2.13 *Boswellia serrata*

2.13.1 Description

Fig.6 *Boswellia serrata* Plant

Boswellia serrata (frankincense) is a moderate-to-large branching tree, found in India, Northern Africa, and the Middle East (Fig.6). Strips of *Boswellia serrata* bark are peeled away, yielding a gummy oleoresin. *Boswellia serrata* contains oils, terpenoids, sugars and volatile oils. Up to 16% of the resins are essential oil, the majority being alpha-thujene and p-cymene. Four pentacyclic triterpene acids are also present, with beta-boswellic acid being the major constituent. Extracts of *Boswellia serrata* gummy exudate have been traditionally used in the Ayurvedic System of Medicine as an anti-arthritic, astringent, stimulant, expectorant, antiseptic etc (Altern Med Rev, 2008).

The main boswellic acids are (Fig.7):

- Boswellic acid
- β -Boswellic acid
- Acetyl-β-Boswellic acid
- Acetyl-Boswellic acid
- 11-Keto-β-Boswellic acid (KBA)
- Acetyl-11- Keto-β-Boswellic acid (AKBA)

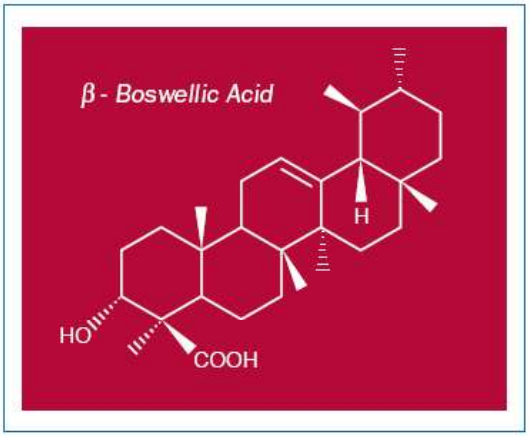

Fig.7 Structure of β-Boswellic Acid

2.13.2 Taxonomical Hierarchy (Pharmacog Rev, 2007)

(a) Classification

Kingdom	**:**	**Plantae**
Division	**:**	**Angiospermae**
Class	**:**	**Dicotyledoneae**
Order	**:**	**Geraniales**
Family	**:**	**Burseraceae**
Genus	**:**	***Boswellia***
Species	**:**	**s*errata* Roxb**

(b) Vernacular names

English	**:**	**Indian Olibanum or Indian frankincense**
Hindi	**:**	**Kundur, Salai**
Bengali	**:**	**Kundur, Salai**
Gujarati	**:**	**Dhup, Gugali**
Kannada	**:**	**Chitta, Guguladhuph**
Malayalam	**:**	**Parangi, Saambraani**
Tamil	**:**	**Parangi, Saambraani**
Telugu	**:**	**Phirangi, Saambraani**
Sanskrit	**:**	**Ashvamutri, Kundara, Shallkai**

(c) Part used **:** **Gum-resin**

(d) Botanical description: It is a deciduous medium sized tree with ash colored bark, peeling off in thin flakes, shoots are young and leaves pubescent. Leaves are compound, long, opposite, sessile variable in shape ovate or lanceolate, obtuse flowers in auxiliary racemes shorter than leaves. Calyx is pubescent outside. Petals are long and ovate and drupe is tringonous (Prakashanand Ayurveda Research Centre, 1992).

(e) Geographical distribution: The tree is common at the foot of the Western Himalayas, Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Bihar, Orissa, Andhra Pradesh and further south in the peninsular region. A large forest of this tree occurs in the khandesh and Nagpur-Wardha Divisions in Maharashtra, Khandwa-Nimar Division in Madhya Pradesh and Adilabad in Andhra Pradesh (Wealth of Asia, 1998).

2.13.3 Phytochemistry (Oleo-Gum-Resin)

Boswellia serrata contains essential oil, gum and resin. Its essential oil is a mixture of monoterpenes, diterpenes and sesquiterpenes. In addition phenolic compounds and a diterpene alcohol (serratol) is also found in essential oil. Gum portion of the *Boswellia serrata* consist of pentose and hexose sugars with some oxidizing and digestive enzymes. Resin portion mainly composed of pentacyclic triterpene acid of which boswellic acid is the active moiety (Kokate et al., 1999). A new lupane triterpene was isolated from fractionation of

methanol extract of *Boswellia serrata* resin together with Boswellic acids (Pardhy and Bhattacharya, 1978). The fraction on further purification with Ethanol- Hexane (1:1) yielded 3α- hydroxy- lupane 20 (Nicoletti and Forcellese, 1968) ene-24-oic acid whose structure (Fig.8) was confirmed by NMR and mass spectroscopy (Culioli et al., 2003).

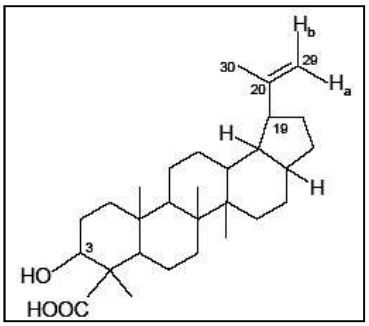

Fig. 8: Structure of boswellic acid (3α-hydroxy-lup-20(29)-en-24 oic acid) by NMR spectroscopy (Culioli et al., 2003)

HPLC analysis of Indian and African samples of *Boswellia serrata* gum-resin yielded 12 different pentacyclic triterpene acids (Gupta et al., 1998; Pardhy and Bhattacharya, 1978; Ganzera and Khan, 2001; Tawab et al., 2001; Schweizer et al., 2000). This method provides differentiation and standardization of gum-resin of different origin and gum-resin phytopharmaceuticals (Buchele et al., 2003). GLC studies with OV-17 and SE-30 (stationary phases) at 69-200°C yielded thirteen components including d-a-thujene (32%) as major and a-pinene, p-cymene and d-limonene as minor constituents in lower boiling fraction where as high boiling fraction yielded α-terpineol, methyl chavicol and four unidentified compounds (Kumar and Saxena, 1979). A highly sensitive reverse phase HPLC method for the detection and analysis of Boswellic acids in *Boswellia serrata* was developed by Ganzera et al in 2001 using acidic mobile phase at 60°C at 210 and 254 nm (Ganzera et al., 2001). Essential oil fraction from steam distillation of n-hexane extract of *Boswellia serrata* on GC-MS analysis revealed 33 components containing esters (62.1%), alcohol (15.4%), monoterpenes (9.9%) and diterpenes (7.1%) (Corsano and Nicoletti, 1977). This essential oil was found comprise of a-thujene, a-pinene, camphene, sabinene, b-pinene, myrcene, o-methylanisole, a-terpinene, hexyl acetate, p-cymene, 1-8-cineole, limonene, cis-b-ocimene, trans-b-ocimene, g-terpinene, 1-octanol, terpinolene, linalool, 1-decanol, terpinen-4-ol, a-terpineol, 1-octylacetate, bornyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, hexyl hexanoate, 1-decyl acetate, hexyl octanoate, isocembrene, cembrene, iso-incensole and incensole (Wahab et al., 1987). *Boswellia serrata* resin upon exhaustive successive extraction with n-hexane and chloroform followed by crystallization and subsequent studies on IR, NMR, Mass, melting point and

specific rotation parameters gave the presence of four pentacyclic triterpene acid i.e. β-Boswellic acid, 11-Keto-β-boswellic acid, Acetyl-β-boswellic acid and Acetyl-11-keto-β-boswellic acid (Pardhy and Bhattacharya, 1978).

Tetracyclic triterpene acids E, F, G and H from resin of *Boswellia serrata* were obtained from acidic fraction of n-hexane extract by column chromatography using silica gel-G with n-hexane and ethyl acetate as eluent with following structures as determined by IR, NMR and Mass studies (Buchele et al., 2003).

2.13.4 Mechanism of Action

A number of *in-vitro* molecular targets of boswellic acids have been reported, such as 5-lipoxygenase, leukocyte elastase (Safayhi et al., 1997) or topoisomerase 1 and 2 (Syrovets et al., 2000). In an *in vitro* study of the effects of beta-boswellic acid on the complement system, the extract demonstrated a marked inhibitory effect on both the classical and alternate complement pathways (Knaus and Wagner, 1996). Boswellic acids have been found to interfere with the key enzyme for the biosynthesis of leucotriens, the 5-lipoxygenase. The enzyme 5-lipoxygenase catalyses the formation of leucotriens from arachidonic acid in inflammatory processes. Leukotriens act as mediators in inflammation causing chemotaxis, chemokinesis and release of phagocyte enzymes. Boswellic acids selectively inhibit the 5-lipoxygenase (Ammon, 2002) and reduce leukotrien biosynthesis in a concentration-dependent manner (Glaser et al., 1999). Among the investigated boswellic acids, Acetyl-11-Keto-β-Boswellic acid showed the strongest inhibitory efficacy. *In vitro* testing reveals boswellic acids, isolated from the gum resin of *Boswellia serrata*, in a dose-dependent manner block the synthesis of proinflammatory 5-lipoxygenase products, including 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B4 (LTB4) (Ammon et al., 1991), which cause bronchoconstriction, chemotaxis and increased vascular permeability (Robertson, 1987). Other anti-inflammatory plant constituents, such as quercetin, also block this enzyme, but they do so in a more general fashion as an antioxidant; whereas, boswellic acids seem to be specific inhibitors of 5-lipoxygenase (Safayhi et al., 1992; Ammon et al., 1996). Animal studies performed in India show ingestion of a defatted alcoholic extract of *Boswellia serrata* decreased polymorphonuclear leukocyte infiltration and migration, decreased primary antibody synthesis (Kaul et al., 1988; Sharma et al., 1989) and almost totally inhibit the classical complement pathway (Wagner, 1989). An investigation of *Boswellia serrata*'s analgesic and psychopharmacological effects noted marked sedative and analgesic effects in

animal models (Menon and Kar, 1971). *Boswellia serrata* inhibits human leukocyte elastase (HLE) which may be involved in the pathogenesis of emphysema. HLE also stimulates mucus secretion and thus may play a role in cystic fibrosis, chronic bronchitis and acute respiratory distress syndrome (Rall et al., 1996; Safayhi et al., 1996). Boswellic acids and triterpenoids from *Boswellia serrata* also have an inhibitory and apoptotic effect against the cellular growth of leukemia HL-60 cells (Shao et al., 1998; Bhushan et al., 2007; Huang et al., 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) can cause a disruption of glycosaminoglycan synthesis and accelerating articular damage in arthritic conditions (Lee and Spencer, 1969; Palmoski and Brandt, 1979; Dekel et al., 1980; Brandt and Palmoski, 1984). An *in vivo* animal study examined *Boswellia serrata* extract and ketoprofen for effects on glycosaminoglycan metabolism and found significantly reduced degradation of glycosaminoglycans whereas ketoprofen caused a decrease in total tissue glycosaminoglycan content (Reddy et al., 1989).

2.13.5 Pharmacological Activities

A lot of research has currently been going on this plant due to its numerous medicinal properties and traditional usage worldwide. Alcoholic (ethanolic or methanolic) extracts of gum, resin and other parts of plant have been studied for various pharmacological activities. The current thrust area where this plant has shown promising actions includes:

Ileitis: Krieglstein et al in 2001, reported that acetyl-11-keto-β-boswellic acid (AKBA) isolated from *Boswellia serrata* functions on leukocyte-endothelial cell interactions in inflammatory bowel disease. It was observed that *Boswellia serrata* extract and both potencies of acetyl-11-keto-β-boswellic acid decreased rolling (up to 90%) and adherent leukocytes (up to 98%), attenuated tissue injury scores, and significantly reduced macroscopic and microscopic inflammation of the gut mucosa.

Ulcerative Colitis: In a study, *Boswellia serrata* extract (350 mg three times daily) was compared to sulfasalazine (1 g three times daily) in ulcerative colitis patients. Patients on *Boswellia serrata* extract showed better improvements than patients on sulfasalazine; 82% of *Boswellia serrata* patients went into remission compared with 75% on sulfasalazine (Gupta et al., 1997). Furthermore, 14 of 20 patients (70%) treated with *Boswellia serrata* gum resin went into remission compared to 4 of 10 patients (40%) treated with sulfasalazine (Gupta et al., 2001).

Crohn's Disease: Chemical mediators of inflammation were addressed in a clinical trial comparing a *Boswellia serrata* extract with mesalazine in the treatment of acute Crohn's disease. The Crohn's Disease Activity Index decreased significantly with both *Boswellia serrata* extract and mesalazine, hence *Boswellia serrata* extract proved to be as effective as the pharmaceutical (Gerhardt et al., 2001).

Asthma: In a study, effect of *Boswellia serrata* on bronchial asthma was studied with 40 patients treated with 300 mg of *Boswellia serrata* preparation thrice daily for six weeks, while another 40 patients took a placebo. Seventy percent of patients taking *Boswellia serrata* demonstrated significant disease improvement, measured by symptomatology and objective measures of lung and immune function (Gupta et al., 1998).

Anti-Arthritic Activity: Kimmatkar et al in 2003, conducted a double-blind, placebo-controlled trial, *Boswellia serrata* demonstrated beneficial effect on knee osteoarthritis. Thirty patients were given 1,000 mg *Boswellia serrata* daily and placebo in three divided doses for eight weeks. Patients in the *Boswellia serrata* group experienced a significant decrease in pain and swelling and increase in range of motion compared to placebo ($p < 0.001$) (Kimmatkar et al., 2003). In a double-blind, placebo-controlled, crossover study, *Boswellia serrata* in combination with ashwagandha, turmeric, and zinc was studied in osteoarthritis patients (Kulkarni et al., 1991). The treatment group experienced significant decreases in pain severity ($p<0.001$) and disability scores ($p<0.05$) compared to placebo. Non-Steroidal Anti-Inflammatory Drug (NSAID) dosage, however, decreased 5.8 percent in the treatment group and 3.1 percent in the placebo group (Sander et al., 1998).

Anticancer Activity: Tsukada et al in1986, on examining the alcoholic extract of *Boswellia serrata* for anti-carcinogenicity in mice with ehrlic ascites carcinoma and S-180 tumor, found inhibition of tumor growth by inhibiting cell proliferation and cell growth due to the interference with biosynthesis of DNA, RNA and proteins (Tsukada et al., 1986). Boswellic acid, 11-Keto-β-Boswellic acid and Acetyl-11- Keto-β-Boswellic acid showed anti-proliferative and apoptotic effect HT-29 on colon cancer cell and extrinsic pathway activation leading to apoptosis (Liu et al., 2002; Green, 1998; Green and Reed, 1998; Ashkenazi and Dixit, 1998). Although both 11-Keto-β-Boswellic acid and Acetyl-11- Keto-β-Boswellic acid increased the amount of cytoplasmic DNA histone complex in a dose dependent manner, the formation of this complex was high due to Boswellic acid. Topical

application of Boswellin (1.2-3.6 mg) with 5 nmol TPA twice daily for 16 weeks to mice previously treated with dimethylbenz-anthracene, caused 87-99% inhibition in the number of tumor/mice (Huang et al., 1997). As per Shao et al (1998) *Boswellia serrata* extract inhibit the synthesis of DNA, RNA and protein in HL-60 cells. Out of boswellic acids, Acetyl-11-Keto-β-Boswellic acid was most potent inhibitor and its inhibitory effect on DNA synthesis was irreversible (Huang et al., 2000). According to Hoernlein et al (2000) Acetyl-11- Keto-β-Boswellic acid caused reduction in thymidine incorporation and cell count in HL-60 and CCRFCEM cells. This effect was pronounced when Acetyl-11-Keto-β-Boswellic acid was cross linked with CD-95 receptor. Flow cytometric analysis of propidium iodide stained cells indicated apoptosis which was confirmed by G1 peak in Acetyl-11-Keto-β-Boswellic acid treated cells and by DNA laddering in DNA relaxation assay, Acetyl-11-Keto-β-Boswellic acid inhibited topoisomerase-1 from calf thymus at low concentration which in-turn induced apoptosis in HL-60 and CCRFCEM cell. Boswellic acid treatment to female Wistar rats inoculated with C-6 tumour cells not only showed significant reduction in brain tumor volume but also enhanced the survival time of animals in dose dependent manner (Hoernlein et al., 1999; Kaplan and Meier, 1958; Benda et al., 1968; Witkin et al., 1961). Boswellic acids induce concentration dependent inhibition of glioma cell proliferation and show anti-edema effect in glioblastoma patients (Boker and Winking, 1997). It was also revealed that boswellic acids induced apoptosis is protein synthesis dependent and not associated with free radical scavenging activity. Acetyl-11-Keto-β-Boswellic acid causes rapid inhibition of phosphorylation of ERK pathways impairing the motility of meningioma cells by impaired signal transduction and tumorigenesis thus causing cytotoxicity against meningioma cells (Park et al., 2002).

Anti-inflammatory Activity: *Boswellia serrata* has a long history of usage as an anti-inflammatory agent and the researchers all over the world are working hard to find out the mode of action of this medicinal plant. Studies on alcoholic extract of *Boswellia serrata* revealed anti-inflammatory activity in carrageenan induced paw edema in rat and mice; Dextran induced edema in rats and also in adrenalectomised rats (Winter et al., 1962; Winter, 1964; Srimal and Dhawan, 1971; Schultzer, 1935). Shrivastava et al in 2003 ascertained that the Boswellic acids exert their action by inhibiting the synthesis of 5-LOX products. They also inhibit topoisomerase, elasase and C-3 convertase enzymes (Shrivastava et al., 2003).

Muscle Relaxant Activity: The oleo-gum-resin obtained from the whole plant of *Boswellia serrata* revealed stimulatory effect on skeletal muscles and spasmogenic effect on smooth muscle of guinea pig ileum. According to an earlier report the essential oil of *Boswellia serrata* has selective action on biological tissues and its activity was not due to non specific action on cell membrane (Lis-Balchin and Hart, 1997).

Hepatoprotective Activity: It is well reported that alcoholic extract of *Boswellia serrata* causes hepatoprotection in galactosamine/endotoxin induced liver damage in mice which was reflected by reduced titer of SGOT, SGPT, aminotransferase and serum enzymes. According to Safayhi et al (1991), the hepatoprotection was most probably through inhibition of 5-LOX activity.

Hypoglycemic Activity: Herbal formulation containing *Boswellia serrata* oleo-gum-resin as one of the ingredient has been reported to produce significant anti-diabetic activity on diabetes mellitus in streptozocin induced diabetic rats where reduction in blood-glucose level was comparable to that of phenformin (Al-awadi et al., 1991).

Anti-diarrhoeal Activity: In a recent study *Boswellia serrata* extract was found effective in treating diarrhoea in patient with inflammatory bowel syndrome without causing constipation. It also found effective against acetylcholine, croton, castor oil and barium chloride induced diarrhoea by inhibiting contraction of intestinal smooth muscles (Borrelli et al., 2006).

Analgesic and Psychopharmacological Activity: Menon and Kar in 1971, found the non-phenolic fraction of *Boswellia serrata* showing sedative and analgesic effects. It produced reduction in motor activity and ptosis in rats. The fraction also potentiated secobarbitone induced hypnosis in rat (Menon and Kar, 1971).

Clastogenic Activity: Clastogenic effect of dietary supplements used in stress relief, memory enhancement and memory boost was demonstrated by Ghoshal et al in 2001 using Swiss Albino mice (Ghosal et al., 2001).

Immunomodulatory Activity: *Boswellia serrata* extract showed anti-anaphylactic and mast cell stabilizing or inhibiting mast cell degranulation activity in passive paw anaphylaxis and

induced mast cell degranulation (Pungle et al., 2003). Activity in Autoimmune Encephalitis of crude Acetyl-boswellic acid (ABA) inhibited ionophore stimulated release of leukotrienes from polymorphonuclear leukocytes (PMLNs). The pure drug was found more potent and even the intraperitoneal administration of this compound reduced the symptoms of autoimmune encephalitis (Wildfeurer et al., 1998).

2.13.6 Side Effects and Toxicity

Clinical trials were conducted on 175 patients who were suffering with musculoskeletal rheumatism inducing rheumatoid arthritis and ankylosing spondylitis of moderate to severe type 1-6 years duration in the age group of 10 to 50 years of either sex. None of these patients complained of any undesirable side effects at therapeutic doses (Pachnanda et al., 1982; Sharma et al., 1984; Singh and Atal, 1986). The acute LD_{50} in primates has been established at >2000 mg/kg for the plant (Atal, 1982).

2.14 *Withania somnifera*

2.14.1 Description

Withania somnifera, also known as ashwagandha, Indian ginseng, and winter cherry, has been an important herb in the Ayurvedic and indigenous medicinal systems for over 3000 years. Ashwagandha is a small, woody shrub in the Solanaceae family (Fig.9). The roots are the main portion of the plant used therapeutically. Historically, the plant has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent, and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia, chemopreventive and senile dementia. Clinical trials and animal research support the use of ashwagandha for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease. Ashwaganda is also used therapeutically as an adaptogen for nervous exhaustion, insomnia, and debility due to stress, and as an immune stimulant in patients with low white blood cell counts. The herb is termed a rasayana in Ayurvedic drug, which means it acts as a tonic for vitality and longevity (Altern Med Rev, 2004).

Fig.9 *Withania somnifera* Plant

The major biochemical constituents of ashwaganda root are steroidal alkaloids and lactones in a class of constituents called withanolides. Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A (Fig.10) and withanolide D (Elsakka et al., 1990).

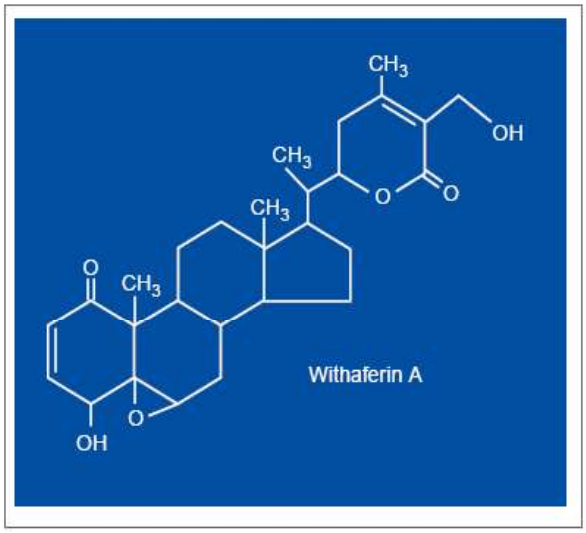

Fig.10 Structure of Withaferin A

2.14.2 Taxonomical Hierarchy (Rajeswara et al., 2012)

(a) Classification:

Kingdom	**:**	**Plantae**
Division	**:**	**Angiospermae**
Class	**:**	**Dicotyledoneae**
Order	**:**	**Solanales**
Family	**:**	**Solanaceae**
Genus	**:**	***Withania***
Species	**:**	***somnifera***

(b) Vernacular names:

English	**:**	**Indian ginseng, Winter cherry**
Hindi	**:**	**Asgandh**
Bengali	**:**	**Ashwagandha**
Gujarati	**:**	**Ghodaakun, Asuth**
Kannada	**:**	**Amangura, Sogadeberu**
Malayalam:		**Amukkiram, Pevetti**
Tamil	**:**	**Amkulan-kalang, Achuyagandhi**
Telugu	**:**	**Penneru gaddalu**
Sanskrit	**:**	**Ashvagandha**

(c) Part used : Roots and leaves

(d) Botanical description:

Withania somnifera is a small, erect, branched, evergreen, tomen-tose woody shrub that grows up to 150-170 cm tall and is found throughout the drier parts of India in waste places and on bunds. Roots are stout, fleshy and whitish brown in colour. Leaves simple, petiolate, elliptic-ovate to broadly ovate, entire, exstipulate, cunate or oblique, glabrous, up to 10 cm long, those in the floral region are smaller and opposite. Flowers are inconspicuous, greenish or lubrid-yellow, pedicellate, 4-6 mm in diameter, axillary, umbel-late cymes occurring in 5-25 clusters. Berries are small, globose, bright orange-red when mature, 5 mm in diameter, enclosed in the persistent calyx containing numerous seeds. Seeds are small, smooth, yellow, reniform, 2 mm long, 1.5-2 mm wide and 0.5 mm thick (Rajeswara et al., 2012).

(e) Geographical distribution:

In India, Ashwagandha is commercially cultivated as rain fed crop in Kota (Rajasthan); Bhanpura, Manasa, Neemuch, Jawad tehsils of Mandsaur district of Madhya Pradesh; Anantapur, Kurnool, Mahabubnagar, Warangal and Prakasam districts of Andhra Pradesh. Cultivation has also been initiated at few locations in Karnataka (Kattimani et al., 1999; Kattimani et al., 2001; Rajeswara et al., 2006; Sastry and Rajeswara, 2007).

2.14.3 Phytochemistry

Laboratory analysis has revealed over 35 chemical constituents contained in the roots of *Withania somnifera* (Rastogi and Mehrotra, 1998). The biologically active chemical constituents are alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X). *Withania somnifera* is also rich in iron. The roots of *Withania somnifera* consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (Panax ginseng) known as ginsenosides. Ashwagandha's withanolides have been researched in a variety of animal studies examining their effect on numerous conditions, including immune function and even cancer (Grandhi, 1994). Chemical analysis of Ashwagandha shows its main constituents to be alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine andanahydrine.

Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, with a six membered lactone ring (Padmawar, Anruta Herbals). Twelve alkaloids, 35 withanolides, and several sitoindosides from *Withania somnifera* have been isolated and studied. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. Further chemical analysis has shown the presence of the following: Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferiene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A-Y(Steroidal lactones) (Bone,1996; Elsakka et al., 1990).

2.14.4 Mechanism of Action

The major biochemical constituents of ashwagandha from which its primary medicinal properties emanate, are based upon the actions of certain steroidal alkaloids and steroidal lactones in class of constituents called withanolides (Budhiraja and Sudhir, 1987). These serve as important hormone precursors which the body is then able, as needed, to convert into human physiological hormones. If there is an excess of a certain hormone, the plant based hormone precursors occupy the so-called hormone receptor sites, without converting to human hormones, to block absorption. In this way, ashwagandha, like other adaptogenic tonic herb, is amphoteric and can serve to regulate important physiological processes, increasing or decreasing as needed. Ashwagandha is also considered to be an adaptogen, facilitating the ability to withstand stressors, and has antioxidant properties as well. Other studies have shown ashwagandha to have an immunostimulatory effect. Its leaves are used in Ayurvedic and Unani systems for treatment of tumors and tubercular glands (Chopra, 1994). A number of withanolide steroidal lactones have been isolated from the leaves of *Withania somnifera* (Glotter et al., 1973) and exhibit antibacterial, anti-fungal and antitumor properties (Devi, 1993). There are a number of reports elucidating the chemical and pharmacological properties of *Withania somnifera* (Nittala and Lavie, 1988; Kandil, 2009). Ayurvedic practitioners have used the roots of this plant for centuries with success as a tonic to increase vitality and longevity, as well as to treat health conditions as diverse as tumors and arthritis. Recent laboratory studies have begun to confirm what Ayurvedic practitioners have known for years – that *Withania somnifera* deserves attention as an herbal therapy to ease or even eliminate many of today's common health problems.

2.14.5 Pharmacological Activities

Anti-ageing actions: Bone in 1996, revealed in a double-blind clinical trial, ashwagandha improved Hemoglobin, red blood cell count and hair melanin. Serum cholesterol was decreased and nail calcium was preserved. Erythrocyte sedimentation rate decreased significantly and 71.4% reported improvement in sexual performance (Bone, 1996). Hormone regulation is directly or indirectly linked to the erythropoiesis which is the marker of ageing. Improved level of blood count explains the mechanism of hormone production in the body (Archives of internal medicine, 2006).

Immunomodulation and Hematopoiesis Activity: A series of animal studies show ashwagandha to have profound effects on the hematopoietic system, acting as an immunoregulator and a chemoprotective agent (Kuttan, 1996; Ziauddin et al., 1996). In a mouse study, administration of root extract from ashwagandha was found to enhance total white blood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group (Davis and Kuttan, 2000). Recent research suggests a possible mechanism behind the increased cytotoxic effect of macrophages exposed to *Withania somnifera* extracts (Iuvone, 2003). Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganisms and tumor cells. Iuvone et al in 2003, demonstrated *Withania somnifera* increased Nitric Oxide production in mouse macrophages in a concentration-dependent manner. This effect was attributed to increased production of inducible nitric oxide synthase, an enzyme generated in response to inflammatory mediators and known to inhibit the growth of many pathogens (Bogdan, 2001). Ashwagandha exhibited stimulatory effects, both *in vitro* and *in vivo*, on the generation of cytotoxic T lymphocytes, and demonstrated the potential to reduce tumor growth (Davis and Kuttan, 2002). The chemopreventive effect was demonstrated by ashwagandha, a significant decrease in incidence and average numbers of skin lesions were found. The chemopreventive activity is thought to be due in part to the antioxidant/free radical scavenging activity of the extract (Prakash et al., 2002). An *in vitro* study showed *Withania somnifera* inhibited growth in human breast, central nervous system, lung, and colon cancer cell lines comparable to doxorubicin. These results suggest *Withania somnifera* extracts may prevent or inhibit tumor growth in cancer patients, and suggest a potential for development of new chemotherapeutic agents (Jayaprakasam et al., 2003).

Anti-Anxiety and Anti-Depression Actions: In an animal study assessing the anxiolytic and antidepressive actions of *Withania somnifera* compared to a group administered the

benzodiazepine lorazepam for anxiolytic activity, and the tricyclic antidepressant imipramine for antidepressant investigation. Both the ashwagandha group and the lorazepam group demonstrated reduced brain levels of a marker of clinical anxiety (tribulin-an endocoid marker). *Withania somnifera* also exhibited an antidepressant effect comparable to that induced by imipramine in the forced swim-induced "behavioral despair" and "learned helplessness" tests (Bhattacharya et al., 2000). Other similar studies confirm these results, lending support to the use of *Withania somnifera* as an antistress adaptogen (Bhattacharya et al., 2001; Singh et al., 2001; Archana and Namasivayam, 1999; Dhuley, 2000).

Cardiovascular Protection Activity: Hypoglycemic, diuretic and hypocholesterolemic effects of *Withania somnifera* were assessed. A decrease in blood glucose comparable to a standard drug was observed. Significant increases in urine volume, urine sodium, and decreases in serum cholesterol, triglycerides, and low-density lipoproteins were seen (Andallu and Radhika, 2000).

Hypothyroidism Actions: Animal studies reveal *Withania somnifera* has a thyrotropic effect (Panda and Kar, 1999; Panda and Kar, 1998). An aqueous extract of dried *Withania somnifera* root was given to mice and serum was collected for T3 and T4 concentrations. Significant increases in serum T4 were observed, indicating the plant has a stimulatory effect at the glandular level. *Withania somnifera* extract significantly decreased lipid peroxidation in the liver homogenate and significantly increased catalase activity, promoting scavenging of free radicals that can cause cellular damage. These results indicate *Withania somnifera* may be a useful botanical in treating hypothyroidism.

Antioxidant Actions: The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species (Halliwell and Gutteridge, 1989). Since traditional Ayurvedic use of *Withania Somnifera* has included many diseases associated with free radical oxidative damage, it has been considered likely the effects may be due to a certain degree of antioxidant activity. The active principles of *Withania somnifera*, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels in the rat brain frontal cortex and striatum. Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting

degenerative effects. An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue. This implies that *Withania somnifera* does have an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties (Bhattacharya et al., 1997).

Anti-inflammatory Activity: *Withania somnifera* and withanolides being a potent inhibitor of pro-inflammatory transcription factors NF-kB and AP-1 holds promise as a novel agent for the treatment of inflammatory diseases (Kaileh et al., 2007).

Adaptogenic Activity: *Withania somnifera* possess a potent antistressor effect and reported to alleviate stress induced changes and provide cardioprotection like properties ascribed to adaptogens like *Panax ginseng*. It also increases heart weight and glycogen in myocardium and liver indicating intensification of the anabolic process and enhances the duration of contractility as well as coagulation time (Dhuley, 2000; Dhuley, 1998).

Anti-Atherogenic Activity: *Withania somnifera* has profound hypocholesteremic and antiatherogenic activity (Visavadiya and Narasimhacharya, 2007; Hemalatha et al., 2006; Andallu and Radhika, 2000; Mary et al., 2003). Mary et al in 2003 demonstrated the antiatherogenic activity of Caps HT2, a botanical medicine comprising of several plants including *Withania somnifera* against vascular intimal damage and atherogenesis which leads to various types of cardiovascular diseases. The formulation scavenges free radicals, inhibited lipid peroxidation, delayed the plasma re-calcification time and enhanced the release of lipoprotein lipase enzyme. The formulation altered atherogenic index and reduced the body weight with rise of high density lipoprotein cholesterol levels in hyperlipidemic rats. In a clinical study, a formulation of *Withania somnifera* used as an adjunct to conventional anti-ischemic drugs has been found to reduce total cholesterol, triglycerides and increase high density lipoprotein cholesterol in the post myocardial infarction patients (Dwivedi et al., 2000). The hypolipidemic and antiatherogenic potential is an additional benefit of its usefulness in cardiovascular diseases.

Positive Inotropic Activity: *Withania somnifera* reduced blood pressure due to autonomic ganglion blocking action and myocardial depressant effects as well as positive inotropic and chronotropic effects (Budhiraja et al., 1983). The alkaloids had prolonged hypotensive, bradycardiac and respiratory-stimulant action (Malhotra et al., 1981).

Hypoglycemic Activity: Hyperglycemia is a major risk factor of cardiovascular diseases. *Withania somnifera* favorably alters blood and urine glucose levels, glycated hemoglobin and liver enzymes in diabetic rats (Andallu and Radhika, 2000; Udayakumar et al., 2009).

Chronic Stress: In a rat model of chronic stress *Withania somnifera* and *Panax ginseng* extracts were compared for their ability to attenuate some effects of chronic stress. Both botanicals were able to decrease the number and severity of CS-induced ulcers, reverse CS-induced inhibition of male sexual behavior, and inhibit the adverse effects of CS on retention of learned tasks. Both botanicals also reversed CS-induced immunosuppression, but only *Withania somnifera* extract increased peritoneal macrophage activity in the rats. *Withania somnifera*, however, has an advantage over *Panax ginseng* in that it does not appear to result in ginseng-abuse syndrome, a condition characterized by high blood pressure, water retention, muscle tension, and insomnia (Bhattarcharya and Muruganandam, 2003).

2.14.6 Side Effects and Toxicity

Withania somnifera is generally does not show adverse effect when taken in the crude form in dosage range (Aphale et al., 1998). Large doses have been shown to cause gastrointestinal upset, diarrheoa and vomiting. An important consideration when investigating the medicinal properties of an unknown compound is diligent evaluation of its potential for harmful effects, usually evaluated through toxicity studies. For *Withania somnifera*, no systematic study was found which included acute, sub-acute, sub-chronic or chronic toxicity of *Withania somnifera* root powder, whole plant powder, or different extracts of the plant (e.g., water, alcohol, petroleum ether, purified alkaloids, and glycosides). Although one preliminary toxicity study of *Withania somnifera* was conducted, it was of insufficient quality to support its findings as too few animals were used, body weight data was not collected, and survival data was not reported (Arseculeratne et al., 1985). In one central nervous system study, a suspension of ashwagandholine (total alkaloids from the roots of *Withania somnifera*) prepared in propylene glycol using two-percent gum acacia as suspending agent was used to determine acute toxicity (Malhotra et al., 1965). The acute LD_{50} was 465 mg/kg in rats and 432 mg/kg in mice. In one long-term study, *Withania somnifera* was boiled in water and administered to rats in their daily drinking water for eight months while monitoring body weight, general toxicity, well being, number of pregnancies, litter size, and progeny weight (Sharma et al., 1986). The estimated dose given was 100 mg/kg/day. In the second part of the study, the estimated dose was 200 mg/kg/day given for four weeks while monitoring body

temperature, body weight, cortisol value in heparinized plasma, and ascorbic acid content of the adrenals. The liver, spleen, lungs, kidneys, thymus, adrenals, and stomach were examined histopathologically and were all found to be normal. In the four-week study, the weight gained in the treated group was comparable to that of the control group (Sharma et al., 1986). The results concluded the decoction of *Withania somnifera* promoted growth especially during the active growth period and helped produce healthier progeny (Mishra, 2000).