CHAPTER II

LITERATURE REVIEW

Asparagus racemosus

A. racemosus is among 300 species classified in the family Asparagaceae. Its medicinal usages have been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicines such as Ayurvedic medicines, Tibetan medicines and early Roman medicines (Bopana, et al., 2007). Steroidal saponins and sapogenins were commonly found in various parts of the plants in this genus (Bopana, et al., 2007).

1. *A. racemosus*: the plant species

A. racemosus Willd. (family Asparagaceae), is commonly called Satavari, Satawar or Satmuli in Hindi; Satavari in Sanskrit; Shatamuli in Bengali; Shatavari or Shatmuli in Marathi; Satawari in Gujarati; Toala-gaddalu or Pilli-gaddalu in Telegu; Shimaishadavari or Inli-chedi in Tamil; Chatavali in Malayalam; Majjigegadde or Aheruballi in Kannada; Kairuwa in Kumaon; Narbodh or atmooli in Madhya Pradesh; and Norkanto or Satawar in Rajasthan (Anonymous, Wealth of India, 1987). In Thai, this plant is called Samroyrak, Samsib, Sawnoyroyphau.

The plant was commonly found in tropical and subtropical forests such as India Australia and Thailand. The plant is a spinous under-shrub, with tuberous, short rootstock bearing numerous succulent tuberous roots (30–100 cm long and 1–2 cm thick) that are silvery white or ash coloured externally and white internally. The roots (Figure 1) are finger-like and clustered. These roots are the part that finds use in various medicinal preparations. The stem (Figure 2) is woody climber growing to 1-2 m in length, whitish grey or brown coloured with small spines. The leaves (Figure 3) are like pine-needles, small and uniform. The inflorescence has tiny white flowers (Figure 4), in small spikes. The plant flowers during February–March leaving a mild fragrance in its surrounding and by the end of April, fruits (Figure 5) can be seen with attractive red berries (Anonymous, Wealth of India, 1987).

Figure 1 Roots of *A. racemosus*

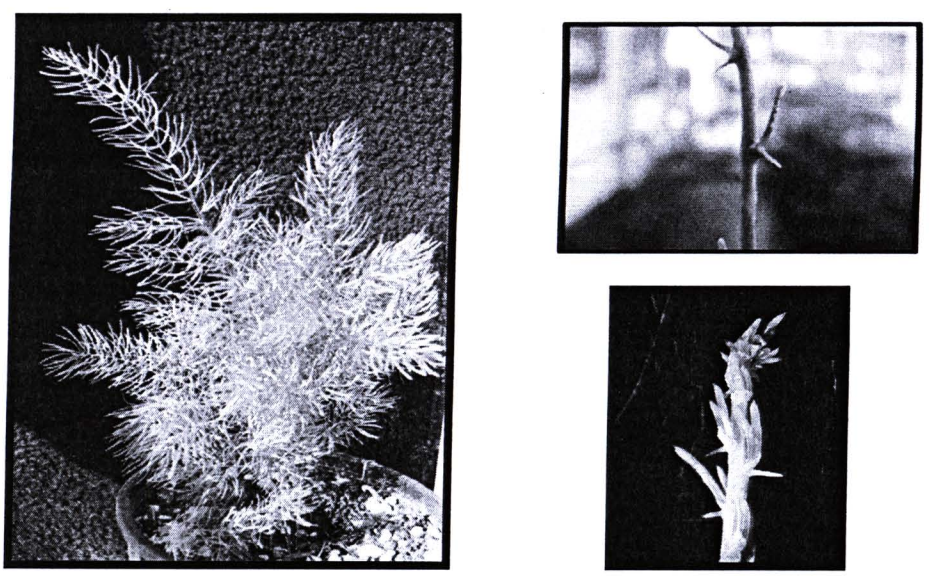


Figure 2 Stems of *A. racemosus*

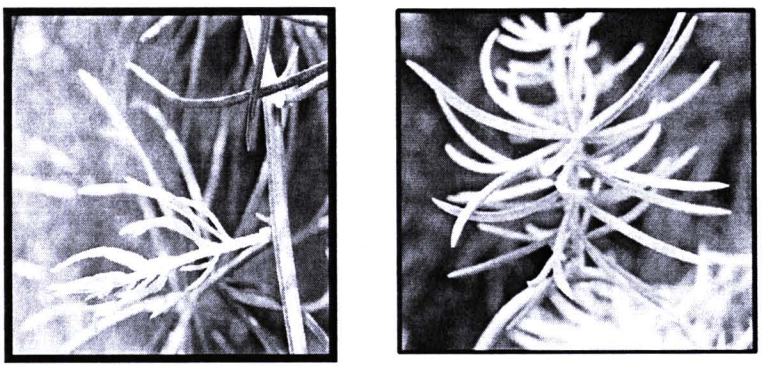


Figure 3 Leaves of *A. racemosus*

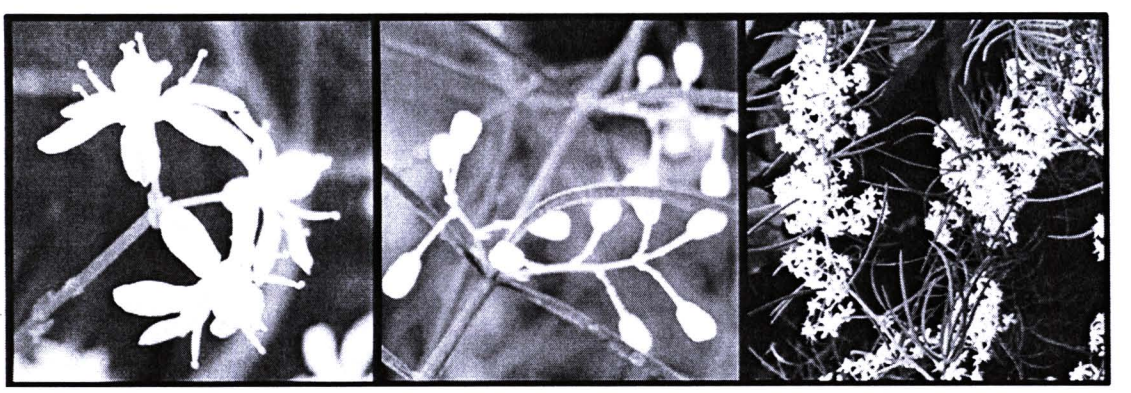


Figure 4 Flowers of *A. racemosus*

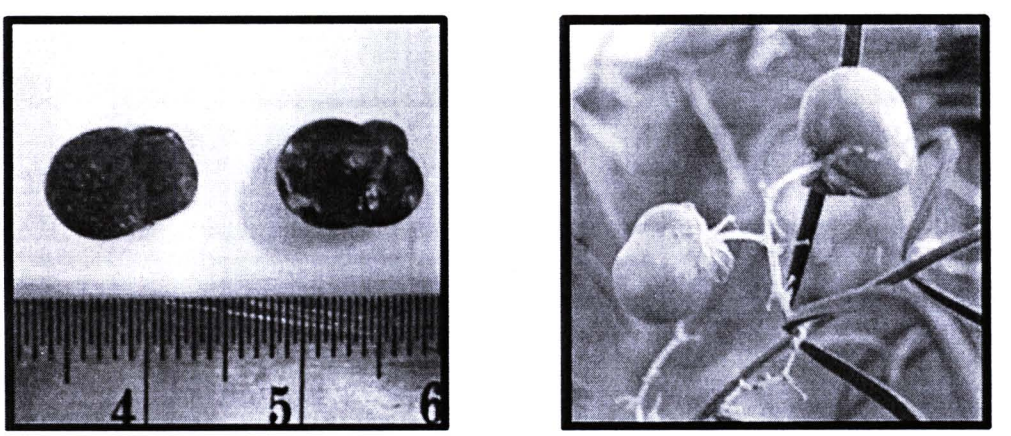
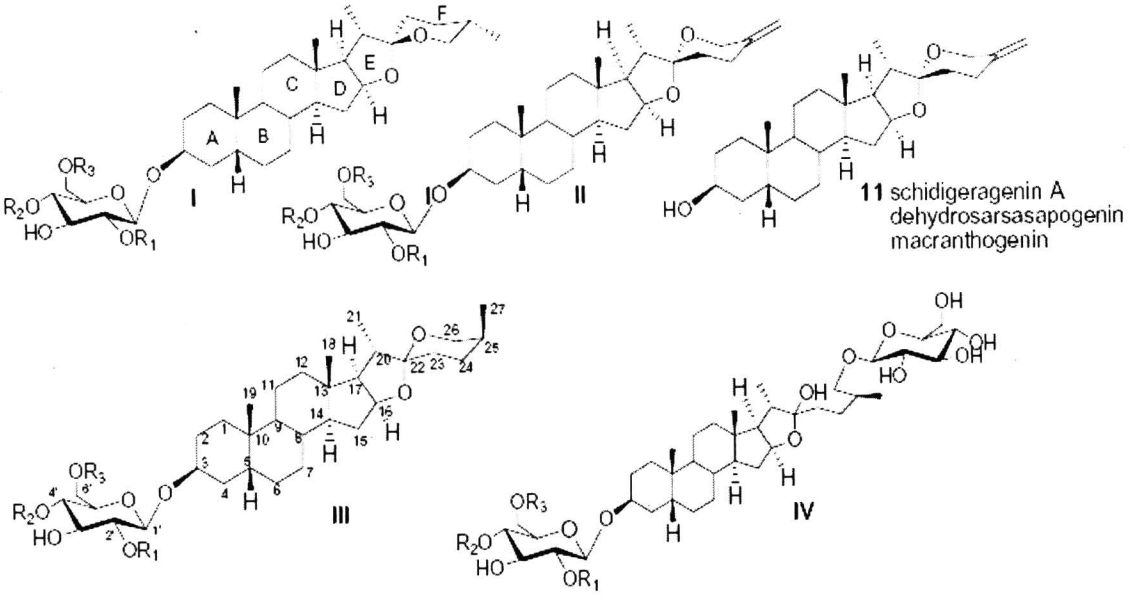


Figure 5 Fruits of *A. racemosus*

2. Active constituents of *A. racemosus*

Ten steroidal saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparanin A) and shatavarins VI–X have been isolated from the roots of *A. racemosus*. Shatavarins I and IV are the main active constituents in this part. The molecular structures of the steroidal saponins isolated from roots of *A. racemosus* are presented in Figure 6 (Hayes et al., 2007).



	Type	R1	R2	R3	
1	I	β-D-Glc	α-L-Rha	H	shatavarin VI
2	II	β-D-Glc	α-L-Rha	H	shatavarin VII
3	III	β-D-Glc	α-L-Ara	β-D-Glc	shatavarin VIII
4	III	β-D-Glc	β-D-Glc	H	shatavarin IX
5	III	α-L-Rh	β-D-Glc (6-OAc)	H	shatavarin X
6	III	α-L-Rh	β-D-Glc	H	shatavarin V
7	III	β-D-Glc	α-L-Rha	H	shatavarin IV
8	III	β-D-Glc	H	H	asparinin A
9	III	α-L-Rha	α-L-Rha	H	immunoside
10	IV	β-D-Glc	α-L-Rha	H	shatavarin I

Figure 6 Structures of steroidal saponins 1–10 isolated from *A. racemosus* roots, divided in four major structural types (I–IV)

Source: Hayes, et al., 2007

In addition, 'Racemofuran' a new antioxidant compound was identified from roots of *A. racemosus* and separated by DPPH (α,α-diphenyl-picrylhydrazyl) autography-directed separation (Wiboonpun, et al., 2004). One isoflavone, 8-methoxy-5-6-4-trihydroxyisoflavone 7-*o*-d-glucopyranoside, was reported from the roots of the plant (Saxena and Chourasia, 2001). The isolation and characterization of a polycyclic alkaloid called 'Asparagamine' from *A. racemosus* that exhibited a unique cage-type structure and remarkable antioxytocic activity was reported by Sekine, et al. (1994). Later, 'Racemosol' was isolated from the ethanol extract of roots (Sekine, et al., 1997).

Other compounds such as quercetin, rutin and hyperoside are found in the flowers and fruits while diosgenin and quercetin-3 glucuronide are present in the leaves (Anonymous, Wealth of India, 1987). Sarsasapogenin glycosides and kaempferol have been isolated from the woody portion of tuberous roots of *A. racemosus*. SD1XThe seeds of 16 species of the genus *Asparagus* were surveyed and phytoecdysteroids were found in roots of *A. racemosus* (Dinan, et al., 2001).

3. Pharmacological applications of *A. racemosus*

A. racemosus is recommended in Ayurvedic text as a galactogogue, aphrodisiac, anodyne, diuretic, antispasmodic and nervine tonic (Sharma, et al., 2000, p. 418-430 as cited in Bopana and Saxena, 2007, p. 3). It can also used for prevention and treatment of gastric ulcers and dyspepsia. Shatavari means 'who possesses a hundred husbands'. It is considered as both a general tonic and a female reproductive tonic. *A. racemosus* is the main Ayurvedic rejuvenative tonic for the female, as is Withania for the male. *A. racemosus* is, however, used for sexual debility and infertility in both sexes. It is also used for menopausal symptoms and to increase lactation. It is a sweet and bitter herb which is said to be particularly balancing to Pitta Dosha. Recent research has shown it to be an immunomodulator with antioxidant, healing and adaptogenic properties.

3.1 Phytoestrogenic effects

The root extract of *A. racemosus* has also been traditionally used in Ayurveda to increase milk secretion during lactation. The aqueous extract of *A. racemosus* roots increased the weight of mammary glands in post-partum and oestrogen-primed rats and the uterine weight in the oestrogen-primed group (Sabnis,

et al., 1968). This effect could be attributed to the action of released corticoids or prolactin. Oral administration of the alcoholic extract of *A. racemosus* rhizome (30 mg/100 g body weight, daily for 15 days) to adult pregnant female albino rats had an oestrogenic effect on the female mammary glands and genital organs (Pandey, et al., 2005). An increase in milk secretion after administration of *A. racemosus* in the form of Ricalex® tablets (Aphali Pharmaceuticals; 40 mg concentrated root extract per tablet) to women suffering from deficient milk secretion (Joglekar, et al., 1967). Randomized controlled trial to evaluate the effect of *A. racemosus* as a lactogogue in lactational inadequacy among women who had delivered at term without complications was reported. Each 100 g dose of the medicine contained 15 g *A. racemosus* root extract. However, after 4-week treatment with *A. racemosus* extract, any lactogogue effect was not observed (Sharma, et al., 1996). On the other hand, the effect of *A. racemosus* extract to increase both the weight of mammary lobuloaveolar tissue and the milk yield was reported. This effect was attributed to the action of released corticosteroids or an increase in prolactin (Bharatiya, et al., 1992). *A. racemosus* was also found to stimulate milk production in buffaloes (Patel, et al., 1969).

Interestingly, Satavari has been studied for its influence on the male reproductive system by Ghumare, et al. (2004). They found that rats fed with *A. racemosus* root powder (0.5 g/kg rat feed) for 21 consecutive days exhibited significantly high testes weights compared to untreated controls. This, however, is an isolated report and has to be investigated further to broaden our understanding regarding the effect of Satavari on the male reproductive system.

3.2 Adaptogenic Activity

Six rasayana plants from Ayurveda, were studied for their adaptogenic potential. The whole, aqueous, standardized extracts of selected plants (*Tinospora cordifolia, A. racemosus, Emblica officinalis, Withania somnifera, Piper longum* and *Terminalia chebula*) were administered orally to experimental animals, in a dose extrapolated from the human dose, after which they were exposed to a variety of biological, physical and chemical stressors. The plant extracts were found to offer protection against the stressors, as measured by markers of stress responses and objective parameters for stress manifestations. Using a model of cisplatin induced

alterations in gastrointestinal motility, the ability of the plants to exert a normalizing effect, irrespective of direction of pathological change was tested. All the plants reversed the effects of cisplatin on gastric emptying, while *A. racemosus* also normalized cisplatin-induced intestinal hypermotility. All the plant drugs were found to be safe in both acute and subacute toxicity studies. Studies on the mechanisms of action of the plants revealed that they all produced immunostimulation (Rege, et al., 1999). A traditional Ayurvedic formulation, Siotone, a rasayana formulation with adaptogenic properties contains *Withania somnifera, Ocimum sanctum, A. racemosus, Tribulus terristris* and shilajit (a mineral-rich, composted plant exudate scraped off rocks). All ingredients are classified in Ayurveda as rasayanas which are reputed to promote physical and mental health, improve defense mechanisms of the body and enhance longevity. An *in vivo* study has shown that Siotone improved glucose tolerance, libido, depression, cognitive dysfunction and immunosuppression caused by chronic stress (Bhattacharya, 2000).

3.3 Effect on neurodegenerative disorders

Alzheimer's and Parkinson's diseases, excitotoxicity and oxidative stress are the major mechanisms of neuronal cell death. *A. racemosus* is a well-known nervine tonic in the Ayurvedic system of medicine. The methanolic extract of *A. racemosus* roots against kainic acid (KA) - induced hippocampal and striatal neuronal damage in mice (Parihar and Hemnani, 2004). The mice treated with *A. racemosus* extract showed an enhancement in GPx activity and GSH content, and reduction in membranal lipid peroxidation and protein carbonyl. It was concluded that the plant extract plays the role of an antioxidant by attenuating free radical induced oxidative damage.

'EuMil', a polyherbal formulation containing the standardized extracts of *Withania somnifera*, *Ocimum sanctum*, *A. racemosus* and *Emblica officinalis* was evaluated for its anti-stress activity in rats (Bhattacharya, et al., 2002). Chronic electroshock stress for 14 days was found to increase the rat brain tribulin activity and decrease the monoamine neurotransmitter levels. 'EuMil' treatment normalized the perturbed nor-adrenalin, dopamine and 5-hydroxytryptamine concentrations and also attenuated the tribulin activity.

'Mentat', a herbal psychotropic preparation containing *A. racemosus* has been found to be effective in the treatment of alcohol abstinence induced withdrawal symptoms such as tremors, convulsions, hallucinations and anxiety in ethanol administered rats (Kulkarni and Verma, 1993) due to its anticonvulsant and anxiogenic action.

3.4 Anti-diarrhoeal effects

Diarrhoea has long been recognized as one of the most important health problems in the developing countries (WHO, 2005). The ethanol and aqueous extracts of *A. racemosus* showed significant ($p < 0.05$) inhibitor activity against castor oil induced diarrhoea and PGE2 induced enteropooling in rats when tested at 200 mg/kg. Both extracts also showed significant ($p < 0.001$) reduction in gastrointestinal motility in charcoal meal test in rats. The results point out the possible anti-diarrhoeal effect of the plant extracts and substantiate the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine (Nanal, et al., 1974)

3.5 Anti-dyspepsia effects

Shatavari is used in Ayurveda for dyspepsia (amlapitta) and it has been shown to improve digestion by increasing the levels of amylase and lipase (Dange, et al., 1969). An Indian study with eight healthy male volunteers compared shatavari with the drug metoclopramide which is used in dyspepsia to reduce gastric emptying time. Metoclopramide and shatavari did not differ significantly in their effects. It was found that shatavari reduced gastric emptying time by 37% ($p<0.001$) (Shakila, et al., 1996).

3.6 Cardio protective effects

Increase in serum lipid levels especially cholesterol along with the generation of reactive oxygen species are the major reasons for the development of coronary artery disease and atherosclerosis. Chronic administration of Abana, an Indian herbomineral preparation containing 10 mg *A. racemosus* extract per tablet, showed a significant hypolipidaemic activity in rats and therefore demonstrated a potential for use as a cardio-protective agent (Khanna, et al., 1991). They found that the total cholesterol, phospholipids and triglyceride levels were significantly lower (37–45%) comparing to the control. *A. racemosus* root powder supplements decreased lipid peroxidation and caused a dose-dependent reduction in lipid profiles in

hypercholesteremic rats (Visavadiya and Narasimhacharya, 2005). They found that the total lipids, total cholesterol and triglycerides in plasma and liver as well as plasma LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein)-cholesterol decreased by more than 30%.

3.7 Immunoadjuvant effects

Shatavari is an immunomodulator. The immunoadjuvant potential of *A. racemosus* was studied in experimental animals immunized with diphtheria, tetanus, and pertussis (DTP) vaccine (Gautam, et al., 2004). After challenge, animals treated daily with *A. racemosus* aqueous root extract (100 mg/kg body weight) showed a significant increase ($p = 0.0052$) in antibody titres to *Bordetella pertussis* as against the untreated animals. Reduced mortality coupled with overall improved health status was observed in treated animals and this indicated the development of a protective immune response. Extracts and formulations prepared from *A. racemosus* exhibited various immunopharmacological actions such as increases in white cell counts, haemagglutinating and haemolytic antibody titers in cyclophosphamide (CP) treated mouse ascitic sarcoma (Diwanay, et al., 2004). Animal studies found that shatavari was capable of producing leucocytosis with neutrophilia and, furthermore, was able to prevent myelosuppression by reducing cyclophosphamide-induced leucopenia (Thatte, et al., 1998). Shatavari has also been shown to inhibit drug induced mammary carcinogenesis (Rao, 1981). The hypothesis that macrophages play a pivotal role in the development of intraperitoneal adhesions and that modulation of macrophage activity, therefore, may prevent adhesions, was tested in an Indian study. The effect of shatavari was evaluated in an animal model of intraperitoneal adhesions (Rege, et al., 1989). Shatavari reduced the severity of the adhesions and this correlated with a significant increase in the activity of the macrophages. An vitro study found that shatavari increased phagocytic activity of macrophages (Rege, et al., 1993) while an *in vivo* study showed that *A. racemosus*, *Tinospora cordifolia*, *Withania somnifera* and *Picrorhiza kurrooa* inhibited drug-induced suppression of chemotactic activity and production of interleukin-1 and TNF-alpha by macrophages (Dhuley, 1997).

3.8 Antitussive effects

The methanol extract of *A. racemosus* root (200 and 400 mg/kg, p.o.) showed significant antitussive activity on sulfur dioxide-induced cough in mice. The

cough inhibitions (40.0 and 58.5%, respectively) were comparable to that of 10-20 mg/kg of codeine phosphate (36.0 and 55.4%, respectively) (Mandal, et al., 2000a).

3.9 Antioxidant Activity

Membrane damage induced by free radicals generated during gamma-radiation was examined in rat liver mitochondria. The crude extract and a purified aqueous fraction of *A. racemosus* were shown *in vitro* to have potent antioxidant properties in mitochondrial membranes of the rat liver. Both the crude extract as well as a polysaccharide-rich fraction significantly inhibited lipid peroxidation and protein oxidation. Both fractions also partly protected against radiation-induced loss of protein thiols and inactivation of superoxide dismutase (Kamat, et al., 2000).

3.10 Anti-microbial effects

Different concentrations (50, 100, 150 μg/ml) of the methanol extract of the roots of *A. racemosus* showed considerable *in vitro* antibacterial efficacy against *Escherichia coli, Shigella dysenteriae, S. sonnei, S. flexneri, V. cholerae, Salmonella typhi, S. typhimurium, Pseudomonas putida, Bacillus subtilis* and *Staphylococcus aureus*. The effects produced by the methanol extract were compared with chloramphenicol (Mandal, et al., 2000b). The antibacterial effect of *A. racemosus* may also be playing a secondary role in its action with respect to other functions of the plant as well and therefore needs to be studied in greater detail.

The methanol extracts of *A. racemosus* roots and tubers showed high anticandidal activity against, *Candida albicans, C. tropicalis, C. krusei, C. guillermondii, C. parapsilosis* and *C. stellatoida* isolated from vaginal thrush patients. The zone of inhibition ranged from 13 to 16 mm. The MIC values were between 2.5 to 0.312 mg/ml, while MFC values were between 5 to 0.625 mg/ml. The detailed chemical nature of the active principle(s) responsible for the antifungal activity is not known. However, the preliminary screening has shown the presence of glycosides, steroids, saponins and flavonoids in the active extract (Uma, et al., 2009).

Some saponins have been reported for antibacterial and antifungal activities. Three saponins, namely minutoside A, minutoside B, minutoside C, and two known sapogenins, alliogenin and neoagigenin, were isolated from the bulbs of *Allium minutiflorum* Regel. The isolated compounds were evaluated for their antimicrobial activity. All the novel saponins showed a significant antifungal activity depending on

their concentration with the following rank: minutoside B > minutoside C >> minutoside A (Elisa, et al., 2007).

Six steroidal saponins were isolated from the roots of *Asparagus acutifolius* L., together with a spirostanol glycoside. Four compounds, the spirostanol derivatives demonstrated antifungal activity against the human pathogenic yeasts *C. albicans, C. glabrata* and *C. tropicalis* with MICs values between 12.5 and 100 µg/ml (Marc, et al., 2007).

Antifungal activity was detected in the crude saponin fraction obtained from the bottom cut of *A. officinalis* L. The activity was specific to certain fungi, for example *Candida, Cryptococcus, Trichophyton, Microsporum* and *Epidermophyton*. The MIC ranged from 0.5 µg/ml to more than 8 µg/ml depending upon the nature of the fungi. On the basis of their work, it is possible that *A. officinalis* will contain additional antifungal principles (Shimoyamada, et al., 1990).

From the above mentioned reports, *A. racemosus* might be good source for antifungal activity. This study is aimed to determine the antimicrobial activity of the various extracts from *A. racemosus*.

Overview of fungi

Normal flora is the term used to describe the various bacteria and fungi that are permanent residents of certain body sites, especially the skin, oropharynx, colon, and vagina. The viruses and parasites, which are the two other major groups of microorganisms, are usually not considered members of the normal flora, although they can be present in asymptomatic individuals. The members of the normal flora vary in both numbers and kinds from one site to another. Although the normal flora extensively populates many areas of the body, the internal organs usually are sterile. Areas such as the central nervous system, blood, lower bronchi and alveoli, liver, spleen, kidneys and bladder are free of all but the occasional transient organism.

Because fungi (yeasts and molds) are eukaryotic organisms whereas bacteria are prokaryotic, they differ in several fundamental respects (Table 1). Two different properties between fungal and bacterial cell structures are as follows:

1. The fungal cell wall primarily consists of chitin (not peptidoglycan as in bacteria); thus, fungi are insensitive to antibiotics that inhibit peptidoglycan synthesis

such as penicillin. Chitin is a polysaccharide composed of long chains of *N*-acetylglucosamine. The fungal cell wall contains other polysaccharides of which the most important is β-glucan, a long polymer of D-glucose. The medical importance of β-glucan is that it is the site of action of the antifungal drug, caspofungin.

2. The fungal cell membrane contains ergosterol, in contrast to the human cell membrane which contains cholesterol. The selective action of amphotericin B and azole drugs, such as fluconazole and ketoconazole, on fungi is based on this difference in membrane sterols.

Table 1 Comparison of fungi and bacteria

Feature	Fungi	Bacteria
Diameter	Approximately 4 μm (*Candida*)	Approximately 1 μm (*Staphylococcus*)
Nucleus	Eukaryotic	Prokaryotic
Cytoplasm	Mitochondria and endoplasmic reticulum present	Mitochondria and endoplasmic reticulum absent
Cell membrane	Sterols present	Sterols absent (except *Mycoplasma*)
Cell wall content	Chitin	Peptidoglycan
Spores	Sexual and asexual spores for reproduction	Endospores for survival, not for reproduction
Thermal dimorphism	Yes (some)	No
Metabolism	Require organic carbon; no obligate anaerobes	Many do not require organic carbon; many obligate anaerobes

Source : Warren, 2008

This study was focused on fungi (*C. albicans, Malassezia furfur and M. globosa*) because the preliminary study indicated that *A. racemosus* extracts had antifungal activity while any antibacterial activities were not found.

1. *Candida albicans*

C. albicans, the most important species of *Candida,* is an oval yeast with a single bud. It is one of the normal flora found in mucous membranes of the upper respiratory, gastrointestinal, and female genital tracts. In tissues, it may appear as yeasts or as pseudohyphae. Pseudohyphae are elongated yeasts that visually resemble hyphae but are not true hyphae. Carbohydrate fermentation reactions differentiate it from other species, e.g., *C. tropicalis, C. parapsilosis, C. krusei,* and *C. glabrata.*

C. albicans causes thrush, vaginitis, esophagitis, and chronic mucocutaneous candidiasis. It also causes disseminated infections such as right-sided endocarditis (especially in intravenous drug users) and blood stream infections (candidemia). Infections related to indwelling intravenous and urinary catheters are also important.

When local or systemic host defenses are impaired, disease may result. Overgrowth of *C. albicans* in the mouth produces white patches called thrush. Vulvovaginitis with itching and discharge is favored by high pH, diabetes, or use of antibiotics. Skin invasion occurs in warm, moist areas, which become red and weeping. Fingers and nails become involved when repeatedly immersed in water; persons employed as dishwashers in restaurants and institutions are commonly affected. Thickening or loss of the nail can occur.

In immune suppressed individuals, *Candida* may disseminate to many organs or cause chronic mucocutaneous candidiasis. Intravenous drug abuse, indwelling intravenous catheters, and hyperalimentation also predispose to disseminated candidiasis, especially right-sided endocarditis. *Candida esophagitis*, often accompanied by involvement of the stomach and small intestine, is seen in patients with leukemia and lymphoma. Subcutaneous nodules are often seen in neutropenic patients with disseminated disease. *C. albicans* is the most common species to cause disseminated disease in these patients, but *C. tropicalis* and *C. parapsilosis* are also important pathogens.

The drug of choice for oropharyngeal or esophageal thrush is fluconazole. Caspofungin or micafungin can also be used for esophageal candidiasis. Treatment of skin infections consists of topical antifungal drugs, e.g., clotrimazole or nystatin. Mucocutaneous candidiasis can be controlled by ketoconazole. Treatment of disseminated candidiasis consists of either amphotericin B or fluconazole. These two drugs can be used with or without flucytosine. Treatment of candidal infections with antifungal drugs should be supplemented by reduction of predisposing factors (Warren, 2008).

2. *Malassezia species*

There are several different species of *Malassezia* recognized, and various animals may serve as the natural hosts for specific species of the fungi. For example, most domestic carnivores harbor *M. pachydermatis* as part of their natural cutaneous microflora, while human beings primarily harbor *M. furfur*. Although *Malassezia* are a part of the normal microflora, under certain conditions they can cause superficial skin infection. Because of *Malassezia* dependent on lipids for survival. Their most often found in sebum-rich areas of the skin such as the trunk, back, face, and scalp. Less frequently, they may also be found on other areas of the body including of arms, legs, and genitalia. *Malassezia* nomenclature has evolved over the last century, but the genus now consists of 10 distinct species: *M. globosa, M. restricta, M. furfur, M. sympodialis, M. slooffiae, M. obtusa, M. nana, M. dermatis, M. japonica,* and the sole non-lipid-dependent species, *M. pachydermatis*. All except *M. pachydermatis* can be found on human skin, but the most common species on human scalp are *M. restricta, M. furfur and M. globosa* (Gemmer, et al, 2002).

M. furfur is a polymorphic lipophilic microorganism characterized by a thick, multilayered cell wall. Colonies are small and yellowish-brown with an intact margin. *M. furfur's* reproduction occurs by monopolar budding yeast cells (Takeo, et al., 1986), sometimes capable of forming filaments. Cultures were generally short-lived.

The yeast form of *M. globosa* cells are spherical measuring 2.5-8.0 μm. Colonies on sabouraud dextrose agar overlaid with olive oil are cream to yellowish. *M. globosa* and *M. furfur* likely initiates dandruff formation due to its high lipase activity. *M. globosa* lacks the ability to synthesize fatty acids: it is highly adaptive but

niche dependent and is commonly found on where the highest levels of sebum are produced. *M. globosa* excretes more than 50 different enzymes to help metabolize hair and scalp. *M. globosa* is capable of mating. Although its mating ability may seem irrelevant at first glance, it may affect the ability of *M. globosa* to change and adapt in response to its environment and future needs.

The fungi of the genus *Malassezia* have been associated with a number of diseases affecting the human skin, such as pityriasis versicolor, Malassezia (Pityrosporum) folliculitis, seborrheic dermatitis and dandruff, atopic dermatitis, psoriasis, and - less commonly - with other dermatologic disorders such as confluent and reticulated papillomatosis, onychomycosis, and transient acantholytic dermatosis. Dandruff is perhaps the most common disease associated with *Malassezia spp.*, occurring in 1% to 3% of the general population (Ashbee and Evans, 2002; Warner, et al., 2001). The incidence of dandruff is much higher in patients who are immunocompromised, especially those with AIDS, ranging from 30% to 33% (Farthing, et al., 1985; Smith, et al., 1994). Dandruff has recently received much attention, as its presence can lead to loss of self-esteem and a negative social image (Warner, et al., 2001).

Malassezia spp. are susceptible to a wide range of nonspecific and specific antifungal topical treatments, and several effective oral agents. Older treatments tend to lack antifungal activity and generally possess keratolytic properties. These agents include selenium sulfide, propylene glycol, and sulfur- and tar-containing compounds. However, the activity of selenium sulfide and propylene glycol can be accounted for by their antimicrobial activity (Kinnunen, et al., 1991; McGinley and Leyden, 1982). Zinc pyrithione is particularly effective in dandruff, because of both potent antimicrobial (effective against bacteria and fungi) and anti-inflammatory activities, killing *Malassezia* and causing a decrease in IL-1 release from cultured keratinocytes (Warren, et al., 2003). Specific antifungal agents used for the topical treatment of *Malassezia* infections, particularly pityriasis versicolor and seborrheic dermatitis, include the azoles (Gupta, et al., 2003a; Gupta, et al., 2003b; Uchida, et al., 2003), hydroxypyridones (Vardy, et al., 2000), allylamines (terbinafine) (Faergemann, et al., 1997), benzylamines (butenafine) (Gupta, et al., 2002), tacrolimus (Ling, 2001;

Nakagawa, et al., 1996) and pimecrolimus (Ling, 2001). Several oral agents have also been used successfully to treat *Malassezia* infections.

Traditionally, seborrheic dermatitis has been treated with either topical or oral steroids (Johnson and Nunley, 2000). However, the renewed interest in the role of *Malassezia* yeasts in this condition has made antifungal medications an increasingly popular choice. Tacrolimus has been shown to have potent antifungal activity against *M. furfur in vitro* (Nakagawa, et al., 1996). It has been suggested that topical tacrolimus and pimecrolimus may be superior alternatives to corticosteroids, as they exhibit anti-inflammatory activity but do not have the side effects associated with long-term corticosteroid use (Ling, 2001).

Soponin

Saponins are generally known as non-volatile, surface-active compounds that are widely distributed in plants. They dissolve in water to form colloidal solutions that foams when shaken with water. They consist of nonpolar aglycones coupled with one or more monosaccharide moieties (Oleszek, et al., 2002). This combination of polar and non-polar structural elements in their molecules explains their soap-like behavior in aqueous solutions. Saponins have a diverse range of properties, which include foaming and emulsifying properties (Price, et al., 1987), pharmacological and medicinal properties (Attele, et al., 1999), as well as antimicrobial and antifungal activities (Shimoyamada, et al., 1990; Marston, et al. 1988). Saponins have found wide applications in cosmetics and pharmaceutical products (Sparg, et al., 2004).

Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid (Figure 7(a)) or steroid (Figure 7(b)) in nature. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the C3 position (monodesmosidic), but many saponins have an additional sugar moiety at the C26 or C28 position (bidesmosidic) (Francis, et al., 2002). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone.

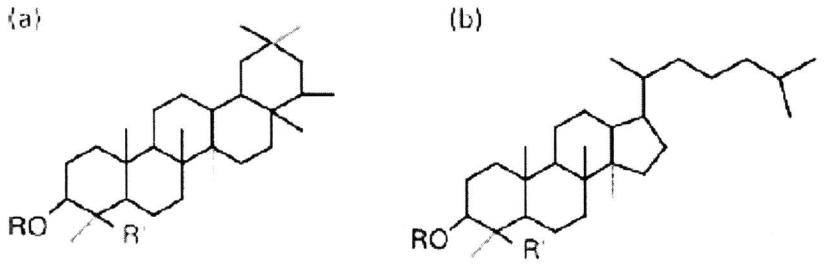


Figure 7 Basic structures of sapogenins: a triterpenoid (a) and a steroid (b)

Saponins are common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and mollusks, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents.

1. Saponin with synergistic effect

Saponin are one of the natural compounds that have antifungal activity, even though they appear to have relatively mild activities against human pathogenic fungi compared to commercial synthetic antifungal drugs. Consequently, the antifungal activities of plant saponins have been evaluated after administration in combination with synthetic drugs to evaluate for synergism (Lucca, et al., 2006; Avijgan, et al., 2010).

Synergy is a potential benefit of combination antibiotic therapy for fungal infections. While numerous methods used to detect *in vitro* synergy between antibiotics have been described, the checkerboard is the most widely used techniques. The checkerboard test, a gauge of inhibitory activity, is a relatively easy test to perform, therefore this method was provided to determine synergistic effect of the *A. racemosus* extracts with antifungal agent.

2. Antifungal mechanism of saponin

The action mechanisms of saponins may lie in damage to the membrane and leakage of cellular materials, ultimately leading to cell death (Mshvildadze, et al., 2000). This activity has been documented in a number of saponins, and the damaging effects have been shown against a variety of fungi, including of *C. albicans*, *Saccharomyces cerevisiae*, *Trichodemta viride*, *Acremonium* spp. and *Cryptococcus neoformans* (Lalitha andVenkataraman, 1991; Polacheck, et al., 1991). For example, medicagenic acid 3-*O*-beta-dglucopyranoside, an antimycotic saponin from alfalfa root, formed stable complexes with ergosterol, causing lethal leakage of ions out of yeast cells (Polacheck, et al., 1991).

Synthetic steroid saponins prepared by Takechi, et al. (1999) were both antifungal and haemolytic but in many cases haemolytic triterpenoid saponins show little antifungal activity. It was observed that those saponins having a branched-chain trisaccharide moiety without any oxygen-containing groups at C2 and C12 exhibited the anti-yeast activity, while saponins with 2b-hydroxyl or 12 keto groups showed very weak or no activity (Miyakoshi, et al., 2000). A saponin with a disaccharide moiety exhibited relatively low activity and the aglycones or bidesmodic furostanol saponins showed no activity. The antifungal activity of food-originated substances has attracted an applicational research. Some reports describe the anti-yeast activity of saponins as having an anti-food deteriorating effect (Miyakoshi, et al., 2000).