2.1 Selection of herbs for polyherbal formulation

An important step in the development of herbal medicine is development of its formulations. Herbs and herbal extracts pose typical problems in the development of formulations such as hygroscopic nature, microbial contamination, stickiness, slipperiness and stability. Hence, to get a proper formulation, it is necessary to tackle all these problems [1].

In recent years, the immunomodulating properties of plants are being studied extensively with greater interest due to the growing awareness on immune system modulation and to achieve the desirable effects on disease prevention. Most of the well-known plant remedies directly affect the pathogen by exerting their anti-infective effects, as well as by stimulating the innate and adaptive defence systems of the host. Hence, by improving immunotherapy these plants have become a versatile means. The natural resistance of the body against infection has been found to be improved by natural remedies and several plants are reported to show immunomodulatory activities [2].

Table 2.1: List of plants traditionally reported for immunomodulatory activity

Sr. No.	Name of the Plant	Family	Part used	Reference
1.	*Actinida macrosperma*	Actinidiaceae	Fruits	Lu Y et al., 2007
2.	*Aesculus indica*	Sapinddaceae	leaves	Chakraborthy et al., 2009
3.	*Allium sativum*	Liliaceae	Bulb	Chopra RN et al., 1958
4.	*Aloe barbadensis*	Liliaceae	Gel	Duke JA et al., 1992
5.	*Andographis paniculata*	Acanthaceae	Leaves	Kumar RA et al., 2004 & Naik SR et al., 2009
6.	*Argyreia speciosa*	Convolvulaceae	Seeds	Bussing et al., 1999
7.	*Aspargus racemosus*	Liliaceae	Roots	Dahankur S et al., 1986

8.	*Azadirachta indica*	Meliaceae	Leaves	Ray A et al., 1996 & Haque E et al. ,2006
9.	*Baliospermum montanum*	Euphorbiaceae	Roots	Patil KS et al., 2009
10.	*Boerhaavia diffusa*	Nyctaginaceae	Roots	Mugantiwar et al., 1997
11.	*Boswellia carterii*	Burseraceae	Resin	Chevrier MR et al., 2005
12.	*Caesalpinia bonducella*	Caesalpiniaceae	Seeds	Shukla S et al., 2009
13.	*Capparis zeylanica*	Capparidaceae	Leaves	Ghule BV et al., 2006
14.	*Centella asiatica*	Apiaceae	Leaves	Patil JS et al., 1998
15.	*Centrosema pubescens*	Fabaceae	Seeds	Da Silva et al., 2000
16.	*Chlorella vulgaris*	Chlorophyceae	Leaves	Morrisa HJ et al., 2007
17.	*Chlorophytum borivilianum*	Liliaceae	Roots	Thakur M et al., 2007
18.	*Clerodendrum phlomidis*	Verbanaceae	Roots	Gokini RH et al., 2007
19.	*Curcuma longa*	Zingiberaceae	Rhizomes	Ranjan D et al., 2004
20.	*Eclipta alba*	Asteraceae	Whole herb	Upadhayay RK et al., 2001
21.	*Emblica officanlis*	Euphorbiaceae	Fruits	Sai Ram M et al., 2002
22.	*Entada africana*	Mimosaceae	roots	Diallo et al., 2001
23.	*Epilobium angustifloia*	Onagraceae	Fruits	Schepetkin IA et al., 2009
24.	*Ficus benghalensis*	Moraceae	Roots	Khan T et al., 2008

25.	*Glycyrrhiza glabra*	Fabaceae	Roots	B.H.Kroes et al., 1997
26.	*H. rhamnoides*	Elaeagnaceae	Leaves, fruit	Geetha et al., 2002
27.	*Isatis cappadoica*	Brassicaceae	Whole plant	Rezaeipoor et al., 2000
28.	*Lawsonia alba*	Lythraceae	Leaves	Kulkarni and Karande 1998
29.	*Leucas aspera*	Lamiaceae	Whole plant	Singh et al., 2002
30.	*Mahonia aquifolium*	Berberidaceae	Stem Bark	Kostalova et al., 2001
31.	*Mangifera indica*	Anacardiaceae	Stem bark	Makare et al., 2001
32.	*Nelumbo nucifera*	Nelumbonaceae	Rhizomes and Seeds	Mukherjee D, *et al*. 2010
33.	*Nigella sativa*	Ranunculaceae	Seeds	Swamy and Tan et al., 2000
34.	*Ogbignya phalerata*	Palmae	Fruits	Da Silva and Parente et al., 2001
35.	*Panax quinquefolium*	Arliaceae	Aerial part	Wang et al., 2001
36.	*Picorhiza scrophulariiflora*	Scrophulariaceae	Rhizomes	Smit et al., 2000
37.	*Platycodon grandiflorum*	Campanulaceae	Roots	Choi et al., 2001
38.	*Prunella vulgaris*	Lamiaceae	Aerial parts	Harput et al., 2006
39.	*Psoralea corylifolia*	Fabaceae	Seeds	Latha et al., 2000
40.	*Saussurea lappa*	Compositae	Roots	Kulkarni and Desai at al., 2001
41.	*Tinospora sinensis, Tinospora cardifolia*	Menispermaccae	Stems	Manjrekar et al., 2000
42.	*Trigonella*	Leguminosae	Whole plant	Hafeez et al., 2002

	foenum graecum			
43.	*Tripterygium wilfordii*	Celastraceae	Roots	Duan et al., 1999
44.	*Withania somnifera*	Solanaceae	Roots	Davis L et al., 2000
45.	*Uncaria tamentosa*	Rubiaceae	Bark	Aguilar et al., 2002
46.	*Zizyphus jujuba*	Rhamnaceae	Fruit	Benammar et al., 2010

In the light of above background, the present study was aimed to screen Indian medicinal plants and their combinations in the form of polyherbal formulations for the potential immunomodulatory activity and synergistic effects of these combinations. The plant drugs present in the formulation have been studied to some extent and their medicinal nature is well known. The following is the description of all the plant drugs present in the formulation.

2.2 Glycyrrhiza glabra

Glycyrrhiza is derived from the ancient Greek word 'Glykos', means sweet, and 'Rhiza', means root. *Glycyrrhiza glabra* is also known as liquorice and sweetwood. It is native to the Mediterranean and certain areas of Asian countries. The dried rhizomes and roots of this plant were used medicinally by the Chinese, Greek, Egyptian, Indian and Roman civilizations as an expectorant and carminative by tribal peoples. Liquorice root extracts are often used as a flavouring agent in modern dosage forms to mask bitter taste in preparations and also as an expectorant in cough and cold preparations [3].

(a) (b)

Fig 2.2 (a) Picture of Glycyrrhiza glabra plant (b) Picture of dried Glycyrrhiza glabra root

2.2.1) Definition:

Glycyrrhiza glabra is commonly known as *Yashtimadu,* which has been used worldwide in various systems of medicine viz, Ayurvedic, Siddha, Allopathic and other traditional systems of medicine [4, 5].

2.2.2) Classification: [6]

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionata (Vascular Plants)

Superdivision: Spermatophyta (Seed plant)

Division-Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Fabales

Family: Leguminosae (Fabaceae)

Genus: Glycyrrhiza

Species: Glabra

Botanical name: Glycyrrhiza glabra

2.2.3) Synonyms: [6-12]

Table 2.2 Synonyms of Glycyrrhiza glabra

Sr. No.	Language	Names
1.	Sanskrit	Yashtimadhuh, Madhuka
2.	Kannada	Yastimadhuka, atimaddhura
3.	Hindi	Jothimadh, Mulhatti
4.	Gujarati	Jethimadhu
4.	Malayalam	Iratimadhuram
5.	Tamil	Atimaduram
6.	Telugu	Atimadhuranu, Yashtimadhukam
7.	English	Liquorice, Liquorice, Sweet wood
8.	Bengali	Jashtimadhu, Jaishbomodhu
9.	Marathi	Jeshtamadha
10.	Oriya	Jatimadhu
11.	Arab	Aslussiesa
12.	France	Boisdoux
13.	Germany	Sussholz
14.	Persia	Ausareha mahaka

2.2.4) Habitat:

Glycyrrhiza glabra is a hard herb or under shrub having a height up to 6 ft. Leaves are imparipinnate, multifoliate, flowers are in axillary spikes, paplionaceous, dark purple to violet in colour, pods are compressed, and containing reniform seeds. Liquorice consists of the dried, peeled or unpeeled underground stems and roots. Fruits are borne in August while Flowers are in March [13, 14].

2.2.5) History:

The genus *Glycyrrhiza* includes about 20 species natively belongs to Asia, Europe North and South America along with Australia. The scientific generic name for the plant group is “Liquiritia,” which was the ancient name of Glycyrrhiza from which its English name ‘Liquorice’ has been named. European liquorice *Glycyrrhiza glabra* is the most popular liquorice [15].

2.2.6) Ehanopharmacolgy:

The roots of *G. glabra* (liquorice) were known to Roman physicians as Radixdulcis and to Arab physicians as a medication for cough, and the plants have been cultivated

in Europe since the 18th century for its particular taste. *Glycyrrhiza glabra* is listed in the British Pharmaceutical Codex (1973 ed.) and contains triterpenoids glycyrrhizin (6-13%) and glycyrrhizic acid, which have anti-inflammatory activity [15].

2.2.7) Description:

Glycyrrhiza glabra is a perennial shrub, slightly hard and attains height up to 2.6 m. The leaves are compound, imparipinnate, alternate, having 5-7 pairs of oblong, lanceolate or elliptical leaflets. The flowers are slightly narrow, typically papilionaceous, born in axillary spikes, of violet to purplish shade. It consists of a short and campanulate calyx; with glandular hairs and lanceolate tips. The fruit comprises of a 1.5 cm long, glabrous, erect, slightly reticulately pitted compressed pod or legume, containing about 3-5 brown and reniform seeds. A 1.5 cm taproot, subdivided into 3-5 subsidiary roots upto 1.25cm length is characteristic feature of the plant, from which the horizontal woody stolons upto 8 m may arise. When these are dried and cut into small pieces, along with the root, constitutes the commercial liquorice. This may either be peeled or unpeeled. Fragments of roots have fibrous fracture revealing the yellowish interior within it. These roots possess a characteristic odour and sweet taste [16-18].

2.2.8) Microscopical Features [19, 20]:

The most diagnostical features of liquorice roots are as shown below:

1. Unpeeled drug shows the presence of polyhedral tubular brownish cork cells.
2. Fibres are thick, lignified or partially lignified, in the groups of 10-15 in phloem and xylem.
3. Vessels are large and closely arranged with broader pits. Also the starch and calcium oxalate crystals are present in parenchymatous cells.
4. In case of stolon, the parenchymatous pith is present whereas root is characterised xylem vessels and absence of pith.

2.2.9) Cultivation:

It is native to Mediterranean regions but now it is also grown in Punjab, Jammu and Kashmir and south India [21]. The plants usually grow well in deep, sandy but fertile soil is required, near streams. The usual method of propagation is by replanting young pieces of stolon, but it can be grown from seed. The underground organs are sufficiently developed by the end of the third or fourth year at which stage they are dug up and washed. Most of this part remains underground and renews itself during

three years as it grows. Most of these are peeled and cut into smaller pieces followed by drying, yet the unpeeled part is also sometimes used. [22]

Varieties for cultivation

Almost 30 species of the genus Glycyrrhiza exists which includes *G. uralensis, G. eurycarpa G. inflata, ,. G. glabra G. Korshinskyi and, G. aspera.* Moreover, three varieties are included: Spanish and Italian liquorices are *G. glabra var. typical, Russian liquorice is G. glabra var. gladulifera,* while Persian and Turkish liquorices are assigned to *G. glabra var. violacea.* All these three varieties were cultivated in Kashmir. These were cultivated at Iran, Ceria, Greece Russia and Turkey. Similarly it is also in trade at Aleczandreta, Spain, Italy, Ceria, Egypt, Iraq, Barcelona, France, Belgium and Germany. It is cultivated as major crop in Spain. Liquorice cultivated in China is considered to be of good quality [21].

Herbal trade: Yastimadhu is amongst the significant medicinal plant species in high trade sourced largely through imports to India. It is fourth highest (amongst 141 formulations available in market) used medicinal substance ranked for frequency of occurrence of medicinal plants in herbal formulations in India. Liquorice is imported from Iran, Afghanistan, and China to India. In India, every year approximately 5000 Tones of Yastimadhu is (i.e. 100% of Indian requirements) imported from Pakistan, Iran, Afghanistan and UAE. It is top selling herbal extracts (standardized) in world market. The demand of this crop in 2001-02 was 873.4 tonnes which reached up to 1359.8 tons in 2004-05 with a growth rate of 15.9 per annum amongst 32 major medicinal plants cultivated in India. It is speculated that Liquorice has a shelf life of approximately 10 years [23-27].

2.2.10) other varieties of Glycyrrhiza [28, 29]:

American Liquorice: Only one species is native to United States i.e. *Glycyrrhiza lepidota*. This wild variety ranges from western Ontario to Washington DC, south to Texas, Mexico and Missouri. There are scattered populations in east region. It has never been developed as a commercial source of liquorice. In Texas, it is called ‘Amolillo’, which refers to the foaming produced by stirring the root in water. The leaves are chewed for toothaches and applied as a poultice. The roots were also used for treating fever in children. It has a strong bitter taste, which then becomes sweet.

European Liquorice: In Europe, it is found in dry open habitats in the south and east regions, and has been cultivated throughout the continent, except Scandinavia. It is used for dry cough, asthma and all diseases of lungs. They can also be chewed to impart sweet flavour.

Chinese Liquorice: The Chinese liquorice 'Gan cao' means 'sweet herb'. Chinese liquorice mainly comes from *G. uralensis.* It is found in dry grassy plains, and sunny mountain sides from much of North China, especially the Asian steppes to the west. This variety along with *G. inflata,* are official drug plants in Chinese Pharmacopiea. Liquorice is used in many Chinese herbal prescriptions as a main drug to enhance the activity of other ingredients, reduce toxicity, as well as improve flavour. It is said that liquorice is used in as many as half of all traditional Chinese medicine prescriptions.

2.2.11) Substitute:

Dhataki (*Woodfordia fruticosa L.*) is suggested as a substitute for *G. Glabra* [30].

2.2.12) Adulterants: [15, 31]

Wild liquorice: It is the most common adulterant, also known as Indian Liquorice which is derived from the roots of *Abrus precatorius* of Leguminosae family. The roots of this adulterant are highly toxic due to presence of an alkaloid 'abrine' and thus it should not be used instead of liquorice. It possesses a disagreeable odour and bitter acrid flavour leaving faintly sweet after taste which distinguishes from the main drug. Stone cells are the characteristic feature in its microscopy.

Manchurian liquorice: It is obtained from *G. uralensis*. It is dark brown in colour. The distinguishing peculiarities are that the medullary rays are curved and presence of lacunae can be seen in the wood. It contains the active constituent glycyrrhizin, but in very less quantity and that too free of sugars.

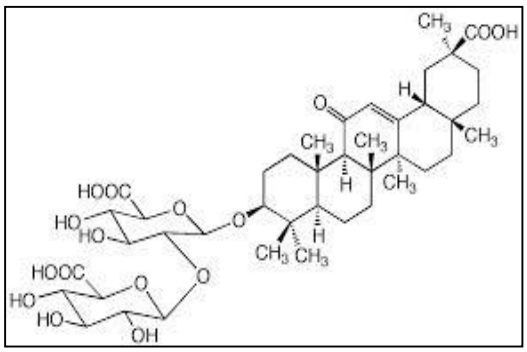
Russian liquorice: It is obtained from *Glycyrrhiza glabra var. glandulifera.* It is purple in colour with numerous long roots but stolons are not found.

2.2.13) Chemical Constituents:

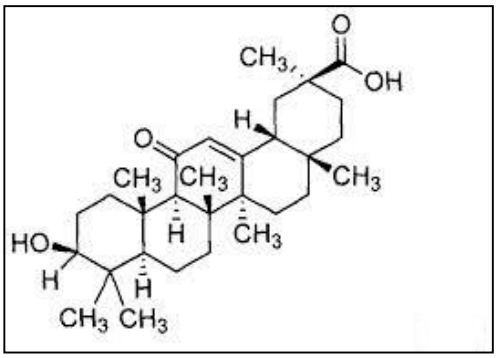
A number of components have been isolated from liquorice, including a water-soluble, biologically active complex that accounts for 40-50% of total dry weight. It contains triterpene saponins, polysaccharides, flavonoids, pectins, simple sugars, amino acids, mineral salts, and various other substances. A triterpenoid compound

named Glycyrrhizin, is responsible for the sweet taste of liquorice root. Glycyrrhizin represents a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid that varies within a range of about 2-25%. Among the various natural saponins that are known, glycyrrhizic acid is composed of a hydrophobic fragment, glycyrrhetic acid and a hydrophilic part, two molecules of glucuronic acid, and. The yellow colour of liquorice is on account of its flavonoid content, which includes isoliquiritin (a chalcone), liquiritin, and some other compounds. The isoflavones hispa-glabridins A and B along with Glabridin possess a significant antioxidant activity, and both glabridin and glabrene possess estrogen like activity [15, 32, 33].

2.2.14) Structures of important chemical constituents [15]



Glycyrrhizin or Glycyrrhizic acid



Glycyrrhetinic acid

2.2.15*)* Side Effects and Toxicity

One of the most commonly reported side effects with liquorice supplementation is elevated blood pressure. This is thought to be due to the effect of liquorice on the renin angiotensin aldosterone system. It is suggested that saponins are capable of potentiating aldosterone action while binding to mineralocorticoid receptors in the kidneys. 'Pseudo aldosteronism' is the term used for this phenomenon. Patients may experience hypokalemia (loss of potassium) and sodium retention, along with hypertension resulting in edema. But all of its symptoms disappear as the therapy is discontinued.

Many studies report no side effects during the course of treatment. Generally, the onset and severity of symptoms depend on the dose and duration of liquorice intake, as well as individual susceptibility. Patients with delayed gastrointestinal transit time

may be more susceptible to these side effects, due to entero-hepatic cycling and reabsorption of liquorice metabolites. Pregnant women should avoid using liquorice as a supplement or consuming large amounts of liquorice as food, as some research suggests it could increase the risk of preterm labor. The amount of liquorice ingested daily by patients with mineralocorticoid excess syndromes appears to vary over a wide range, from as little as 1.5 to 250 g daily [34-36].

2.2.19) Pharmacokinetics

After oral administration of liquorice in humans, the main constituent, glycyrrhizin, is hydrolyzed to glycyrrhetic acid by intestinal bacteria possessing a specialized ß-glucuronidase. Glycyrrhetic acid is 200-1,000 times more potent an inhibitor of 11-ß-hydroxysteroid dehydrogenase (involved in corticosteroid metabolism) than glycyrrhizin. So, its pharmacokinetics after oral intake is more significant. Glycyrrhetic acid is rapidly absorbed after oral dosing and then it is transported via carrier molecules to the liver where it gets metabolized to glucuronide and sulfate conjugates. These hydrophilic metabolites are further rehydrolyzed to glycyrrhetic acid followed by its reabsorption, resulting in a significant delay in its plasma terminal clearance. After oral administration of 100 mg glycyrrhizin in healthy humans, no glycyrrhizin was found in the plasma but glycyrrhetic acid was found at less than 200ng/ml. glycyrrhizin was found in the urine after 24 hour of oral dosing. This suggests that it is partly absorbed as an intact molecule [37, 38].

2.2.20) Mechanisms of Action

A number of mechanisms exist to which the advantageous effects of liquorice can be attributed. glycyrrhizic acid and Glycyrrhizin have reported to inhibit cytopathology and growth of various RNA and DNA viruses, including hepatitis A and C, HIV, *Herpes simplex* and Herpes zoster. Glycyrrhizin and its metabolites inhibit hepatic metabolism of aldosterone and suppress 5-ß reductase, this properties responsible for the well documented pseudo aldosterone syndrome. The similarity is found in structure of glycyrrhetic acid to the structure of hormones secreted by the adrenal cortex accounts for the mineralocorticoid and glucocorticoid activity of glycyrrhizic acid. Liquorice constituents also exhibit anti-inflammatory activity which is very much corelated to the action of steroid- hydrocortisone. *In vitro* research has also suggested that cyclo-oxygenase activity and prostaglandin formation (especially

prostaglandin E2) is inhibited by liquorice. Moreover, it also indirectly inhibits platelet aggregation which is a contributing factor in process of inflammation. Certain Liquorice constituents possess significant antioxidant and hepatoprotective activities. Glycyrrhizin and glabridin inhibit the generation of Reactive Oxygen Species (ROS) by neutrophils at the inflammatory site. The isoflavones of Liquorice, hispaglabridin A and B, inhibit Fe3+-induced mitochondrial lipid peroxidation in rat liver cells, which has been confirmed by the *In vitro* studies . Other research indicates glycyrrhizin lowers lipid peroxide values in animal models of liver injury caused by ischemia reperfusion. Liquorice constituents also exhibit hepatoprotective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients. Glycyrrhizin and other liquorice components appear to possess anti-carcinogenic properties as well. Although the exact mechanisms are still under investigation, research has suggested that abnormal cell proliferation and the tumor formation and growth in breast; skin and liver cancer is being inhibited. Deglycyrrhizinated liquorice formulations are used in the treatment of ulcers. It does not suppress gastric acid release like other anti-ulcer medications. Rather, they promote healing by increasing the mucous production and blood supply to the damaged stomach mucosa, thereby enhancing mucosal healing [39-46].

2.2.21) Pharmacological Activity:

Although a lot of pharmacological investigations have been carried out based on the constituents present but a lot more can still be exploited, explored, and utilized. Given below is the summary of some findings.

Anti-bacterial Activity

The in-vitro inhibitory effects of *G. glabra* extract against the growth of *S. paratyphi B, S. flexneri Shigella sonnei, Salmonella typhi,* and enterotoxigenic E. coli determined its anti-bacterial activity, which was investigated using well and disc diffusion method. *S. paratyphi B* showed no susceptibility to liquorice with concentrations lower than 7.5%, though all tested bacterial strains exhibited susceptibility to high concentration of liquorice [47]. The extracts of liquorice root were tested *in-vitro* against 13 bacterial species and strains by the agar diffusion method. The extracts of liquorice roots showed various antibacterial activities (7-11 mm/20µl inhibition zone) against the microorganisms tested. The alcoholic extracts

did not inhibit, *B. brevis, L. monocytogenes, E. faecalis, P. aeruginosaand B. subtilis var. niger Y. enterocolitica.* the acetone extracts did not inhibit *Y. enterocolitica E. faecalis, P. aeruginosaor and L. monocytogenes,* The ethyl acetate extracts did not inhibit *B. subtilis or Y. enterocolitica,* and the chloroform extracts showed no inhibition effect against *P. aeruginosaand Y. enterocolitica* [48].The glycyrrhizin is commonly used as a vehicle in some oral formulations. It acts by inhibiting the growth of some bacteria, as well as formation of dental plaque. inhibitory effects for liquorice aqueous and ethanolic extracts on *Staphylococcus aureus* and *Streptococcus pyogenes* cultures has been demonstrated by in-vitro studies which proves its anti-bacterial action. Of this, the first one shows the strongest inhibition with a 10-15mm halo diameter [49]. Antimicrobial activity against both gram-positive and gram-negative bacteria has also been reported [50].

Antioxidant Activity

The relative reducing activity in terms of antioxidant activity of extracts was determined by using individual extract (15mg) as well as its combination with equal amount of ascorbic acid. 1.0 ml of deionised water with phosphate buffer was used to dissolve the extract and ascorbic acid separately. The mixture was incubated at 50ºC for 20 min. aliquot of trichloroacetic acid were added to the mixture and centrifuged for 10 min at 3000 rpm. This was followed by mixing the upper layer of solvent with distilled water and a freshly prepared Ferric chloride solution. 500µg/ml extract aliquot was prepared and its absorbance was measured at 700nm. Increased in absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid [51]. The antioxidant activity of *G. glabra* root extracts using *in vitro* models was studied. scavenging activity against nitric oxide was demonstrated by the dose dependent ethanolic and aqueous extracts (concentration that caused 50% inhibition of nitric oxide radicals) (IC_{50} =72 and 62.1µg/ml, respectively), superoxide (IC_{50}=64.2 and 38.4µg/ml, respectively), hydroxyl (IC_{50}=81.9 and 63µg/ml, respectively) radicals. Further, both extracts showed strong reducing power and iron-chelating capacities. In the $Fe^{2+/}$ascorbate system, both extracts were found to inhibit mitochondrial fraction peroxidation of lipids. both these extracts significantly (P<0.05) lengthened the lag phase along with a decline in the oxidation rate, lipid hydroperoxides conjugated dienes and thiobarbituric acid reactive substance formation in copper catalyzed human

serum and low-density lipoprotein oxidation models,. Considerable antioxidant activity and protective effect against the human lipoprotein oxidative system was demonstrated by ethanolic extract of G.Glabra [52]. Glycyrrhetic acid is used in cosmetics as a anti- inflammatory, decongestant agent and wound healer; it is applied as talcum powder, emulsion, or toothpaste. The antioxidant characteristics of G.glabra are through its protective action on mitochondrial functions under oxidative stress and its lipid peroxidation inhibition in rat liver. A marked decrease in the catalase enzyme activity was detected in the blood of these animals. Thus, Liquorice-eco extract is helpful to formulate cosmetic products for the protection of skin and hair against oxidative processes [49]. The hydro-alcoholic extracts of *Glycyrrhiza glabra Linn* which exhibited different anti-inflammatory activities were evaluated for the possible mode of action by studying their antioxidant properties. In the present study we investigated if the respiratory burst of human activated neutrophils, as a result of their antioxidant capacity could be modulated by the standardized hydro alcoholic extracts of plants such as *G. glabra* produced by Hofigal Stock Company [53].

Enzyme Inhibitory Activity

The methanolic extract of liquorice had shown *in vitro* inhibition of the tyrosinase enzyme, with 21.2µg/ml inducing 50% inhibition. Active principles are able to inhibit tyrosinase act by modifying the action site of the enzyme, thus reducing its activity. Liquorice root extract is known to inhibit tyrosinase. Most of the tyrosinase inhibitors are produce their effects through their reducing capacity and thereby act as reducing agents. Thus, liquorice-eco extract is of great use to formulate cosmetic products with depigmenting activity [54]. Isoliquiritigenin inhibited rat lens aldose reductase using DL-glyceraldehyde as substrate. licopyranocoumarin, Licochalcones A and B, licoarylcoumarin, glycyrrhisoflavone glisoflavone and licocoumarone inhibited xanthine oxidase enzyme [55].

Anti fungal activity

Antifungal compounds from liquorice (*Glycyrrhiza glabra Linn*) extracts with 80% methanol (oil-based extract of liquorice-OEL) were screened for their antifungal effects and they were found to have high antifungal activity against *Chaetomium funicola* M002 and *Arthrinium sacchari* M001, and its active compound was identified to be glabridin (3-(2', 4'- dihydroxyphenyl)-8-dimethylpyranochroman). OEL was effective against not only filamentous fungi but also some of the bacteria

which are thermo resistant bacilli such as various genera of *Bacillus* and *Alicyclo bacillus* [56]. Glabridin to be active against both yeast and filamentous fungi and also showed resistance modifying activity against drug resistant mutants of *Candida albicans* at a minimum inhibitory concentration of 31.25 - 250µg/ml [57] . *in-vivo* inhibition of *Mycobacterium smegmatis* and *Candida albicans* has been reported in regards to its anti fungal activity. Various iso-flavonoids such as glabridin, glabrol and other derivatives were responsible for this action. The inhibitory effects of aqueous extract of liquorice root (CIM=1.56 mg/ml) on cultures of *Candida albicans* was demonstrated by a recent study, which had been obtained from mouth lesions of 5 months old infants. These results suggest that liquorice based mouthwash was used to treat *Candida albicans* induced lesions in HIV patients [49]. So, liquorice extract is of great use to formulate cosmetic products with purifying and antiseptic activities.

Anti hyperglycemic Activity

The anti-hyperglycemic activity of 18β-glycyrrhetinic acid and aglycone of glycyrrhizin were reported on streptozotocin induced diabetic rats. Adult male albino rats of the Wistar strain, weighing 180-200gm were used to induce diabetes, by administration of streptozotocin (40 mg/kg of body weight) intraperitoneally (i.p). Diabetic rats showed increase of plasma glucose and glycosylated haemoglobin (HbA1c) and a decrease of plasma insulin and haemoglobin (Hb). Activities of some gluconeogenic enzymes such as fructose 1, 6-bisphosphatase, glucose 6-phosphatase increased and decrease in level of glucose 6-phosphate dehydrogenase and glucokinase in the liver along with glycogen. Oral administration of 18β-glycyrrhetinic acid (50, 100 and 200 mg/kg/body weight) or glibenclamide (600µg/kg/body weight) in 5% dimethyl sulfoxide (DMSO), for 45 days, prevented the above changes and improved towards normalcy [58, 59].

Anti malarial Activity

The Chinese pharmacopoeia accepts three species of *Glycyrrhiza* such as *G. glabra, G. uralensis* and *G. inflata,* as sources of Gan Cao. The chalcone licochalcone A can be isolated from all *Glycyrrhiza* species in different quantities and has been shown to exhibit good anti-malarial activity. In *in-vivo* activities against *P. yoelii* in mice, oral doses of 1000 mg/kg resulted in the complete eradication of the malaria parasite and no toxicity was noted [60]. The burden of this disease is getting worse, mainly because

of the increasing resistance of *Plasmodium falciparum* against the widely available anti-malarial drugs. There is an urgent need for new, more affordable and accessible anti-malarial agents possessing original mode of action. Natural products have played a leading role in the discovery of leads for the development of drugs to treat human diseases. The nonalkaloidal natural compounds isolated from plants with antiplasmodial and antimalarial properties, belonging to the classes of limonoids, terpenes, flavonoids, xanthones, chromones, anthraquinones, miscellaneous and related compounds [61]. Licochalcone A inhibited *in-vitro* growth of both chloroquine susceptible and chloroquine resistant strains of *Plasmodium falciparum* to same extent in hypoxanthine uptake assay [55].

Anti-viral and Immunostimulatory effects

Ammonium glycyrrhinate (amide of glycyrrhetinic acid) showed antiviral properties against herpes simplex 1, vesicular stomatitis and vaccinia virus. Saponins from Glycyrrhiza roots inhibited the development of type A influenza virus in chicken embryos, possibly due to interferon production, because it was found in other studies about the saponins-glycyrrhizin. An *in- vitro* study suggested the inhibitory action of glycyrrhizin on HIV cultures. An *in- vitro* studycarried out in Germany, established that glycyrrhizin inhibited the SARS virus (corona virus, which causes a typical pneumonia), more efficiently than synthetic drugs such as ribavirin, 6-azauridine, pyrazofurin, myco-phenolic acid. Even when the action mechanism is still unclear, evidences indicated that glycyrrhizin acts by stimulating nitric oxide (NO) synthesis, via nitric oxide synthase [49]. Most of the *Glycyrrhiza* phenols reduced the viable cell number of mock infected and HIV infected MT-4 cells to comparable extents [62]. *Glycyrrhiza glabra* showed immune-stimulatory effects *in-vitro* (at 100µg/ml concentration) by increasing TCD-69 lymphocytes production and macrophage production from human granulocytes. Moreover, decrease of IgG and IgA ($P<0.01$) has been reported due to glycchrizin, which plays an important role in hypersensitivity mechanisms. glycyrrhizin inhibited the Arthus phenomenon and the Schwartzman reaction which was demonstrated by a recent study in rats, and that the alcoholic extract of liquorice root inhibited type I allergic reactions induced by injection of *Ascaris lumbricoides* IgE containing serum.

In addition, *in-vivo* assays have shown that liquorice root extracts prevented the rise in the amount of immune complexes associated with some auto immune conditions, such

as systemic lupus erythematosus. It is identified that the activation of the immune system slows down with age, which results in decreased in cell regeneration. So, liquorice root extract is helpful to treat aged skin [49]. The immune stimulatory activities of glycyrrhizin were also studies in the mice infected with influenza virus A2 (H2N2) were unable to survive 10 times the mean lethal dose (LD_{50}) of virus [63]. The studies reported that the transplanting of splenic T-cells from glycyrrhizin treated mice conferred resistance to infected mice that had not been treated with glycyrrhizin. survival of infected mice was not improved by the transplanting of other splenic cells, indicating that glycyrrhizin was a specific inhibitor of the cell mediated immune-logical response. The administration of γ-interferon monoclonal antibody to infected mice blocked the anti-viral properties of glycyrrhizin treatment. These results confirm that the anti-viral activity of glycyrrhizin is because of its stimulating properties of γ-interferon production by T-cells. Glycyrrhizin has also been demonstrated that it is effective in the treatment of herpes simplex virus induced encephalitis in mice [64]. Liquorice and glycyrrhizate compounds have long been used in treatment of chronic viral hepatitis in China and Japan but the possible mechanism of anti-viral activity remains unidentified. *In-vitro* assays have demonstrated that glycyrrhizin is effective at inhibiting the growth of a host of viruses under culture conditions like pathogenic flavi-viruses [65], alpha viruses [66] and herpes simplex virus (HSV) [67]. A direct effect of glycyrrhizin on viral growth, possibly through replication mechanisms, an inhibition of viral particles to cell membrane binding, or through cellular signal transduction mechanisms has been suggested by some studies. *In vivo* and human studies also supportive of the anti-viral activity of glycyrrhizin, but the mechanism of action is somewhat complex and endorses an immune response. Inhibition of the spinach mosaic virus is reported by the aqueous extracts of the roots [68].

Memory Enhancing Activity

Memory enhancing activity was studied to investigate the effects of *G. glabra Linn* (commonly known as liquorice) on memory and learning in mice. In order to test the learning and memory elevated plus maze and passive avoidance paradigm were employed. Administration of Three doses (75, 150 and 300 mg/kg p.o.) of aqueous extract of *G. glabra Linn* in separate groups of animals for 7 successive days was done. The aqueous extract with a dose of 150mg/kg of liquorice significantly improved learning and memory of mice. Additionally, this dose significantly reversed

the amnesia induced by diazepam (1 mg/kg i.p.) and scopolamine (0.4 mg/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice contribute significantly in improving memory. Since liquorice reverses the scopolamine-induced amnesia effect, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic transmission in mouse brain. The present investigation shows promising memory enhancing effect *Glycyrrhiza glabra Linn* in all the laboratory models used [69].

Expectorant Activity

The specific mechanism of action remains unknown, liquorice has been shown to work in the throat as effectively as codeine, it decreases irritations and also shows expectorant activity. One of the author proposed explanation is that gastric mucus secretion could be stimulated by carbenoxolone, a semi synthetic compound derived from *Glycyrrhiza*,, Moreover it also stimulates tracheal mucus secretions , thereby producing expectorant and demulscent activities [70]. *Glycyrrhiza* is a helpful remedy for coughs because it facilitates the movement of mucus from the respiratory tract [71].

Spasmolytic Activity

It showed that liquiritin present in the roots of *Glycyrrhiza glabra* is inactive as an antispasmodic. However, it exhibited a strong spasmolytic activity when it was hydrolysed by heating and converted into isoliquiritigenin [72].

Anti allergic activity

Glycyrrhiza glabra Linn (Glycyrrhizin, Liquiritigenin and 18β glycyrrhetinic acid have anti-allergic activity, which can relive IgE-induced allergic diseases like dermatitis and asthma [73].

Anti-ulcer Activity

A herbo-mineral formulation (Pepticare) of the Ayurveda medicine consisting of the herbal drugs: *Emblica officinalis Glycyrrhiza glabra,* and *Tinospora cordifolia*, was tested for its antiulcer and anti-oxidant activity in rats. The effects of various doses (125, 250, 500 and 1000 mg/kg, p.o.) of Pepticare were used. It produces gastric secretion and gastric ulcers in pylorus ligation and on ethanol induced gastric mucosal injury in rats. The decrease in ulcer index in both the models along with the reduction in volume and total acidity and an increase in the pH of gastric fluid in pylorus ligated

rats proved the anti-ulcer activity of Pepticare. Reports revealed that in protecting against pylorus ligation and ethanol induced ulcers was more significant by *G. Glabra* alone. The increased levels of enzymes superoxide dismutase, catalase and reduced glutathione and membrane bound enzymes like Ca^{2+} ATPase, Mg^{2+} the anti-oxidant property of the formulation was proved by ATPase and Na^{+} K^{+} ATPase and also decrease in lipid peroxidation in both the models. Thus which can be accrediting the anti-oxidant mechanism of action of Pepticare, it could be concluded that Pepticare possessed significant anti-ulcer activity. Glycyrrhizic acid and the aglycone of Glycyrrhizic acid occurs in the form of the two stereo-isomers, 18β (cis) and 18ά (trans). The 18β GA isomers, extracted from the root of liquorice have anti viral, expectrorant, antitumor and antiulcer activity [75]. Perhaps the most predominant and consistent medicinal use for liquorice had been as a demulcent for the digestive system. Indeed, carbeno-xolone is a synthetic derivative of glycyrrhetic acid, which is a widely used pharmaceutical treatment for gastric ulcers. The anti-ulcer property of deglycyrrhizinated liquorice was demonstrated using a rat model of aspirin induced gastric mucosal damage [76]. The anti-ulcer activity of liquorice extract may be due to reduced gastric secretions caused by an inhibiting release of gastrin. Efficacy of deglycyrrhizinated liquorice could be evaluated from the results from some clinical studies which suggested that several components exist in the extracts which promote gastric healing. Botanical compounds with anti-ulcer activity include flavonoids (i.e. quercetin, silymarin, naringin, anthocyanosides, sophoradin derivatives, etc.) saponins (i.e. from *Kochia scoparia and Panax japonicus*), tannins (i.e. from *Linderae umbellatae*), gums and mucilages (i.e. gum guar and myrrh). Among herbal drugs, liquorice, aloe vera and capsicum (chilli) have been used extensively and their clinical efficacy documented [77, 78].

Hepatoprotective Effects

The study reported that in animal and human models glycyrrhetic acid plays a protective role when liver cells were challenged. It decreases inflammatory states by reducing cytokines like tumor necrosis factor alpha and increasing protective antioxidants such as heme-oxygenase-1. fewer markers were released by these shielded tissues of liver damage, repairing and regenerating them more rapidly. The hepatoprotective activities of glycyrrhizin in hepatotoxin treated mice were studied. In this study, the hepatic carcinogen 3'-methyl-4-dimethylaminoazobenzene was

administered to animals in their diet and injected 1mg glycyrrhizin for three months, twice a week. Incidence of hepatic cells showing morphological evidence of injury was significantly reduced in Animals treated with glycyrrhizin, including degenerated mitochondria, atrophied golgi apparatus, increased number of lysosomes, pseudo nuclear inclusions and increased mitotic cells [79]. Glycyrrhizin (25–200μg/ml) was found to significantly inhibit the CCl_4- induced release of AST and LDH. The authors suggested that this function was due to an alteration of membrane fluidity by the glycyrrhizin, or may be due to an inhibition membrane lipid peroxidation induced by CCl4. 18β- glycyrrhetic acid inhibited free radical generation activity at 1mg/ml while Glycyrrhizin exhibited no significant suppressive activity of such kind. 18β-glycyrrhetic acid, the aglycone of glycyrrhizic acid is responsible for hepatoprotective action by inhibiting both free radical generation as well as lipid peroxidation. The *in-vivo* protection of glycyrrhizin against CCl_4- induced hepatotoxicity was more recently illustrated [80]. Extracts of herbal plants *Glycyrrhiza glabra*, they showed a novel hepatoprotective effects against diclofenac induced hepatotoxicity in rats [81]. Glycyrrhizin reduced the mortality of acetaminophen overdosed mice more effectively; attenuate acetaminophen induced hepatotoxicity, and reduced the number and area of γ-GT positive foci, thus protecting liver function [82].

Anticonvulsant activity

The anticonvulsant activity of alcoholic extract of roots and rhizomes of *Glycyrrhiza glabra* (10, 30, 100 and 500 mg/kg, i.p.) in mice was assessed using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using mice. The lithium pilocarpine model of status epileptics was also used to assess the anticonvulsant activity in rats. The alcoholic extract of *G. glabra* root did not reduce the duration of tonic hind leg extension in the MES test even in the dose of 500 mg/kg. Though, the extract significantly and dose dependently delayed the onset of colonic convulsions induced by pentylenetetrazol. All animals were afforded protection by the dose of 100mg/kg. The extract also protected rats against lithium pilocarpine induced seizures [83].

Anti-inflammatory activity

Glycyrrhizin has long demonstrated its strengthening action on hydrocortisone anti inflammatory activity in rats. Alonso showed anti-inflammatory activity of aglycone

(glycyrrhetic acid) with 1/8th potency as compared to cortisol, this activity reaches 1/5th potency, if glycyrrhetic acid is administered as sodium hemisuccinate (chemical structure is identical to carbenoxolone). Other flavonoid components of liquorice root, such as liquiritoside, have also shown *in-vitro* anti inflammatory activity. The activity of 18-α glycyrrhetic acid was found to be similar to that of glucocorticoids and stronger than that of its β-isomer. Due to the inhibition of hepatic D'-5-β-reductase, both glycyrrhizin and its aglycone have mineralo-corticoid effects. The modifications that glycyrrhetic acid and hydrocortisone produce on the activity of certain enzymes have been correlated with their anti-arthritis activity, due to the structural similarity of both the compounds and their activity at the suprarenal level. Leukocyte migration towards swollen areas in *in-vivo* models were inhibited by both glycyrrhizic acid and its aglycone (glycyrrhetic acid). Activated peritoneal macrophages, phospholipase-A activity and prostaglandin-E2 synthesis were inhibited by Glycyrrhizin. Liquiritoside showed experimental inhibition of cyclooxygenase, lipooxygenase and platelet peroxidase. *In-vivo* assays, unlike other anti-inflammatory drugs, glyderinine (a glycyrrhizic acid derivative) showed stronger antipyretic, analgesic and anti-inflammatory properties than hydrocortisone and amidopyrine. The gastroduodenal mucosa was not damaged by it. When it was applied as an ointment, very good penetration in the skin and tolerability was exhibite. Increased penetration of externally applied diclofenac sodium was observed when a 0.1% concentration of glycyrrhizin in a gel or an emulsion was applied. The enzyme 11-β hydroxy steroid dehydrogenase was inhibited by glycyrrhetinic acid, which is converts cortisol (active form) into its inactive metabolites. Thus, significant increase in the levels of cortisol and stimulation of the activity of the glucocorticoid receptors was observed by inhibition of the enzyme by glycyrrhetinic acid, which in turn potentiated the activity of hydrocortisone, the major glucocorticoid that is secreted by adrenal cortex. Glycyrrhizin and glycyrrhetinic acid's anti-inflammatory are associated and accounted for due to Hydrocortisone. [84].

Anti-carcinogenic Effects

The potential anti-carcinogenic effects of liquorice extract and glycyrrhizate compounds were evaluated [85]. The *in-vitro* anti-mutagenic properties of triterpene compounds, such as glycyrrhizin, have been well documented but the mechanism of this action is still poorly understood. The anti-mutagenic effects of glycyrrhizin and

glycyrrhetic acid demonstrated, that both of these compounds inhibited the mutagenicities of 3-amino-1-methyl-5*H*-pyrido [2, 3-*b*] indol (Trp-p-2), 2-acetyl aminofluorene, and benzo (α) pyrene, in the presence S9 fraction hepatic enzymes by using Ames test [86]. When the assay was repeated using mutagens not requiring metabolic activation, such as methyl glyoxal, glyceraldehyde and glucose pyrolysate, glycyrrhizin inhibited the number of induced *Salmonella typhimurium* TA98 revertants, whereas glycyrrhetic acid promoted the number of revertants per plate. Ikken Y et al. consider that glycyrrhetic acid may act by inhibiting the metabolic activation of some mutagens. Both Liquorice extract and glycyrrhizin inhibited the mutagenic effects of Trp-p-1 and Trp-p-2 in *S. Typhimurium* TA98 whereas liquorice extract exerted a moderate to strong anti-mutagenic effect against several *N*-nitrosamine mutagens [87]. Liquorice extract and glycyrrhizin were also effective at inhibiting the mutagenic effects of metabolically pre-activated Trp-p-1, suggested that the antimutagenic effects are not due solely to the inhibition of the activating enzymes. In order to elucidate the inhibition mechanism of chemically induced mutagenicity by liquorice extract, glycyrrhizin, 18α and 18β glycyrrhetic acid, the effects of each of these was studied. Results found that only the liquorice extract was anti-mutagenic towards ribose–lysine. This suggested that a non-glycyrrhizin compound was the active component of this extract responsible for anti-mutagenic activity. The anti-mutagenic activity of liquorice extract was confirmed in the assay in *Bacillus subtilis* strain M45, which is deficient in the genetic recombination function. However, liquorice extract was not anti-mutagenic to the activities of the frame shift mutagens 9-aminoacridine or acriflavine, suggesting specificity in its mechanism of action. These results indicated a possibility that the root extract of liquorice directly interferes with the mutagen or might be acting as an anti-mutagen by enhancing a DNA repair response. Growth of B16 cells in a concentration dependent manner was inhibited by Glycyrrhetic acid and it caused the total inhibition at concentrations greater than 10μg/ml (approx. 21μM), while 200μg glycyrrhizin/ml (243 μM) inhibited growth by 40%.

Anti Carcinogenic studies

Several studies have been conducted on the effects of liquorice and glycyrrhizin on the growth and acid production of oral bacteria associated with the development of dental caries. Glycyrrhizin could significantly reduce the growth and acid production

of *Streptococcus*, *Actinomyces* and *Bacterionema* species. Liquorice root powder, ammoniated glycyrrhizin, and mono-ammonium glycyrrhetic acid competitively reduced the metabolism of sucrose, glucose and fructose, but were themselves minimally fermentable. In distinguish to these results; Segel R et al. reported that neither liquorice 'juice', nor glycyrrhizin inhibited the growth of seven *Streptococcus mutans* strains. In the presence of sucrose, no effect on growth was seen due to 0.5–1% glycyrrhizin, but bacterial adherence to glass was profoundly inhibited to almost 100% at the highest concentration tested [88]. Similar anti-adherent properties were shown by liquorice juice with concentrations of 5 and 10% exhibiting almost 100% activities. The buffering capacity of glycyrrhizin was not sufficient to affect the decrease in pH caused by bacterial sucrose degradation. In a supplementary study evaluating the mechanism of the anti-adherent property of glycyrrhizin [89], examined the effect on bacterial glucosyl transferase activity, an enzyme required in the formation of insoluble glucans required in plaque development. A crude preparation of *S. Mutans* glucosyl transferase was significantly inhibited by glycyrrhizin in a concentration dependent manner. At 12mM glycyrrhizin there was a 50% inhibition of total glucan formation and a 90% inhibition of adhered glucans formation. Although glycyrrhizin was able to inhibit the activity of the soluble glucan-forming glucosyl transferase, the IC_{50} was 36mM. So it can be concluded that inhibition of bacterial glucosyl transferase activity may be a mechanism by which glycyrrhizin inhibits oral bacterial adherence, but that additional enzyme systems may also be affected.

Miscellaneous Studies

Glycyrrhizin is classified as a saponin compound. Interaction of Glycyrrhizin with cellular membranes of erythrocytes and hepatic lysosomal preparations is determined using this property of glycyrrhizin. Glycyrrhizin was found to protect erythrocytes against the hemolysis induced by other saponin compounds including digitonin, tomatin, and saponin-A [90]. The effect of glycyrrhizin was concentration dependent but it was only effective at preventing hemolysis at concentrations approximately 400 times greater than the hemolysin. Glycyrrhizin was found to be as effective against the sapogenins, sapogenin A, digitogenin, and tomatidine, which indicates that its mechanism of action is not because the membrane glycosidases of erythrocytes are being inhibited. Glycyrrhizin alters membrane fluidic dynamics at these high

concentrations or prevents access of hemolysin to its receptor, could be one of the possibilities. The effects of glycyrrhizin on the activity of acid phosphatases from hepatic lysosomal preparations and its release was investigated by Nakagawa K, in order to test this possibility. Both glycyrrhizin and 18β glycyrrhetic acid attenuated acid phosphatase activity, but did not affect β-*N*-acetyl-glucosaminidase activity. The reduction of lysosomal acid phosphatase activity was due to its release from the lysosomes rather than a direct inhibition of the enzyme suggesting an alteration in membrane fluidity [91].

2.3) Nelumbo nucifera:

The Indian Lotus is also known as the Sacred Lotus. It is a culturally significant plant in many Asian countries in general and Indian culture. The plant native to the Indian subcontinent but now a day is found as an ornamental plant throughout world [92].

(a) (b)

Fig 2.3 (a) Picture of *Nelumbo nucifera plant (b)* Leaves of *Nelumbo nucifera* plant

2.3.1) Definition:

Nelumbo nucifera Gaertn., is a large aquatic herb with stout creeping yellowish white coloured rhizomes, Ancient medical literature assigned the Sanskrit name "Kamala" to *Nelumbo nucifera*, there are two forms one with white flowers commonly called 'Pundarika' or 'Sveta kamala' and the other with pink or reddish pink flowers called 'Rakta Kamala' [93].

2.3.2) Classification: [94]

Kingdom: Plantae

Class: Equisetopsida

Subclass: Magnoliidae

Superorder: Proteanae

Order: Proteales

Family: Nelumbonaceae

Genus: *Nelumbo*

Species: *N. nucifera*

Botanical name: *Nelumbo nucifera* Gaertn

2.3) Synonyms of *Nelumbo nucifera:* [95]

Sr. No	Language	Names
1	English	Water Lily, Lotus, Sacred Lotus, East Indian Lotus
2	Hindi	Kamal, Padma
3	Manipuri	Thambal
4	Marathi	Pandakanda, Kamal
5	Tamil	Ambal
6	Malayalam	Tamara
7	Telugu	Erra-tamara
8	Kannada	Tavare-gadde
9	Oriya	Padma
10	Gujarati	Motunkamal
11	Sanskrit	Sharada

2.3.4) Habitat: [94]

Nelumbo nucifera required warm temperate to tropical climates, in a range of shallow (up to about 2.5 m deep) wetland habitats, including flood plains, lakes, ponds, pools, swamps marshes, lagoons and the back waters of reservoirs.

2.3.5) History: [95]

The Indian Lotus is considered to be a symbol of devotion and purity. It is the seat of Goddess Saraswati, Goddess Lakshmi, Lord Brahma and Lord Vishnu.

2.3.6) Description: [93]

Nelumbo nucifera Gaertn., is a perennial aquatic plant. It is having large, peltate (the leaf-stalk attached to the centre rather than the edge) leaves rise above the water surface on 1 to 2 m long petioles. The leaf surface is water repellent and it has inspired the term "lotus effect". This term describes that leaves' having self cleaning capacity which is a result of dirt particles being picked up by water droplets due to a complex nanostructure of the leaf surface, which minimises adhesion. There are two types of leaves such as aerial and floating, both having moderate to large size, orbicular, 20-90 cm in diameter, abruptly acute to form a short tip. It is entire glaucous, petiolate, non-wettable, strong cupped in case of aerial leaves and flat in case of floating ones. The leaves are radiately nerved and the fresh leaves having leathery features. When dry for short periods, they are nearly membranous and brittle. There is more or less brown to red blotching on the lower surface, petioles of the aerial leaves are erect and stout white those of the floating ones are not strong enough. The usual length varies from 24.00 to 33.00 cm. in case of aerial leaves and 23 to 30 cm in case of floating, petioles are smooth, greenish or greenish to brown in colour with small brown dots sometimes rough with very small, but distinct princkees, odour is distinct, fracture is fibrous. In the transverse section of petioles, it always shows the four distinct and large cavities in the centre and small cavities in the periphery region.

2.3.7) Cultivation: [96]

Sexual and Asexual Propagation

The propagation of lotus is done by seeds or by underground stem division. The lotus seeds may be stored for long periods of time about 1,000 to 2,000 years. Their longevity can be attributed to the hard shells around the seeds which is having an impermeable seed coat. Sometimes small changes in the inner gas composition during long periods of storage and also low changes in the concentration of high dehydro ascorbic acid (200mg/100 g) content. The method of propagation by seeds is mainly used in breeding new cultivars. Because of highly heterozygous nature of seeds, the commercial sexual propagation is unusual. Imported seeds present problems due to low viability in Australia. Additionally, germinated seedlings do not produce a crop until the following season. The buds were micro-propagated from rhizomes by scientists in Thailand. The objective of their research was to determine and compare efficacy of different irradiation methods to produce variegated phenotypes of lotus.

Enlarged rhizomes are usually used for commercial cultivation. It is considered as the simplest method which give a ensure yield and good harvestable crop in only one season. The dormant organs of *N. nucifera* are enlarged rhizomes which are used in the survival of the plant under unfavourable conditions. Parent, son and grandson rhizomes are the three categories which are classifies according to the relationship among rhizomes in China. Chinese farmers are using the entire parent as seed rhizomes. Top buds with rhizomes, free of diseases or pest are selected as seed rhizomes. Asexual propagation allows the original characteristics of the mother plant to be preserved, flowers to be enjoyed, and the lotus root to be harvested in the same season. One of the authors recommended the use of rhizomes with at least two segments sealed at either end by an intact node. Transplanting should be done before rhizomes break dormancy, because plants transplanted after rhizomes have germinated do not establish as well. Although, rhizomes seem to be the preferred propagation method, sometimes apical buds or running stems are also used. The rhizomes are required to plant in a saturated media at an angle of 15° with the shoot meristem buried under 5 cm of media. Propagation material should be grown in separate ponds. At the end of each growing season it is recommended to save 20 % of the rhizomes for next season seed [96, 97].

Soil Conditions

For production in containers and ponds several substrates have been suggested, however, lotus prefers rich and fertile soil. Lake or pond bottoms containing large amounts of organic matter are the most suitable. Some commercial stores suggest the use of a heavy clay loam or formulated soil for the growth of aquatic plants. The very heavy soil leads to the leaves would not float so the recommendation limits the use of most potting soil mixes found at garden centres. Germinated hundreds of years old seed in a 3:1 soil mix of clay soil and greenhouse soil (washed sand, sphagnum moss and sandy loam in equal proportions). The harvesting is more difficult when soil is heavy clay and roots cannot penetrate it. On the other hand, sandy soils lack binding sites for nutrients and have been reported to induce a rough flavour on the rhizomes. Suitable soil can be transported into the ponds. Optimal soil is a soft silt loam, free-form particulate matter. Soils with correct organic matter provide nutrients in an extended manner, a buoyancy to texture, good binding sites for nutrients and prevent light penetration [96, 98, 99].

Fertilization

Lotus field, lake, pond, or paddy deficient in fertility should be supplemented with various organic matters such as an oil press cake or composted and green manure. The maturity stage of the crop is dictated by the sufficient amount of fertilizer e.g., Rhizome formation required more amount of potassium and less amount of nitrogen when plants reaching maturity. The fertilization of ornamental lotus by applying soluble fertilizer (20-10-20) applied once every 20 days (4-8 grams) after rhizomes had sprouted (coin leaves were visible on the water surface). Additionally, lotus plants responded favourably to increased fertilizer rates. The weight of fresh root, number of propagules and expanded internodes are increased by applications of 8g of fertilizer. Young plants are easily burned the fertilizers should be carefully administered. It is advisable to split the dose in 3 to 4 applications. The controlled release fertilizer should be used when seedlings are held for longer than 1 year in their pots. Osmocote tablets are used with 3% water soluble magnesium oxide (MgO), 8-9 months release at a rate of 5gm/lit have been used successfully. In India, growing media is enriched by incorporating well decomposed cattle dung manure at a rate of 5 kg/m^2, Neem (*Azadirachta indica*) cake (100 gm/m^2), di-ammonium phosphate (25 gm/ m^2) and muriate of postas (25 gm/ m^2) as a basal dose 15 days prior to the planting. In the reported literature, manure proved to be fatal to the young seedlings. It showed that clay as an important component for nutrient retention and as a minor nutrient source for lotus culture also. For fertilization of the fields, the different combinations of fertilizers are used by farmers in China. Sometimes, fields are improved by adding 45,000 to 60,000 kg/ha of animal waste and in some cases, they combine 1,500 kg of bean manure and15, 000 kg animal waste / ha. In other instances, they use 600 kg of special lotus formulated fertilizer combined with 375 kg of NH_4HCO_3 / ha [96, 100].

2.3.8) Pharmacological activity

Antioxidant activity

In this study, hydrogen peroxide (H_2O_2) mediated Caco-2 cytotoxicity was employed to investigate the potential antioxidant activity of the methanol extract from the lotus leaf (*Nelumbo nucifera*) A dose-dependent protective effect against reactive oxygen species (ROS) - induced cytotoxicity was observed when Caco-2 cells were treated with 10 mM H_2O_2 in combination with the methanol extract of the lotus leaf (0.1 - 0.3

mg/ml). However, no significant effect was found when co-treating Caco-2 cells with 10 mM H_2O_2 and alpha-tocopherol. *In vitro* models showed that the extract exhibited scavenging activities on free hydroxyl radicals, metal binding ability and also as reducing power which explain the mechanism how the extract's ability to protect cells from oxidative damage. The extract also showed concentration dependent antioxidant properties against fenton reaction mediated plasmid DNA oxidation and haemoglobin induced linoleic acid peroxidation [101].

Cytotoxicity

Sulforhodamin B bioassay (SRB) was used as for cytotoxicity screening. The *in-vitro* cytotoxicity of each compound against four cultured human tumor cells was assessed at the Korean Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adeno-carcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma) and HCT15 (colon cancer cells). Doxorubicin was employed as a positive control for cytotoxicity study. The cytotoxicities of doxorubicin using A549, SK-OV- 3, SK-MEL-2 and HCT cell lines were IC_{50} values 0.007, 0.056, 0.117, and 0.164 μM respectively [102].

Cardiac activity

Asimilobine and lirinidine are two serotonin antagonistic alkaloids which were isolated from leaves of *Nelumbo nucifera*. Both the alkaloids inhibited contraction of the rabbit- isolated aorta induced by serotonin. One another alkaloid nelumbine was also reported to be present in leaves and petioles of the plant which acts as a cardiac poison [103].

Respiratory activity

Leaf, pedicles contains alkaloid nelumbine, alkaloid nupharine in 8mg/kg dose to a dog causes lasting stimulation of respiration impaired respiration is restored and stimulated [104].

Anticancer activity

Hepatocellular Carcinoma: The alkaloid neferine decreases cell viability in a dose and time dependent manner with an IC_{50} of 10μM at 48 hours against isolated HepG2 cells. This was associated with an increase in calcium influx into cells which disturbed mitochondrial membrane permeability, which then induces apoptosis [105].

Osteosarcoma: It appears to suppress proliferation of the two cell lines U2OS and Saos-2 with fewer efficacies against the normal cell line HCO when testing neferine in osteosarcoma cells. The mechanism appeared to be a concentration dependent upregulation of p21 activity causing G1 cell cycle arrest secondary to activation of p38 MAPK [106].

Anti-depressant activity

Isolated neferine at 100mg/kg showed a reduction in locomotion in rats which was independent of serotonin receptors. This study reported that anti-depressive effects were mediated via them. The sedative effect appears to extend to the structurally related compounds such as liensinine, nelumboferine, O-methylneferine and isoliensinine with liensinine and isoliensinine having more potancy than the structures of neferine [107].

Anti-diabetic activity

One of the amino acid, tryptophan was isolated from active fraction of the aqueous and methanolic extract from the node of *N. nucifera* rhizome. Tryptophan reduced the blood glucose level significantly in glucose fed hyperglycaemic mice compared with glucose treated group and exhibited 44.3% of activity compared with tolbutamide [108].

Anti-obesity activity

The anti-obesity effect of *Nelumbo nucifera* leaves extract (NNE) evaluated on digestive enzyme activity, lipid metabolism and theromogenesis. The NNE was treated for 5 weeks to study the anti-obesity activity using high-fat diet-induced obesity in mice. It also caused a concentration dependent inhibition of the activities of α-amylase and lipase. NNE was up-regulated lipid metabolism and expression of UCP3 mRNA in C2C12 myotubes. It also When obesity induced by a high fat diet, it prevented the increase in body weight, adipose tissue weight and liver triacylglycerol levels in mice. Therefore, NNE impaired digestion, inhibited absorption of carbohydrates and lipids, accelerated lipid metabolism and up-regulated energy expenditure. Thus, NNE is beneficial for the suppression of obesity [109].

Anti-malarial activity and anti-fungal activity

One new compound, 24(R)-ethylcholest-6-ene-5a-ol-3-O-b-D-glucopyranoside, along with some known metabolites were isolated from the leaves of *Nelumbo nucifera*. They were identified by spectroscopic methods including 1D- and 2D NMR. Antifungal activity for (R)-roemerine (IC_{50}/MIC = 4.5/10 mg/ml against *Candida albicans*) and antimalarial activity for (R)-roemerine and N-methylasimilobine (5) (IC_{50} = 0.2 and 4.8 mg/ml for the D6 clone, respectively, and 0.4 and 4.8 mg/ml for the W2 clone, respectively) was observed. None of the compounds were cytotoxic to Vero cells up to a concentration of 23.8 mg/ml. An analysis of the structure–activity relationship shows that the substituents in position C-1 and C-2 of aporphine alkaloids are crucial for the antimalarial activity [110].

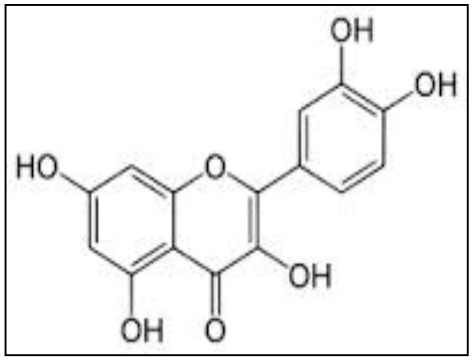
Immunomodulatory activity

The rhizomes and seeds of Nelumbo have been tested independently (both at 100mg/kg or 300mg/kg, compared to 2mg/kg dexamethasone) noted that both the rhizomes and seeds increased total lymphocyte count and the hydro-alcoholic extract of the seeds; at 300mg/kg, increased macrophage phagocytosis [111].

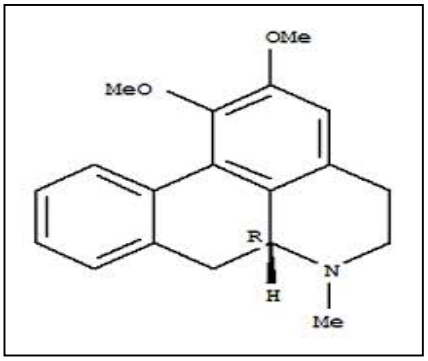
2.3.10) Chemical Constituents:

Leaves and petioles contain nor-nuciferine (m. p 186-189°C), $C_{18}H_9O_2N$, nuciferine (II), (m.p 164-165°C), remerine (HI salts, m. p 224-227°C) and remerine (III), (m. p 100-101°C, (+) armepavine (IV), (m. p 139-140°C, were isolated. Asimilobine and lirinidine are two serotonin antagonistic alkaloids which were isolated from leaves of *N. nucifera*. Both alkaloids inhibited contraction of the rabbit isolated aorta induced by serotonin. Another alkaloid nelumbine was also reported to be present in leaves and petioles of the plant which acts as a cardiac poision. The leaves also contain a glycoside (nelumboside) which on hydrolysis with 5% H_2SO_4 gave one molecule of glucuronic acid, glucose and quercetin on methylation with CH_2N_2 followed by hydrolysis. This glycoside gave 5, 7, 3', 4' tetra-ortho-methyl-I quercetin, m. p 192ºC. The leaves also contain leucoanthocyanidin and iso-quercetin which were identified as leucodelphinidin and leucocyanidin by conversion into corresponding anthocyanidin chlorides or by paper chromatography or by absorption maxima. Leaf, pedicles contains alkaloid nelumbine and nupharine in 8mg/kg dose to a dog causes lasting stimulation of respiration, impaired respiration is restored and stimulates [112].

2.3.11) Structures of important chemical constituents: [112]



Quercetin



Nuciferine

2.4) *Prunella vulgaris*

Prunella vulgaris, known as 'self-heal' or 'heal-all' is a long lived medicinal herb with a worldwide distribution. It has been used as a traditional remedy in Europe and China for thousands of years [113, 114].

(a) **(b)**

(b) Figure 2.4: (a) Picture of *Prunella vulgaris* plant (b) Showing all parts of *Prunella vulgaris* plant

2.4.1) Common names

Self heal, All heal, Hook Heal, Blue curls, Brown wort, Carpenter's weed and Heart-of-the-earth. In Germany, it is known as Kleine Braunelle, In Finland, it is known as Niittyhumala and in Poland it is called Glowienka pospolita [115].

2.4.2) Classification: [116]

Kingdom- Plantae – Plants

Subkingdom -Tracheobionta – Vascular plants

Superdivision- Spermatophyta – Seed plants

Division- Magnoliophyta – Flowering plants

C lass- Magnoliopsida – Dicotyledons

Subclass- Asteridae

Order - Lamiales

Family Lamiaceae – Mint family

Genus Prunella L. – self-heal

Species *Prunella vulgaris* L. – common self-heal

***2*.4.3) Description**

Prunella vulgaris grows 5-30 cm high (about 2-12 inches) with creeping, self-rooting, tough and reddish stems branching at leaf axis. The leaves are lanceoate shaped, serrated margin, and reddish at tip, about 2.5 cm (about 1 inch) long and 1.5 cm (about 0.5 inch) broad and growing in opposite pairs towards the square stem. Each leaf has 3 to7 veins that shoot off of the middle vein towards the margin. The stalks of the leaves are growing up to 5 cm (about 2 inches) long. The flowers grow from a club like structure, slightly square, whirled cluster, immediately below this club are pairs of stalk less leaves standing out on either side like a collar. Flowers are tubular and two lipped. The top of the lip is a purple hood and the bottom lip is often white in colour it has three lobes with the middle lobe being larger and fringed upwardly. Flowers bloom at different times depending on climate and other conditions but mostly in summer (from June to August in the USA). Self-heal propagates both by seed and vegetative by creeping stems that root at the nodes. The flowers are purple in colour which sprouts at top of the stem. Flowers are in full bloom mostly from June to August [117, 118].

2.4.4) Habitat

Self heal is a perennial herb found throughout Asia, North America and Europe as well as most temperate climates. Its origin seems to be Europe, though it has been documented in other countries also. In the Republic of Ireland, it is currently abundant in the west in the counties such as the southwest in Kerry, Galway and Clare, the south coast and is also found around the central basin of Ireland [119].

2.4.5) Cultivation

It is often found growing in moist areas, woodland edges, grassland, waste ground and usually at basic or neutral pH soils. The germination of self heal seed is greater at alternating temperatures of 20 to 30°C than a constant 20°C. Seed germinates better in the light than in the dark condition. Germination is improved by scarification of soil. Seeds sown in a 75 mm layer of soil in the field and cultivated periodically emerged from February-October with peaks of emergence in April and September. Flushes tend to followed by cultivations. Seedling emergence is higher in bare soil than in vegetation or leaf litter covered soil. Shoots usually die after flowering but may regenerate from the base. Self heal overwinters as a rosette of leaves. The new shoots are elongated in late spring. In a cultivated soil, the seeds can persist for at least 5 years. They have been recorded in large numbers in the soil beneath pasture even when the plant was poorly represented in the vegetation cover. Self heal spreads by seeds. The seeds are a common impurity of Swedish, clover and cultivated grass seed of UK and Danish origin. Self heal also spreads vegetatively by using short rhizomes or stolons. It regenerates from shoot fragments also if the plant is disturbed and is able to rapidly colonise cleared areas [120].

2.4.6) Chemical Constituents

Screening of the plant material was revealed the presence of the saponins, terpenes, phenols, alkaloids and tannins. The plant's active chemical constituents are betulinic acid, D-fenchone, cyanidin, D-camphor, delphinidin, manganese, hyperoside, oleanolic acid, lauric acid, myristic acid, rosmarinic acid, linoleic acid, ursolic acid, lupeol, beta-sitostero-l, rutin and tannins. A triterpene, 2a, 3a, 24-trihydroxyolean-12-en-28-oic acid was found in the leaves and stems of *Prunella vulgaris.* Further two hexa-cyclic-triterpenoids such as (12R, 13S)-2a, 3a, 24-trihydroxy-12, 13-Cyclotaraxer-14-en-28-oic acid and (13S, 14R) - 2a, 31, 24- trihydroxy-13, 14-cycol-olean-11-en-28-oic acid as methyl esters were isolated from the roots of plant as well. Urosolic acid and its derivatives were also reported in the plant. Prunellin is an anti-HIV polysaccharide which was isolated from water extracts of *P. vulgaris*. Monosaccharides such as glucose, xylose, galactose, galactonic acid gluconic acid and galactosamine were also reported. Latter on four d-glucopyranosides named stigmsterol, sitosterol, spinasterol and stigmast-7-en3-ol were reported. From methanolic extract of aerial parts of *P. vulgaris Var. Lilacina,* four flavonoids, fifteen

triterpenoic acids and aditerpene were isolated. Ursolic acid (UA) and Oleanolic acid (OA) in *P. vulgaris* were separated by modified HPLC method. Two phenyl propanoids were isolated from the ehtanol extract of the spikes of *P. vulgaris* i.e., etyl rosmarinate, butyl rosmarinate, rosmarinic acid, methyl rosmarinate and 3-4 a trihydoxy-methyl phenyl propionateand p-coumaric acid. Seven compounds from the ethanol extract of the spikes of *P. Vulgaris* were isolated and their structures were reported as rhein, autantiamide acetate, danshensu, tanshinone I, stigmast-7, 22-dien-3-one, butyl rosmarinate and 3-4 alpha-trihydroxy methyl phenyl propionate. A new triterpenoid sapoin 24-dihydroxyloean-12-en-3-o-beta glucuronoside (Prunelloside A), 16-oxo-17-demethyl 1-3 beta and a flavones glycoside acacetin-7-o-beta 0-glucopyranoside were reported as constituents of *P. Vulgaris*. By using HPLC and LC/1v1S analysis the main active compounds obtained were phenols such as rosmarinic acid, caffeic acid quercetin and rutin [121, 122].

2.4.7) Bioavailability

Bioavailability varies from compound to compound and can be considered in the context of a tissue/cell type or in the context of metabolic interactions with host microflora. Qiang et al recently described the permeability of rosmarinic acid and ursolic acid, two major components found in *P. vulgaris*, across human intestinal epithelial Caco-2 cell monolayers. As detected by HPLC, it was found that rosmarinic acid and ursolic acid contained in whole extracts of *P. vulgaris* were able to permeabilize intestinal epithelial monolayers with equal efficiency as purified rosemarinic acid or ursolic acid (UA). Additionally, UA is not subject to intestinal glucuronidation/sulfation which would potentially increase the bioavailability of this compound. It should be noted that this dissertation focuses on intestinal bioavailability, which can be uniquely altered by microflora metabolism and the condition of the intestinal, luminal epithelial barrier in the context of health and homeostasis as well as during onset and maintenance of colitis. The epithelial barrier changes which occur in the intestine in the context of colitis could dramatically alter the cell types and tissues to which target compounds could be exposed. The diversity and quality of the microflora is also altered in the colitic intestinal milieu which could additionally alter bioavailability of these plant compounds [123].

2.4.8) Pharmacological Activity:

Antiviral and immunomodulatory activity

P. vulgaris has been shown to have antiviral and immunomodulatory activities. A polar fraction of *Prunella vulgaris* showed potent antiviral activity against HSV-1, HSV-2 and HIV-1 have been reported. Additionally, it has been shown that the extracts and chemical components can modulate various immune factors including TNF-alpha, histamine, nitric oxide, IgG, IgG1, IgG2b, IFN-gamma, LTB4, IL-2 in T cell activation [124-126].

Anti-HSV activity

The polysaccharide at 100 mg/ml was active against the herpes simplex virus viz. HSV-1 and HSV-2 using a plaque reduction assay. But it was inactive against human influenza virus types A and B cytomegalovirus, the vesicular stomatitis virus or the poliovirus type 1. The dose of the polysaccharide for 50% plaque reduction for HSV-1 and HSV-2 was 10 mg/ml. Clinical isolates and known acyclovir resistant (TK-deficient or polymerase-defective) strains of HSV-1 and HSV-2 were similarly inhibited by the polysaccharide. HSV-1 with the polysaccharide was pre-incubated at 4, 25 or 37°C. It completely abrogated the infectivity of HSV-1 but pre-treatment of Vero cells with the polysaccharide did not protect cells from infection by the virus. The polysaccharide was added at 0, 2, 5.5 and 8 h post-infection of Vero cells with HSV-1 at a multiplicity of infection (MOI) of five reduced the 20 h yield of intracellular infectious virus by 100, 99, 99 and 94% respectively. In contrast, a similar addition of heparin showed 85, 63, 53 and 3% reduction of intracellular virus yield, respectively. These results showed that the polysaccharide may inhibit HSV by competing for cell receptors as well as by some unknown mechanisms after the virus has penetrated the cells. The Prunella polysaccharide in the concentration 0.5 mg/ml of was not cytotoxic to mammalian cells up to the highest concentration tested. It did not show any anti-coagulant property. So, the polysaccharide isolated from *P. vulgaris* has specific activity against HSV and its mode of action appears to be different from other anionic carbohydrates like heparin [127].

Anti-diabetic activity

P. vulgaris extract at dose of 100 mg/kg significantly suppressed the rise in blood glucose after 30 min in the acute glucose tolerance test. It enhanced the anti-

hyperglycemic effects of exogenous insulin without stimulating insulin secretion in streptozotocin-induced diabetic mice. Extract also has a protective effect on IL-1 -induced INS-1 cell apoptosis. It attenuates IL-l -increased NF- KB binding activity and inflammatory cytokine expression in INS-1 pancreatic cells. PVAE may have a benefit for type I diabetic patients [128].

Anti-stress activity

It was reported that the ethanolic extract of leaves of *P. vulgaris* had ability to prolong the swimming time and improve the stress induced changes in animal stress models. So, it has suggested that *P. vulgaris* is having its adaptogenic property [129].

Effect on immune system

The immonosupressive activity of the ethanol extract of *P. vulgaris* on the immune response in mice was studied [130]. One of the polysaccharide fraction (PV21V) was up-regulated the immune response of monocytes [126]. It showed that aqueous extracts of *P. vulgaris* stimulated the proliferation of T-lymphocytes but suppressed nitric oxide production so lipopolysaccharide is stimulated [131]. The immunostimulatory and antitumor property of *P. vulgaris* in murine macrophage (RAW 264.7) cells was reported. Plants extract also stimulated macrophage phagocytic activity, Nitric oxide (NO) production and cytostatic activity. Additionally, induced gene expression and production of macrophage related cytokines such as TNF-*a*, IL-l and IL-6n and rosmarinic acid in ethanol extract of plant inhibited lipopolysaccharide induced prostaglandin E2 and nitric oxide in RAW 264.7 mouse macrophages [132]. 0.1% and 1.0% doses of *P. vulgaris* extracts augmented diets increased the non-specific immune response and disease resistance of *P. olivaceus* against *U. Marinum* [133].

Anti-allergic activity

The effect of aqueous extract of herb on immediate type allergic reactions was studied which showed that extract (0.005 to 1 g /kg) inhibited systematic anaphylactic shock in rats. The serum histamine levels were also reduced when extract was given at concentrations ranging from 0.005 to1 g/kg [134]. The effect of water extract of *P. vulgaris* on the mast cell which was mediated by allergy model, it was revealed that the extract (0.001 to 0.1 g/ kg) dose dependently inhibited systematic anaphylaxis and serum histamine release in mice [135].

Effect on teeth

Herbal based dentifrice such as *P. vulgaris* extract was reported to be effective in reducing symptoms of gingivitis [136]. *P. vulgaris* extract and rosmarinic acid was able to suppress LPS-induced biological changes in gingival fibroblasts by modulating the inflammation process in periodontal disease [137].

Antimicrobial activity

P. vulgaris decoction revealed that strong inhibition and control on diarrhoea *bacilli, E.coli, Vibrio cholerae, salmonella typhi, Proteus vulgaris, Mycobacterium tuberculosis and Staphylococcus aureus.* In addition, alcoholic decoction of *P. vulgaris* revealed that inhibition on *P. aeruginosa and* aqueous decoction showed inhibition on fungi. It also inhibits *Bacillus typhi, Pseudomonas aeruginosa, E. coli and Mycobacterium tuberculosis.* The rosmarinic acid isolated from *P. vulgaris* was found to exhibit a moderate antimicrobial activity on gram-positive bacteria [138]. Antibacterial activity of the methanolic extract of *P. vulgaris* extracts was reported against *Staphylococcus aureus, Escherichia coli, Kleibsella pneumonae* and *Salmonella typhimurium* [139]. Two polyacetylenic acids were isolated from *P. vulgaris* methanolic extract as active principles and were identified as octadeca-9, 11, 13-triynoic acid and trans-octadec-13-ene-9, 11-diynoic acid. These two compounds inhibited the growth of fungal pathogens *Magnaporthe oryzae, Rhizoctonia solani, Phytophthora infestans, Sclerotinia sclerotiorum, Fusarium oxysporum f.* sp. *Raphani and Phytophthora capsici.* The n-hexane fraction of *P. vulgaris* significantly suppressed the development of rice blast, tomato late blight, wheat leaf rust and red pepper anthracnose [140]. The effect of the extract of *P. vulgaris* on Multiple Drugs Resistant Bacillus Tuberculosis (MDR-TB) was showed that it could enhance the cellular immunological function in rats by up- regulating level of genetic transcription. It also provided the basis of healing of MDR-TB [141].

Antioxidant activity

Hydroalcholic extract of *P. Vulgaris* showed significant anti-oxidative activity. The anti-oxidative activity was partly with regard to the rosmarinic acid content. Anti-oxidant activity against superoxide, hydroxyl radicals and preoxidants were reported [142]. Furthermore it was reported that *P. vulgaris* extract showed scavenging activity on DPPH radical, inhibited *in-vitro* human LDL. Extract also inhibited rat erythrocyte

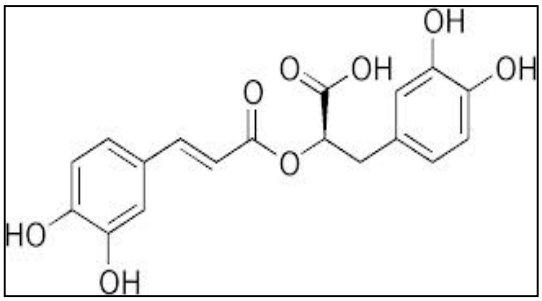
haemolysis and reduced the production of LTB in bovine PMNL generated by the 5-lipoxygenase pathway [143]. The *P. vulgaris* was rich in phenolics which showed positive effect on blood, liver antioxidant status and lipoprotein metabolism. It also affected plasma lipoprotein profile in an experimental *in-vivo* model with induced dietary hyper-triglyceridemia [144]. A rosmarinic acid isolated from *P. vulgaris* extract which significantly suppressed UVA-induced ROS production in a human keratinocyte cell line (HaCaT). It indicated that intracellular lipid peroxidation was decreased [145].

Effect on cell proliferation

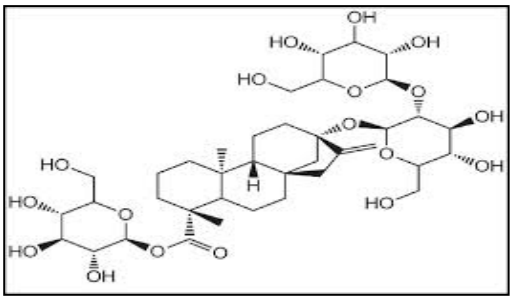
The organic fraction of *P. vulgaris* (25.7% w/w of rosmarinic acid) showed anti-proliferative effects against HaCaT cells and mouse epidermal fibroblasts [143]. Rosmarinic acid was isolated from the methanolic extract of *P. vulgaris* that showed inhibitory activity against lymphocyte cell specific kinase (Lck) Src-Homology 2 (SH2) binding to a synthetic phosphotyrosine-containing peptide (phosphopeptide) of hamster polyomavirus middle-sized tumor. *P. vulgaris* extracts was reported to suppress the proliferation in Raji cells and may be a new anti-lymphoma drug. Immuno cytochemistry showed that after Raji cells were treated by the injection of *P. vulgaris* (50 mg/ml) for 48 h, the expression of bel-l was up-regulated and the expression of bax was down-regulated [146]. *P. vulgaris* displayed significant antiestrogenic activity against endometrial cancer cell line, ECC-1 [147]. A phenolic components present in the *P. vulgaris* ethanolic extract was reported to significantly inhibit the tumor growth in mice [148]. Chemo prevention by 60% ethanolic extract of *P. vulgaris* (P-60) was again reported against non-small cell lung cancer via promoting apoptosis in SPC-A-1cells and regulating the cell cycle [149]. Two polysaccharides (P31 and P32) were isolated from the aqueous extract of herb. Polysaccharides showed anti-lung cancer activity in a C57BL/6 mouse- Lewis Lung Carcinoma (LLC) model that increased the thymus index and the spleen index [150]. Aqueous extract of *P. vulgaris* was reported to affect migration and invasion of human liver carcinoma cells by inhibiting activities of metallo-proteases, MMP-2 and MMP-9, without affecting cell viabilities. *P. vulgaris* showed strong inhibitory effect on the growth of A549 and SPC-A-1 adeno-carcinoma cell lines. Inhibition rate For lung tumor deterioration the inhibition rate were 3.56 ±6. 79% and 33.45± 10.98% for high and low dose extract respectively. It also enhanced thymus index and no effect

was observed on spleen index in tumor-bearing mice. *P. vulgaris* group showed increase content of TNF- in serum and hence *P. vulgaris* prevent lung cancer. Different concentrations of *P. vulgaris* extract inhibited the proliferation of both Raji and Jurkat cells and also cell apoptosis rate increased in the concentration of the P. vulgaris extract [151].

2.4.9) Structures of important chemical constituents [121, 122]



Rosmarinic acid



Prunellin

2.5) *Zizyphus jujuba*

Ziziphus jujuba commonly called jujube or sometimes jujuba. They are red date, Chinese date, Korean date or Indian date is a species of Ziziphus in the buckthorn family (Rhamnaceae), used primarily as a shade tree that also bears fruit. The name *Ziziphus* is related to Greeks and an ancient Arabic word. They used the word ziziphon for the jujube [152].

(a)

(b)

Figure 2.5: (a) Picture of Zizyphus jujuba plant (b) Picture of dried fruits of Zizyphus jujube

2.5.1) Classification: [152]

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Rhamnaceae

Genus: *Ziziphus*

Species: *jujube*

2.4) Synonyms of *Zizyphus jujuba:* [153]

Sr. No.	Language	Names
1.	Sanskrit	Rajabadari
2.	Hindi	Ber
3.	Gujarati	Bordi
4.	English	Berry
5.	Bengali	Kul
6.	Marathi	Bor
7.	Punjabi	Beri
8.	Tamil	Vadari
9.	Telugu	Renu
10.	Urdu	Ber

2.5.2) Habitats:

Woodland Garden secondary and sunny edge, Dappled Shade, Hedge [154]

2.5.3) History:

Ber has been recognized as a useful edible fruit since tradition of Ram and Shabari in India and depicted in Ramayana. Literature mentions both *Z. jujuba* and *Z. mauritiana* and even the wild variety *Z. nummularia.* The 'Ber' is thought to be truly wild in the area of Deccan plateau area. Once cultivated, 'Ber' would be carried with historical migrations of people and their trade [155, 156]

2.5.4) Description:

Jujubes are species of the genus *Ziziphus* which is belongs to the family Rhamnaceae named after the genus *Rhamnus.* The fruits of Rhamnaceae family are closely related

to another family Vitaceae which includes major economic species whose fruits are berries. There are two main types of jujubes, and *Z. jujuba* Mill., the common jujube and *Z. mauritiana* Lam. the Indian jujube or ber. The species has a broad range of morphologies from shrubs to small or medium sized trees which may be erect, semi-erect or spreading on the soil. According to geographical region, height can vary from 3-4 to 10-16 m or more although trees of 20 m are rare. Trees are much branched and semi deciduous. The bark has deep longitudinal furrows and is greyish brown or dark red in colour. Usually the shrub or tree is having spine but infrequently unarmed. Branchlets are much dense, white, pubescent, especially when plant is young. Sometimes branches are in zigzag pattern. Branches spread erect, becoming flexuous and dull brown to grey in colour. Fruit containing branches are not deciduous. The lamina of the leaves is elliptic to ovate or nearly orbicular. The apex is rounded, obtuse or sub acute to emarginated, the base rounded, sometimes cunate, mostly symmetrical. Margins are minutely seriate. There are 3 marked nerves almost to the apex, the nerves being depressed in the upper, light or dark green, glabrous surface. Lower surface is whitish in colour due to persistent dense hairs but may be cream coloured. Infrequently the lower surface is glabrous. Leaves are petiolate 1.1 to 5.8 mm long and stipules are mostly spines, in each pair one hooked and one straight, or both hooked, or more not developed into a spine. Flowers have sepals which are dorsally tomentose, a disk about 3 mm in diameter and a 2-celled ovary, immersed in the disk. Styles are 2, 1 mm long and connate for half their length. Flowers tend to have an acrid smell. Flowers are borne in cymes or small axillary clusters. Cymes can be sessile or shortly pedunculate, peduncles 1-4 mm tomentose. Pedicels are also tomentose and are 2 to 4 mm at flowering and 3 to 6 mm at fruiting. Fruit is having glabrous surface and oval in shape. It is edible drupe varying greatly in size from (1 to 2) cm diameter but some oval varieties can reach up to 5 x 3 cm also. The pulp is acidic and sweet in taste and the colour is greenish when unripe and yellow or sometimes reddish when they are fully ripped. [154].

2.5.5) Cultivation [157]

This species has been cultivated over vast areas of the world. It succeeds in most soils so long as they are well-drained. An open loam and a hot dry position are preferred. It succeeds in an alkaline soil. Plants are fast growing, even in poor soils. Plants are hardy to about -20°c. Another report says that they are hardy to about -30°c when

fully dormant. The different varieties of jujube are often cultivated in warm temperate zones for its edible fruit. The fruits were ripped in trees in hot dry summer. The tree spreads by root suckers which are self-sowing and forming dense thickets. When the climate is not suitable for the optimum growth of the plant, it produces an invasive and problematic weed. Trees are resistant to most pests and diseases. Trees form a deep taproot and should be planted into their permanent positions as soon as possible. The trees are fast growing and quick to mature. It bears the fruits in 3 - 4 years from seed.

Propagation

Seeds are best sown in a cold frame as soon as it is ripe. Stored seed requires three months warm then three months cold stratification. Germination should take place in the first spring, though it might take another twelve months. Prick out the seedlings into individual pots when they are large enough to handle and grow them on in a cold frame for at least their first winter. Plant out in early summer. The Roots are cut in a greenhouse in the winter season. The optimum results are obtained if a temperature is maintained between of 5 to10°C. The cuttings of the mature wood are done mostly from November to January and the do the division of suckers in the dormant season. They are planted out directly into their permanent positions or farm if required.

Harvesting and Preparation

The fruits are harvested when they are ripe in the mid September. They are dried in the sun light or boiled the fruits until the surface skin of the fruit is soft and wrinkled. Then it is dried in oven at low temperature below 35°C. Red jujube is harvested when ripe and dried directly in the sun. Good quality of fruits are used for medicinal purpose which is thick, light to dark red in colour with small seeds. It is having a sweet taste. Black jujube is harvested when ripe and then parboiled before drying in the sun. It should have a glossy, black, wrinkled surface and a very sweet taste.

2.5.6) Chemical constituents:

Alkaloids

Alkaloids are distributed throughout all parts of the plant. Stem bark of *Ziziphus* species contain many alkaloids. A zizogenin and sapogenin has been isolated from Z. *mauritiana* stems. The cyclic peptide alkaloids such as mucronine-D, mauritine-A, nummularine-A and B, amphibine-H, frangulanine, sativanine-A and sativanine-B, nummularine-B and mucronine were isolated from the bark of *Z. jujuba*. The cyclic

peptide alkaloids sativanine-C, sativanine-G, sativanine-H, sativanine-E, sativanine-D sativanine-F and sativanine-K isolated from Z. *jujuba* stem bark. The alkaloids isoboldine, coclaurine, norisoboldine, iusirine, asimilobine and iusiphine were isolated from *Z. jujuba* leaves. Peptide and cyclopeptide alkaloids from *Z. jujuba* were reported to show sedative effects. The seeds of *Z. jujuba* var. *spinosa* also contain cyclic peptide alkaloids sanjoinenine, franguloine and amphibine-D and four peptide alkaloids such as sanjoinine-B-D-F and -G2. The seeds are also used in Chinese medicine as a sedative. Chemical studies of *Z. mauritiana* led to the isolation of the cyclopeptide alkaloids, mauritines A and B, C-F, G and H, frangufoline, amphibines D, E, B and F, scutianin-F, hysodricanin-A and aralionin-C. Mauritine J, cyclopeptide alkaloid, was isolated from the root bark of *Z. mauritiana*. For the first time reported six Cyclopeptide alkaloids isolated from the stem bark of *Z.jujuba* are Mauritine-A; Amphibine-H, Jubanine-B, Jubanine-A, Mucronine-D and Nummularine-B. Latter reported Sativanine-E. Antibacterial peptide alkaloid Frangufoline from Ziziphus species was also reported. Franganine, Melonovine-A, Daechuine-S3, Frangulanine, Nummularine-A Daechuine-S6, and Nummularine-R, all are cyclopeptide alkaloids. Four cyclopeptide alkaloids from the stem bark *Z. jujuba* which are Scutianine-C, Jubanine-C, Scutianine-D and Ziziphine-A reported. Two reports appeared in the literature on isolated ingredients from the root bark of *Z. jujuba.* Both Frangulanine and Adouetine-X are active (sedative) ingredients from the root bark of *Z. jujuba.* Both are cyclopeptide alkaloids isolated and characterized by modern techniques [158-160].

Glycosides

Flavonoid glycosides/spinosins: The structure of spinosin (2"-O- beta-glucosyl swertisin) extracted from *Z. jujuba* var. *Spinosa* seed. They later identified three acylated flavone-C glycosides (6"'-sinapoylspinosin, 6"'-feruloylspinosin and 6"' pcoumaroylspinosin), pharmacologically they have sedative property in rat saponin glycosides. Different parts of *Z. jujuba* that is stem, leaf and seeds also contain glycosides. The saponins isolated from the seeds of *Z .jujuba* such as jujubosides A, A1, B, B1 and C and acetyljujuboside B and the protojujubosides A, B and B1. Ziziphin, a saponins was isolated from the dried leaves of *Z. jujuba* [27]. It is chemically 3-O - a - L- rhamnopyranosyl (1-2) - a - arabinopyranosyl 20- O- (2, 3)- di -O-acetyl - a - L-rhamnopyranosyl jujubogenin. Some other constituents were also excreted from constituents *Z. jujuba* stems and leaves. It was assigned chemically as

3-O- ((2-O- alpha - D - furopyranosyl - 3-Obeta - D -glucopyranosyl) - alpha - L - arabinopyranosyl) jujubogenin. They are being widely researched for cholesterol control and cancer prevention as mentioned by Ogihara [29]. Same compound is also reported by Sharma and Kumar in another species i.e Z. *mauritiana.* Saponins revealed the anxiolytic, hemolytic, sedative adjuvant and sweetness inhibiting activities [27]. Jujuboside A (JuA) is also known to be a non competitive inhibitor of calmodulin and is thought to be linked to its sedative properties [161-164].

Flavonoids

Sedative flavonoids such as Swertish and spinosin were isolated from fruit and seeds of *Z. jujuba* such as 6'''-feruloylspinosin, Puerarin, 6'''-feruloylisospinosin, apigenin-6-*C*-b-D glucopyranoside, isovitexin- 2''-*O*-b-D-glucopyranoside and isospinosin [35]. The other flavonoids are Quercetine 3-*O*-rutinoside, Quercetine 3-O –robinobioside, Quercetine 3-*O*-b-D-xylosyl-(1→2)- α -L-rhamnoside, Quercetine 3-*O*-β-D-xylosyl-(1→2)- α -L - rhamnoside-4'-O-a-L-rhamnoside,Quercetine 3-*O*-α-L-arabinosyl-(1→2)-α -L-rhamnoside Quercetine 3-*O*-β-D –galactoside, Kaempferol 3-O robinobioside and Kaempferol 3-O-rutinoside and Quercetine 3-*O*-β-D-glucoside, 3', 5'-Di-C-β-D-glucosylphloretin. Some of the representative flavonoids are discovered [24, 34] a new flavonoid isolated named zivulgarin. It is chemically 4-beta-D-glycopyranosyl swetisin [165,166].

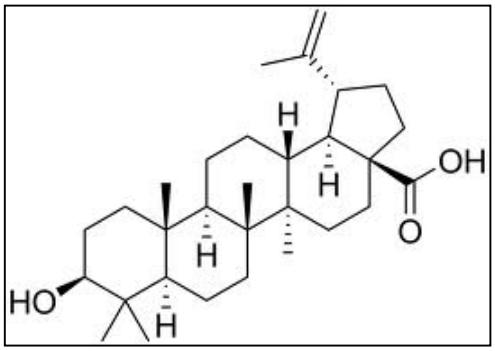
Terpenoids

The triterpenoic acids have been isolated from the fruits of Z. *jujuba.* Some of them are alphitolic acid, colubrinic acid, 3-O-transp coumaroylalphitolic acid, 3-O cis-pcoumaroylalphitolic acid, 3-O-trans-pcoumaroylmaslinic acid, 3-O-cis-p-coumaroylmaslinic acid, betulonic acid, oleanolic acid, oleanonic acid, zizyberenalic acid and betulinic acid. Triterpenoic acids have also been extracted from roots of Z. *mauritiana*. Betulinic acid, Betulin, ursolic acid, Ceanothic acid and 2α-hydroxyursolic acid are triterpenes. Some of them have anticancer and anti-HIV properties. Three triterpene esters were isolated viz. ceanothic aciddimethyl ester, 2-*O*-protocatechuoyl alphitolic acid and caffeoyl alphitolic acid [167-170].

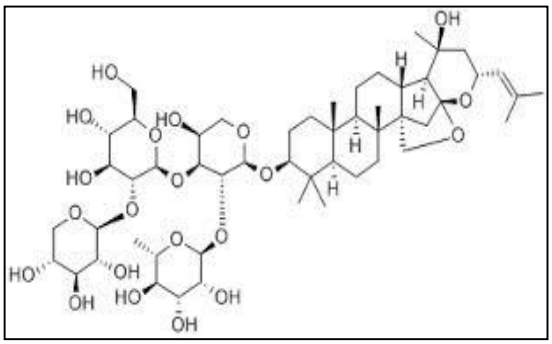
Phenolic Compounds

Phenolic compounds were isolated from the extract of fruit of *Z. jujuba.* Betulinic acid is widely distributed in all parts of *Z. jujuba*. It is having pentacyclic triterpenoid ring structure. It also demonstrated selective cytotoxicity against a number of specific tumour types [166, 171, 172].

2.5.7) Structures of important chemical constituents: [154]



Betulinic acid



Jujuboside

2.5.8) Pharmacological Activity:

Sedative, Hypnotic and Anxiolytic effect

The seeds and leaves of many *Ziziphus* species have been found to have hypnotic, anxiolytic and sedative effects. Zizyphus species reduces anxiety and induces sleep. They are known to depress activity of the central nervous system. It was produced sleep but was not muscle relaxant or anticonvulsant. Jujuboside- A showed inhibitory effect on rat hippocampus. A polyherbal substance containing seed extract of *Z. jujuba* having anxiolytic effects in mice. Both alkaloids sanjoinine A and nuciferine were isolated from fruits which prolonged the sleeping time produced by hexobarbital. When heat was given to sanjoinine, it produced an isomer of even greater sedative effect [173-175].

Sweetness inhibitors

Some triterpenoid sweetness inhibitors were isolated from the leaves of *Z. jujuba.* The leaves extract have been found to suppress sweet taste sensation in rat, hamster and fly *(Pharma regina)*. Anti-sweet substances isolated from *Z. jujuba* included jujuboside B from the leaves, jujubasponins II, III, IV, V and VI from the leaves and seeds and ziziphus saponins I-III from dried fruit. Ziziphin and jujubosaponins II and III were the only anti-sweet saponins from this plant with acyl groups. They were up to 4 times more active in suppressing the sweet taste of sucrose than the other anti-sweet constituents and thereby decreasing obesity in diabetic or overweight people. Ziziphin is a saponin isolated from *Z. jujuba* which suppressed the sweetness induced by D-fructose, D-glucose, glycine, stevioside, sodium saccharin, naringin dihydrochalcone and aspartame. It however showed no suppressive effect on the bitter

taste of quinine and sour taste of hydrochloric acid indicating that ziziphin is highly specific to sweet taste [46]. Ziziphin was also found to inhibit the sweet taste receptors in humans. When ziziphin was comparrd with known gymnemic acids, it showed dissociation of ziziphins from taste receptor membranes and/or inactivation in the membrane. It may be much faster than with gymnemic acids [163, 176-178].

Cancer (chemotherapy)

The triterpenoic acids were extracted from *Z. jujuba* which were tested against tumour cell lines for *in-vitro* cytotoxicities assay. The lupine type triterpenes showed that it had high cytotoxic activities. The cytotoxic activities of 3-O-p- coumaroyl alphitolic acids were found to be better than those of non-coumaroic triterpenenoids. So from this results one can concluded that coumaroyl moiety at the C-3 position of the lupine type triterpene may play significant role in increasing cytotoxic activity. The betulinic acid was extracted from *Z. jujuba* and *Z. mauritiana* which showed selective toxicity against cultured human melanoma cells. It is currently undergoing preclinical development. It may also be effective against other types of cancer also such as small and non small cell lung, ovarian, cervical and head and neck carcinomas. Betulinic acid induces apoptosis in sensitive cells in a p53- and CD95-independent fashion [161, 171, 172, 179, 180].

Antimicrobial activity

Antifungal effects of *Z. jujuba* in ethanol extract of the root showed significant inhibitory activity on fungi *Aspergillus flavus, A. niger Candida albicans, C. tropicalis,* and *Malassezia furfur*. In addition, the extract of root bark of *Z. jujuba* showed antibacterial property against more than 20 bacteria. Leaf extracts of *Z. mauritiana* were also found to exhibit antibacterial activity against *Pseudomonas species., Escherichia coli* and *Klebsiella species, Bacillus subtilis and Proteus vulgaris* when acetone and methanol extracts were used. Betulinic acid was isolated from stem bark of *Z. jujuba* which was retarded the progression of HIV 1 infection i.e. antiviral activity [181-184].

Antiulcer activity

An antiulcer property of *Z. mauritiana* leaf extracts (ZJE) was reported in rat. This extract possesses significant dose-dependent antiulcer property. This activity can be attributed by its antisecretory and cytoprotective action [185].

Anti-inflammatory and antispasmodic effect

"Huangqin Tang", the Chinese market formulation which contains the fruit of *Z. jujuba.* It showed marked antispastic or antispasmodic effect and significant anti-inflammatory activity. Another verity *Z. mauritiana* leaf extracts were found to possess significant anti inflammatory activity against carrageenan-induced rat paw edema [186, 187]

Antiallergic activity

The anti-allergic activity of the aqueous extracts of leaves of Z. *jujuba* was measuring its inhibitory effect *in- vitro* on hyaluronidase activation by bovine testes. Experimental data showed that *Z. jujuba* having strong anti-allergic activity [188].

Permeability enhancement activity

Delivery of certain classes of drugs such as peptides creates problems in transportation across cell membranes and subsequent diminished bioavailability. To overcome this barrier, permeability enhancers can be used to aid the passage of drugs across cell membranes. To assess the permeability enhancing activity of *Z. jujuba,* an aqueous extract of seeds was compared to two members of a known series of permeability enhancement agents belonging to the alkyl glycosides [189].

Cognitive activities

Oleamide was isolated from Z. *jujuba* extract. It could be a useful chemo-preventative agent against alzheimer's disease. The methanolic extract of Z. *jujuba* showed 34.1 % activation effect on choline acetyltransferase *in vitro* which was an enzyme that controls the production of acetylcholine. So it appears to be depleted in the brains of Alzheimer patients. The active ingredient was found to be cis-9 octadecenoamide (oleamide) which showed 65% activation effect using sequential fractionation [190].

Antifertility/contraceptive property

The ethyl acetate extract of *Z. jujuba* bark showed antisteroidogenic property so it produced fertility in adult female mice. It was found that it arrest the normal estrus cycle of adult female mice at diestrus stage. Hence it reduced the weight of ovaries significantly. When extract of *Z. jujuba* given to mice, it did not alter the biochemical estimations of whole blood and serum and hematological profiles. Ovarian steroidogenisis and normal estrus cycle were restored after withdrawal of extract

treatment. Anti-fertility properties of crude extracts were found to be reversible in rat [191].

Hypotensive and Antinephritic effect

In the kidney tissues of rats, *Ziziphus jujuba* has been found to stimulate nitric oxide release *in vitro,* in cultured endothelial cells and *in vivo.* By increasing renal blood flow, *Z. jujuba* may contribute to its antinephritic (reduction of inflammation of the kidney) and hypotensive (reduction of blood pressure) action [192].

Cardiovascular activity

A neo-lignan isolated from *Z. mauritiana* leaves was found to increase the release of endogenous prostaglandin I2 (the most potent natural inhibitor of platelet aggregation yet discovered and a powerful vasodilator) from the rat aorta by up to 25.3 % at 3 µg/ml [193].

Immunostimulant effects

The leaf extract of *Z. jujuba* was found to stimulate phagocytic, chemo tactic and intracellular killing potency of human neutrophils at the concentration ranging from 5-50 µg/ml [194].

Antioxidant effects

Recently, a comprehensive and an exhaustive account on 70 antioxidant Korean medicinal plants have been reported and they confirmed *in vitro* antioxidant effect of Z. *Jujube* [195, 196].

Wound healing activity

In the book of "Herbal Drugs",it is reported that the root of *Z. jujuba* was used as a wound healer. There were not much experimental data on wound healing activity of the root of *Z. jujuba* available in literature. The root of *Z. jujuba* was used in the form of ointment at the dose of 0.5% to 1% in rat models [197-199].

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