CHAPTER 2

REVIEW OF LITERATURE
REVIEW FOR COMMIPHORA WIGHTII (ARN.) BHANDARI

Commiphora wightii (Arn.) Bhandari (Burseraceae) plant is known as Indian bdellium. It is distributed in the arid, rocky tracts of Rajasthan, Gujarat, and Maharashtra states of India, and the Sindh and Baluchistan States of Pakistan. In Gujarat, this species is mainly found in Kachchh and in some parts of Saurashtra regions (Sabnis and Rao, 1983; Shah, 1978). Earlier the plant was found abundant in Rajasthan and Gujarat but currently threatened because of significant declines in population sizes (IUCN, 2004) due to faulty extraction methods employed by traditional guggul resin collectors. It came in vulnerable list in the 1997 IUCN Red List as vulnerable (VU) and in the 2004 IUCN Red List as data deficiency (DD) (IUCN, 2004). The Government of India has recently banned the export of the gum (IUCN, 2004), due to its high market price in international trade.

Generally the gum resin is collected by tribal people using traditional tapping methods involving making several deep incisions on the stem to extract the maximum amount of gum. They then apply a paste around the incision consisting of horse or wild ass urine, oleo gum resin and copper sulphate. Whilst this crude method increases the amount of gum three to four times over that obtained under normal tapping procedures, the shrub becomes subsequently unfit for tapping for the next couple of years and ultimately plants may die due to the injurious effect of copper sulphate (Kshetrapal and Sharma, 1993). It is now believed that such tapping methods to increase gum yield causes mortality of plants.

Guggul gum is a source of guggulsterones and has many medicinal properties. It lowers hepatic cholesterol levels by acting as an antagonist of the FXR bile acid receptor, important in metabolism of cholesterol (URIZAR, 2002). It takes 8 to 15 years to become commercially exploitable through tapping and yields 700 to 900 g resin. After which the plant invariably dies (Sabinsa Corp., 2000). Moreover, seeds are a result of apomixes so their formation is very irregular. Apomixis is non-pseudogamous (Gupta et al., 1996, Gupta et al., 1998). According to one report, the germination rate is as low as 1.4% (Yadav et al., 1999) and it is a slow growing species (Soni, 2010). Hence, natural regeneration rate of this species is very low.
For centuries, guggul has been used extensively by Ayurvedic physicians to treat a variety of afflictions, including arthritis, inflammation, bone-fractures, obesity and disorders of lipid metabolism. It provides ‘guggul’, an oleo gum resin who’s medicinal and curative properties are mentioned in the classic Ayurvedic medical text, the Sushruta Samhita 3000 years ago. The plant has become endangered because of over exploitation for its gum-resin, associated with slow growth of the plant, poor seed set and excessive tapping for gum-resin, which causes mortality of the plant. Gum-resin yield guggulsterones effective against high blood cholesterol and lipids.

Guggul was first introduced to the scientific world by an Indian Medical Researcher, G. V. Satyavati, in 1966 (Deng, 2007). In 1986, guggul was approved for marketing in India as a hypolipidaemic drug (Deng, 2007). Guggul gum is pharmacologically active in controlling rheumatoid arthritis, obesity and peptic ulcer (Atal et al., 1975). The pharmacological and clinical studies on its crude drug constituents and various extractives have revealed its significant hypocholestermic, hypolipidimic, antiinflammatory, antirheumatic and antifertility activities (Nityanand and Kapoor, 1971 a, b; Kapoor et al., 1979; Kakrani, 1981a,b; Tajuddin et al., 1994; Singh et al., 2001). Guggulsterone – Z and E, the active constitute of resin are responsible for lipid lowering properties in human blood (Satyavati et al., 1969; Tripathi et al., 1968; Singh et al., 1994). Despite having so many activities no reports found on the antibacterial activity of guggul gum up to structural identification of active compound. It is also reported for the treatment of thrombosis (Tripathi et al., 1968) and chronic bronchitis (Sinha et al., 2001), nodulocystic acne (Thappa et al., 1994), spongy gums, chronic tonsillitis and teeth car-ries (Raghunathan et al., 1999).

SOIL ANALYSIS

Soil is one of the factors affecting the growth of the plants. It has been found that variation in various parameters like Chloride, magnesium, phosphate, calcium etc does affect the growth of the plant. Penetration resistance has been found to have a profound effect on wheat yield by Oguz et al., 2012. Similarly a high adverse effect of soil parameters on spring wheat spike number was reported in northeastern China by Zhang et al., 2006.
Geographical variation leads to variation in soil characteristics like calcium, sodium, magnesium, chloride and sulphate has been noted by Sehgal, 1980 in different samples collected from IRAQ. Yadav and Girdhar (1981) reported increased dispersion of clay particles and reduced hydraulic conductivity when the Mg/Ca ratio was increased at a given SAR and electrolyte concentration of the leaching water. Studies by Rowell and Shainberg (1979) and Alperovitch \textit{et al.}, (1981) similarly have shown that magnesium has a specific effect on clay dispersion and loss of hydraulic conductivity of non-calcareous soils. In some studies, the presence of calcium chloride has caused a reduction in nitrate uptake as well as nitrate reductase activity. This action is attributed to the role of chloride in plants (Richharia \textit{et al.}, 1997). Hence the site selected for the sample collection were analyzed for the presence of basic components like chloride, calcium, magnesium, COD and phosphate and variation in their levels were recorded. The soil parameters of the forest and non-forest area from where the plants were collected will may change the \textit{in vitro} potential of the plant and it will also alter the phytochemical profiling of the plant of the two different regions.

TISSUE CULTURE AS A CONSERVATION STRATEGY

Tissue culture technology is a powerful tool for the conservation and rapid multiplication of many threatened plant species (Fay, 1992). It has been particularly useful for the conservation and rapid propagation of valuable, rare and endangered medicinal species. The plant is an important medicinal plant and is used in large number of ayurvedic preparations to treat variety of diseases. Identification and preclinical/clinical development of novel antiangiogenic agents continues to be a topic of intense research (Dhanabal \textit{et al.}, 2005; De Smet \textit{et al.}, 2006). \textit{In vitro} raised callus are being widely used. Hopgood and Ferrel (1977) cultured shoot apices of M.26 root stock on MS medium supplemented with BA and reported axillary shoot proliferation. It was followed by a report published by Huth (1978) who observed that BA (8.9µM) and NAA (1.1µM) were best for the establishment of very small (8.9mm) ‘Jonathen’ meristem tips and reduced NAA concentration (0.3µM) favored optimal shoot growth. Rooting (70%) was achieved using NAA (5.4 µM) and results improved with GA3 (0.3µM). To overcome difficulties in acclimatizing the rooted shoots he developed a successful technique of grafting the \textit{in vitro} produced shoots into apple seedlings in the green house. The callus was raised from explants of cotyledonary leaf and
root segments of *Carthamus tinctorius* (Rani *et al*., 1996) and *Allium sativum* (Myers and Simon, 1998) were efficient for differentiation.

Mei-Chun Lu (2002) succeeded in obtaining plantlets by using axillary buds from 15 year old tree of *Morus latifolia*. Optimum shoot multiplication was obtained by using MS medium supplemented with 2% fructose and (2 mg/l) BAP. A high frequency rooting 85% was achieved using half strength MS medium supplemented with 2% fructose and (1mg/l) BAP. A rapid and efficient propagation system was developed, using (2mg/l) BAP, 2% fructose and (1.0 mg/l) IBA in this economically important *Morus* species. With a slight increase or decrease in hormonal concentrations a decline in shoot as well in root number was observed.

Zong *et al*., (2000) developed a micropropogation system for mass propagation of „Fargo a newly released cultivator of Asian white birch (*Betula platyphylla*). Shoot tips from the mature, 7-year-old tree were established on 75% strength Murashige and Skoog medium supplemented with (0.1 μM) benzyladenine (BA), solidified with (6.5 g/liter) agar, and cultured at 24 °C. Microshoots were rooted *in vitro* or *ex vitro* followed by establishment in the greenhouse. A system to regenerate plantlets from leaves of aseptically cultured shoots was also developed. The generated shoots proliferated on the micropropagation medium were divided and the resulting shoots were rooted *ex vitro* and acclimated in greenhouse conditions.

Sivanesan (2007) compared different media (MS, SH and B5) for the shoot multiplication from the shoot tip explants of mature plants of *W. somnifera* L. MS medium was found superior to SH and B5 medium. Similar observation was made in *Eclipta alba*L. (Baskaran and Jayabalan, 2005). Liang and Keng (2006) developed a protocol for a rapid production of *P. niruri* L plantlets using nodal segments. Rapid and efficient propagation of *P. niruri* L. using shoot tip culture for providing a better source for continuous supply of plants in the manufacturing of drugs (Karthikeyan *et al*., 2007). Kalidass and Mohan (2009) developed an efficient micropropagation protocol for the medicinal plant *P. urinaria* Linn. using nodal segment for axillary shoot proliferation. Callus cultures were established from the immature stem of *C.wightii* which was then optimized for the production of anthocyanin (Dass *et al*., 2008). The selected tree of
C. wightii were micropropagated through forced axillary branching on Murashige and Skoog's (MS) medium supplemented with benzyladenine (BA) and kinetin which were later subjected to shoot elongation and root development later establishing successfully in the soil (Durga et al., 1993). In situ conservation of C. wightii in Rajasthan has been conducted where in C. wightii have been conserved in its natural habitat (in situ protection) through the development of protected area networks. Local communities (rural and tribal peoples) as well as local authorities were involved in the project. Proper education was provided on the value of medicinal succulent plants, and the need for their conservation and sustainable use (Vineet, 2008).

**PHYTOCHEMISTRY.**

There is an increasing interest in natural plant products as a source of new pharmaceuticals and other biologically-active compounds. Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. Ecology nowadays includes an increased amount of chemistry because the communication between a plant and its environment depends to a large extent on secondary metabolites (Zobel, 1986).

Medicinal plants continue to be a major source of drugs and natural products on the basis of their therapeutics (Lown, 1993) in virtually all cultures (Anwannil and Atta, 2006). The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases, diabetic, cancer (Dahanuka et al., 2000) and have often been used in folkloric medicine (Wang et al., 2007). In developing countries, the use of medicinal plants in the treatment of infectious disease and diabetic are rife and reasons include the high cost of effective drugs (Okeke et al., 1999). However, potential indigenous plants exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as first step towards the ultimate development of drugs (Odebiyi and Sofowora, 1998).

Ku et al., have isolated eight known compounds – oleanolic acid 3-acetate, apigenin, apigenin-7-O-α-D- (6”-p-coumaroyl)glucoside, cirsimaritin (5,4’-dihydroxy-6,7-dimethoxyflavone), mixture of β-sitosterol and stigmasteryl, and mixture of β-sitosterol-3-O-β-D-glucoside and stigmasteryl-3-O-β-D-glucoside from the n-hexane, chloroform and ethyl acetate fractions of the methanolic extract of the whole herbs of *Leucas mollissima* (Ku
et al., 2000). Saponins and their aglycone such as 3-methoxy-lanost- 9(11)-ene, diosgenin, yamogenin, 3-O-Dglucopyranosyl-24(S)-ethyl-22E-dihydrocholesterol, 3-O-D-glucopyranosyl-24(R)-ethyl-22E-dihydrocholesterol, dioscin were isolated from butanolic extract of *Brachiaria decumbens* by Viviane et al., (2002). Phytochemical screening of 10 Nigerian medicinal plants revealed of medicinally active compounds like alkaloids, tannins, flavanoids and glycosides (Edeoga et al., 2005). New steroidal saponin like diosgenin- 3-O-d-glucopyranosiduronic acid methyl ester, diosgenin, diosgenin 3-O-d-glucopyranosiduronic acid, diosgenin 3-O-a-rhamnopyranosyl-(152)-glucopyranosiduronic acid, diosgenin-3-O-a-l-rhamnopyranosyl-(152)-d-glucuroniduronic acid methyl ester from *Solanum lyratum* were isolated and characterized by Li et al., (2006). The phytochemical screening on qualitative and quantitative analysis shows that the leaves of the *Azadirachta indica*, *Centella asiatica*, *Embillica officinalis*, *Hibiscus rosasinensis*, *Imperata cylindrica* and *Moringa oleifera* are rich in alkaloids, tannins, saponins, terpenoids and flavanoids (Krishnaiah et.al., 2008). Dettrakul has isolated a new meroterpene from the root extract of *C.globifera* (Dettrakul et al., 2009).

Antioxidant flavonoids have been isolated from the flower of *Rhododendron yedoense*. One new flavonoid and three known flavonoids, quercetin-5-O-β-D-glucopyranoside, quercetin and quercitrin, were isolated from the butanol and ethyl acetate extracts of the plant. The new flavonoid was identified as myricitrin-5-methyl ether (Santosh, 2008).

Seasonal effects on the phytochemistry of the plant have been noted in various instances. To access the optimal cultivation time of *Vaccinium angustifolium*, a medicinal plant used for the treatment of diabetes, which produces a variety of phenolic metabolites with putative anti-diabetic activities, seasonal changes in the concentration of the phenolic compounds were examined and significant seasonal variations helped in indicating the collection time indicating optimal activity (McIntyre et al.,2009). A range of seasonal phytochemical variations was noted in *Calotropis procera* by Falguni, 2011. Seasonal and geographical variations in cellular characters and chemical contents in *Desmodium gangeticum* (L.) DC. – an ayurvedic medicinal plant was also noted (Jayanthy et al.,2013). The seasonal metabolic changes in *Phillyrea angustifolia* and the effects of them on the biological activity were noted. A variation in the iridoid content was noticed throughout the year by Scognamiglio et al., 2014.
Anthocyanin are natural pigments belonging to the flavonoid family and are responsible for the red, blue and purple colours of many fruits, berries and flowers. The golden yellow oleo-gum-resin is a complex mixture of over two dozen ketones, several phenolics, diterpenoids and sterols (Dev, 1989). Tender stems are red but anthocyanin contents are never reported in in vivo or in vitro cultures. A stable and high yielding cell line is an important component for scaling up of a process. In the study conducted by Dass et al., 2008, the selection of highly pigmented cells by visualization followed by medium manipulation resulted in a stable cell line, which on repetitive selection and subculture yielded high anthocyanin containing cell clusters.

With the discovery of the hypolipidemic activity for the gum resin, several chemical investigations have been undertaken. It was found that guggul resin is a complex mixture of various classes of chemical compounds such as lignans, lipids, diterpenoids and plant steroids. A waxy solid comprising a mixture of esters based on homologous long chain tetraols and ferulic acid with a unique structure, was identified in the benzene phase (Satyavati, 1991). In a more detailed study by Kumar et al., (1987), the stereochemistry of these tetraols was been determined. Guggulsterone is the resin of Mukul myrrh tree, a small, thorny plant found predominantly in the rocky, dry regions of India. Gum Guggulu, the yellowish resin produced in the stem of the Commiphora mukul tree is obtained by tapping the tree throughout the year. This resin is the source of the active components, Z-guggulsterone and E-guggulsterone (Antonio, 1999) as well as another eight steroids, acids, several aromatic acids, terpenes, and fatty acid alcohols. Some components seem to focus on cholesterol reduction activity, some are anti-inflammatories (Arora, 1972, Duwiejua, 1993) and some appear to work synergistically. Cell suspension cultures of C.wightii was produced on MS medium containing 0.5 mg/l 2,4-D and 0.25 mg/l kinetin which produced 5µg guggulsterone dry weight which was further produced in bioreactor for biomass production.

HIGH PERFORMANCE THIN LAYERED CHROMATOGRAPHY (HPTLC)

HPTLC is employed to analyze commercial samples to illustrate their application in qualititative (fingerprint) and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from the Herbal drugs and formulations. This technique will help to induce to come out uniform standard products, which will restore faith of
product and Alternative herbal medicine therapy (Kasar et al., 2013). HPTLC screening of *Costus Speciosus* (Koen.) showed the presence of different type of anthra-glycosides, arbutin, bitter principle, flavanoids, alkaloids, saponin, cardiac glycosides, essential oils, coumarins, phenols, carboxylic acids, valepotriates, anthraquinones, steroids and sterols (Aparna, 2010).

A simple high performance thin layer chromatographic (HPTLC) method was developed for the simultaneous determination of the pharmacologically important active curcuminoids viz. curcumin, demethoxy curcumin and bis-demethoxycurcumin in *Curcuma longa* L. The assay combined the separation and quantification of the analytes on silica gel 60 GF<sub>254</sub> HPTLC plates with visualization under UV and scanning at 425 nm (Paramasivam et al., 2008). Optimization of Extraction Conditions and HPTLC - UV Method was determined for Determination of Quinine in different extracts of *Cinchona species* Bark by Misra et al., in 2008.

By using analytical technique like HPTLC quantitative estimation of gallic acid, quercetin and rutin along with method validation was carried out in *Eruca sativa* by Sajeetha, 2010. Secondary metabolite β-sitosterol was quantified from *Caesalpinia bonduc* (Linn.)Roxb. by using HPTLC( Sunita et al., 2010). Flavanoid β-sitosterol from *Caesalpinia bonduc* (linn.) Roxb.Emend. Dandy & exell. seeds collected from different geographical locations have been found to vary in quantity by quantifying it using HPTLC (Shailajan et al., 2010). The HPTLC method was found to be best for quantitative and qualitative analysis of pentacyclic triterpenes by Rais et al., in 2010.

Chemical Fingerprinting Analysis of *C. wightii* and quantitation of E- and Z-guggulsterone was carried on by HPLC-UV method by Ahmed et al., 2010. No literature has been found on qualitative or quantitative analysis of *C. wightii* by HPTLC.