Rats were given MAR in the doses of 100, 200 and 400 mg/kg daily for 7 days and then subjected to forced swim test (FST) and learned helplessness test (LH). The results show that MAR decreases immobility in FST and increases avoidance response in LH indicating antidepressant activity (Singh et al., 2009).

1.5.1.8.14. Antiulcerogenic activity

A. racemosus (Shatavari) crude extract (100 mg/kg/day orally) for fifteen days significantly reduced ulcer index when compared with control group. The reduction in gastric lesions was comparable to a standard antiulcer drug Ranitidine (30 mg/kg/ day orally). Crude extract also significantly reduced volume of gastric secretion, free acidity and total acidity. A significant increase in total carbohydrate (TC) and TC/total protein (TP) ratio of gastric juice was also observed (Bhatnagar and Sisodia, 2006).

1.5.1.8.15. Antioxidant activity

Methanolic extract of A. *racemosus* (100 mg/kg BW/day p.o.) given orally for 15 days significantly increased in antioxidant defense, that is, enzymes superoxide dismutase, catalase, and ascorbic acid where as a significant decrease in lipid peroxidation was observed (Visavadiya and Narasimhacharya, 2009).

1.5.1.8.16. Anti-diarrhoeal activity

Ethanol and aqueous extracts of Asparagus racemosus root 200 mg/kg was shown Anti-diarrhoeal activity on castor oil-induced diarrhoea model in rats (Venkatesan et al., 2005).

1.5.1.8.17. Antiparkinsonian activity

Extract of *A. racemosus* shows antiparkinsonian effect on Excitotoxic kainic acid (KA) induced of neuronal cell death in neurodegenerative disorders that occurs in both Alzheimer's and Parkinson's diseases. The results showed impairment of hippocampus and striatal regions of brain after KA injection marked by an increase in lipid peroxidation and protein carbonyl content and decline in glutathione peroxidase (GPx) activity and reduced glutathione (GSH) content. The *A. racemosus* extract supplemented mice displayed an improvement in GPx activity and GSH content and reduction in membranal lipid peroxidation and protein carbonyl. We show that the minimizing effect of AR extract on oxidative damage in addition to the elevation of GPx activity and GSH content could eventually result in protective effect on the KA-induced excitotoxicity (Parihar and Hemnani, 2004).

1.5.1.8.18. Antitussive activity

The methanol extract of *A. racemosus* root (200 and 400 mg/kg, p.o.) showed significant antitussive activity on sulfur dioxide-induced cough in mice, the cough inhibition (40.0 and 58.5%, respectively) being comparable to that of 10-20 mg/kg of codeine phosphate (36.0 and 55.4%, respectively) (Mandal et al.,2000).

1.5.1.8.19. Prokinetic activity

A. racemosus, shows reduce gastric emptying time and gastric emptying half- time (GE t1/2) . The basal GE t1/2 in volunteers was 159.9 +/- 45.9 min (mean +/- SD) which was reduced to 101 +/- 40.8 min by Shatavari (p less than 0.001) and to 85.3 +/- 21.9 by metoclopramide (p less than 0.001). Metoclopramide and Shatavari did not differ significantly in their effects (Dalvi et al., 1990).

1.5.1.8.20. Adaptogenic activity

A. racemosus is described in Ayurveda as a 'rasayana' herb. 'Rasayana'is a group of plant drugs known to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. These attributes are similar to the modern concept of 'adaptogens' which are the agents that increase the non-specifi c resistance of organisms against a variety of stresses (Dahanukar et al., 2010).

1.5.1.8.21. Cerebroprotective activity

Methanolic extract of root of *A. racemosus* at dose 200mg/kg and 400 mg/kg effective in global cerebral ischemia which is induced by bilateral carotid artery occlusion method (Nandgopal, et al., 2011).

1.5.1.8.22. Antineoplastic activity

Chloroform or methanolic (1:1) extract of fresh root of *A. racemosus* has been reported to retard the tumor incidence in female rats treated with DMBA (7, 12 dimethyl benz (a) anthracene) (Rao, 2011).

1.6.2. Plant profile of *Euphorbia hirta*

It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color (Kirtikar and Basu,2003).

1.6.2.1. Synonyms.

Euphorbia pilulifera Linn.

Chamaesyce pilulifera Linn.

Family: *Euphorbiaceae*

1.6.2.2. Vernacular Names

Bengali : Barokhervi

English : bearing spurge, asthma herb, snakeweed

Gujarati : Dudeli

Hindi : Dudhi

Marathi : Dudnali, govardhan

Tamil : Amumpatchaiyarissi

Telagu : Reddinanabrolu, Bidarie, Nanabala, Nanabiyan

Malayalam : Nelapalai

Fig. 1.2. Exomorphic feature of *E. hirta*

1.6.2.3. Botanical classification

Kingdom : Plantae

Clade : Angiosperms

Order : Malpighiales

Family : Euphorbiaceae

Subfamily : Asparagoideae

Genus : *Euphorbia*

Species : *hirta*

1.6.2.4. Geographical sources

Euphorbia hirta is distributed throughout the hotter parts of India and Australia, frequently found in waste places along the road sides (Sood et al., 2005).

1.6.2.5. General Description

It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color.

Leaves- are opposite, elliptic - oblong to oblong-lanceolate, acute or subacute, dark green above; pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge (Linfang, et al., 2012)

Fruit- are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter.

Stem- are hairy branched from base to top and 40 cm in height.

1.6.2.6. Phytoconstituents

The whole plant contains quercitrin, quercetin, rutin, myricitrin, shikmic acid, tinyatoxin and choline. Afzelin have been isolated from methanolic extract of *E. hirta.* Other constituents of the whole plant are euphorbin-ABCD, 2, 4, 6-tri-*O*-galloyl-β - d-glucose, 1, 3, 4, 6-tetra-*O*-galloyl-β -d-glucose, kaempferol, gallic acid, protocatechuic acid, β -amyrin, 24-methylenecycloartenol β -sitosterol. Leaf of plant contains flavonoids, tannins, sterols and alkaloids (Linfang et al., 2012).

1.6.2.7. Traditional medicinal uses

Euphorbia hirta is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.), and in conjunctivitis. Hypotensive and tonic properties are also reported in *Euphorbia hirta*. The aqueous extract exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities. The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils (Narayanan et al., 2011).

Extracts of *Euphorbia hirta* have been found to show anticancer activity. The aqueous extract of the herb strongly reduced the release of prostaglandins I 2, E 2, and, D 2. The aqueous extract also inhibits aflatoxin contamination in rice, wheat, maize, and mustard crops (Subramanian et al., 2011). Methanolic extract of leaves have antifungal and antibacterial activities. The leaves pounded with turmeric and coconut oil are warmed and rubbed on itchy soles. The latex of *E. hirta* is applied on lower eyelids, like *surma* to cure eye sores. The root exudates exhibit nematicidal activity against juveniles of meloidogyne incognita (Subramanian et al., 2011).

Decoction of dry herbs is used for skin diseases. Decoction of fresh herbs is used as gargle for the treatment of thrush. Root decoction is also beneficial for nursing mothers deficient in milk. Roots are also used for snake bites. The polyphenolic extract of *Euphorbia hirta* has antiamoebic and antispasmodic activity (Bangou et al., 2011). Quercitrin, a flavanoid glycoside, isolated from the herb showed an antidiarrheal activity (Basma et al., 2011). It is reported to have a relaxation effect on respiration.

1.6.2.8. Pharmacological literature review

1.6.2.8.1. Antibacterial activity

E. hirta extracted using the chloroform, methanol, acetone, and ethanol and saponification procedure. The efficacy of the extracts on the uropathogens was tested by agar disc diffusion method in order to analyses the inhibitory activity of plant extract on the organisms, Euphorbia hirta Linn. exhibited high inhibitory activity against most of the tested pathogens. Among the tested organisms, P. aeruginosa and Staphylococcus epidermidis were the most susceptible and Serratia marcescens, Enterobacter cloaceae, Citrobacter koseri, and Citrobacter freundii were the least inhibited by most of the extracts of *E. hirta*. It is concluded that revised antibiotic policies and more importantly the

development of herbal medicine as an alternative may be incorporated in urological practice (Narayanan et al., 2011).

1.6.2.8.2. Anti-diabetic activity

Oral administration of *E. hirta* leaves extract (300 mg/kg b.w./rat/day) for a period of 30 days indicated the antidiabetic nature of the leaves extract. On the basis of determination of the lipid peroxides, hydroperoxides, and both enzymatic and non-enzymatic antioxidants evidenced the antioxidant potential of the leaves extract (Subramanian et al., 2011).

1.6.2.8.3. Nephroprotective activity

The nephroprotective activity of the ethanol extract of *E. hirta* (400 mg/kg body weight) was studied in nitrobenzene-induced albino rats (1000 mg/kg body weight). The activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and the levels of reduced glutathione (GSH), total thiols and vitamin C in the kidney tissues were determined. Histopathologic investigation was performed in the kidney tissue samples. The results indicate that the ethanol extract of *E. hirta* ameliorates renal dysfunction and could be used as an effective protector against nitrobenzene-induced nephrotoxicity, primarily through its antioxidant capacity (Subramanian et al., 2011).

1.6.2.8.4. Enzymes inhibition activity

Methanolic extract of *E. hirta* were used for their Glutathione-s-transferase (GST), Acetyl-cholinesterase (AChE), Carboxyl esterase (CES) and Xanthine Oxidase (XO) inhibitory activities at concentration of 100 micro gram/ ml activ (Bangou et al., 2011).

1.6.2.8.5. Antioxidant activity

The leaves extract of *E. hirta* exhibited a maximum DPPH scavenging activity of (72.96±0.78) % followed by the flowers, roots and stems whose scavenging activities were (52.45±0.66) %, (48.59±0.97)%, and (44.42±0.94)%, respectively. The standard butylated

hydroxytoluene (BHT) was (75.13±0.75) %. The IC (50) for leaves, flowers, roots, stems and BHT were 0.803, 0.972, 0.989, 1.358 and 0.794 mg/mL, respectively (Basma et. al., 2011).

1.6.2.8.6. Immunomodulatory Activity

Methanolic extract of *E. hirta* shows immunomodulatory activity, which has been proved using simple techniques like the macrophage activity testing, carbon clearance test and mast cell de granulation assay (Ramesh and Padmavathi, 2011).

1.6.1.8..7. Molluscicides activity

Euphorbia hirta Linn latex powder were evaluated against the freshwater snails Lymnaea (Radix) acuminata and Indoplanorbis exustus in pond. These combinations showed significant time and dose dependent effect against both the snails (Yadav and Singh, 2011).

1.6.2.8.8. Anti -cytotoxicity activity

The alcoholic extract of *E. hirta* shows protective effect of against antitubercular drug-induced cytotoxicity in freshly isolated hepatocytes. It normalized the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triacylglycerol (TAG), cholesterol, total protein, albumin, total and direct bilirubin, which were altered due to antitubercular drug intoxication (Brindha et al., 2010).

1.6.2.8.9. Antidote activity

Fish poisoning (CFP) is an illness caused by eating tropical coral fish contaminated with ciguatoxins (CTXs). The clinical management of patients with CFP is generally supportive Ciguatera and symptomatic in nature as no antidote exists, methanolic extract of *E. hirta* has shown protective effect against Ciguatera fish poisoning (Kumar et. al., 2011).

1.6.2.8.10. Antifungal activity

Methanolic extracts of *E. hirta* leaves, flowers, stems and roots were evaluated against yeast using the agar disc diffusion method; one yeast (Candida albicans) species was screened. Inhibition zones ranged between 16-29 mm. Leaves extract inhibited the growth of microorganisms with large zones of inhibition, followed by that of flowers, it shows it's have fungicidal activity (Rajeh et al., 2010).

1.6.2.8.11. Anthelmintic activity

Ethanolic extract of *E. hirta* were assessed for their in vitro anthelmintic activity by using the bovine filarial parasite Onchocerca ochengi and the free living nematode Caenorhabditis elegans, a model organism for research on nematode parasites. Worms were incubated in the presence of different concentrations of extracts and inhibitory effects were monitored at different time points. Ethanolic extract of *E. hirta* shows affected the growth and survival of C. elegans and O. ochengi significantly. (Ndjonka, 2011).

1.6.2.8.12. Anti-anxiety activity

Hydro alcoholic extract of *E. hirta* (EH) shows anxiolytic property in chronically stressed rats subjected to elevated plus maze (EPM) and open field test (OFT). EH treatment (200 mg/kg, p.o., seven days) showed marked anti-anxiety activity in chronic immobilization stress. In contrast, the forced swim stress-induced anxiety was only partially decreased by EH. Co treatment of rats with flumazenil (0.5 mg/kg), bicuculline (1 mg/kg, i.p.) or picrotoxin (1 mg/kg, i.p.) resulted in a significant reduction of anxiolytic effect of EH indicating that its actions are mediated through GABA(A) receptor-benzodiazepine receptor-Cl(-) channel complex (Anuradha, et al., 2011).

1.6.2.8.13. Anti-inflammatory activity

The ethanol extract of *E. hirta* were shows anti-inflammatory action on lipopolysaccharide (LPS) induced inflammation. The ethanolic extract of *E. hirta* and its active component

were studied in lipopolysaccharide (LPS)-activated macrophage cells (RAW 264.7) as an established inflammation model. After activation, nitric oxide (NO) production and expression of iNOS protein and iNOS mRNA were measured by using a colorimetric assay (Griess reagent), western blotting, and reverse transcription polymerase chain reaction (RT-PCR), respectively. The alteration in the content of PGE (2), TNF-alpha, and IL-6 was concurrently monitored by ELISA. In results, we found that in the concentration range without showing cytotoxicity, EH produced a remarkable anti-inflammatory effect via its active component of beta-amyrin and showed a dose-related inhibition of LPS-induced NO production (Shih, et al., 2011)

1.6.2.8. 14. Anti-mutagenic activity

Aqueous and methanolic extracts of *E. hirta* was shows anti-mutagenic activity (Loh et al., 2011).

1.6.2.8.15. Antiviral activity

Aqueous extract of *E. hirta* shows antiviral activity direct effects of the aqueous extract on HIV-1, HIV-2 and SIV (mac251) reverse transcriptase (RT) activity were determined. A dose-dependent inhibition of RT activity was observed for all three viruses (Gyuris et al., 2011).

1.6.2.8.16. Anti-arthritics activity

Water extracts of *E. hirta* at low doses shows beneficial in reducing cartilage degeneration in cases of arthritis (Lee et al., 2008).

1.6.2.8.17. Larvicidal activity

Ethyl acetate, butanol, and petroleum ether extracts *E. hirta*, were tested against the early fourth instars larvae of Aedes aegypti L. and Culex quinquefasciatus (Say). The larval mortality was observed after 24 h of exposure. However, the highest larval mortality was found in petroleum ether extract were, 272.36 against A. aegypti and 424.94 against C

quinque fasciatus. Of the various ratios tested. This is an ideal ecofriendly approach for the control of the dengue vector, A. aegypti, and the lymphatic filariasis vector, C. quinquefasciatus (Sharma et al., 2009).

1.6.2.8.18. Anti-Helicobacter pylori activity

Methanol plant extracts *E. hirta* tested demonstrated anti-Helicobacter pylori activity by antimicrobial activity with zone diameters of inhibition ranging from 0-30mm. of these, showed very potent antibacterial activity on the isolates H. pylori (Ndip et al., 2007).

1.6.2.8. 19. Anti-anaphylactic activity

Ethanolic extract of *E. hirta* was found to possess a prominent anti-anaphylactic activity. A preventive effect of *E. hirta* given by oral route at dose from 100 to 1000 mg/kg was observed against compound 48/80-induced systemic anaphylaxis. At the same range of dose, *E. hirta* inhibited passive cutaneous anaphylaxis (PCA) in rat and active paw anaphylaxis in mice. A suppressive effect of EH was observed on the release of TNF-alpha and IL-6 from anti-DNP-HSA activated rat peritoneal mast cells (Youssouf et al., 2007).

1.6.2.8.20. Anti-microbial activity

The ethanolic extracts of the, aerial parts of *E. hirta* were tested for antimicrobial activity. This plant exhibited a broad spectrum of antimicrobial activity, particularly against Escherichia coli (enteropathogen), Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus (Sudhakar et al., 2007).

1.6.2.8.21. Anti-diarrheal activity

The aqueous leaf extract of *E. hirta* decreased the gastrointestinal motility in normal rats and decreased the effect of castor oil-induced diarrhea in mice (Tona et al., 2000).

1.6.2.8.22. Anti-malarial activity

Ethanolic extracts *E. hirta* whole plant were observed antiplasmodial activity may be related to the presence of terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans,

xanthones and anthraquinones. ethanolic extract of *E. hirta* used in Congolese traditional medicine for the treatment of malaria were submitted to a pharmacological test in order to evaluate their effect on P. falciparum growth in vitro of these plant species, 14 (70%) extracts including EtOH from *E. hirta* whole plant produced more than 60% inhibition of the parasite growth in vitro at a test concentration of 6 microg/ml. Extracts from *E. hirta* showed a significant chemo suppression of parasitaemia in mice infected with P. berghei berghei at orally given doses of 100-400 mg/kg per day (Tona et al., 1999).

1.6.2.8. 23. Antifertility activity

The aqueous crude extracts of *E. hirta* were administered to thirty eight-week old sexually mature male albino to determine the effects of these extracts on the male reproductive organs of these animals. The results from this study revealed that the aqueous crude extracts of *E. hirta* caused varying degrees of testicular degeneration as well as reduction in the mean semniferous tubular diameter (STD) in the treated rats. It thus shows that the aqueous crude extracts of *E. hirta* have potentially deleterious effects on the testes and accessory organs of rat's cause's infertility (Mathur et al., 1995).

1.6.2.8.24. Anti-amoebic activity

Three major extracts from some traditional preparations, based on medicinal plants, used as antidiarrhoeal agents were investigated for their putative ant amoebic and spasmolytic activities in vitro. Results indicated that both biological activities are concentrated in the polyphenolic fraction, and not in the saponin or alkaloid containing fractions. The most active polyphenolic extracts were those from *E. hirta* whole plant inhibiting Entamoeba histolytica growth with MAC < 10 micrograms/ml. The same extracts, at a concentration of 80 micrograms/ml in an organ bath, also exhibited more than 70% inhibition of acetylcholine and/or KCl solution-induced contractions on isolated guinea-pig ileum (Tona et al., 2000).

1.6.2.8..25. Diuretics activity

The water and ethanol extracts (50 and 100 mg/kg) of the *E. hirta* plant produced time-dependent increase in urine output. The water extract increased the urine excretion of Na+, K+ and HCO3-. In contrast, the ethanol extract increased the excretion of HCO3- decreased the loss of K+ and had little effect on renal removal of Na+ Acetazolamide, like the water extract, increased urine output and enhanced the excretion of Na+, K+ and HCO3-. The high-ceiling diuretic, furosemide increased the renal excretion of Na+ and Cl-; but had no effect on K+ and HCO3- loss. This study suggests that the active component(s) in the water extract of *E. hirta* leaf had similar diuretic spectrum to that of acetazolamide (Johnson et al., 1999).

1.6.2.8. 26. Analgesic and antipyretic activity

Lyophilized aqueous extract of *E. hirta* from the doses of 20, 25 mg/kg shows analgesic action against chemical (writhing test) and thermic (hot plate test) stimuli and anti- pyretic action and antipyretic activity was obtained at the sedative doses of 100 and 400 mg/kg, on the yeast-induced hyperthermia in rat and mice (Shih et al., 2011).

1.6.2.8.27. Antiasthmatic activity

E. hirta is reported to have an antiasthmatic activity due to the relaxation effect on the bronchial tubes and a depressant action on respiration (Blanc et al., 1963).

1.6.2.8. 28. Galactogenic activity

The powdered *E. hirta* showed a galactogenic activity in guinea pigs before puberty by increasing the secondary sexual organ and induction of milk secretion (Wei et al., 2004).