Chapter – II

Review of Literature
2. Review of Literature

2.1. Ashwagandha

Ashwagandha (*Withania* Sp) is one of the vital ancient drugs gaining importance nowadays and is drawing more attention of the herbal industrialists and farmers because of the increasing demand for its roots in pharmaceutical industries. But the cultivation of this crop is not being done in a scientific manner to utilize the full potential of this plant. In India, Ashwagandha is being cultivated in about 4000 hectares especially in Mandaur district of Madhya Pradesh with an annual production of about 1500 tonnes of dry root per year (Nigam, 1984).

2.2. *Withania somnifera*

Vakeswaran (2001) has reported that *W. somnifera* commonly known as belongs to the family of Solanaceae, is an important medicinal plant in India. It is a shrub, evergreen, tomentose. Roots are stout, fleshy, cylindrical and 1-2 cm thick. Leaves are simple, alternate, petiolate, elliptic ovate to broadly ovate, entire, exstipulate, the tip of the leaf is acute, cuneate or oblique and glabrous up to 10 cm long. Inflorescence is cymose cluster of 5-25 with inconspicuous pale green flowers. Flowers are shortly pedicillate and 4-6mm in dia. Seeds are smooth, discoid, 20-25 seed per fruit. Alkaloids such as ‘withanine’ and ‘somniferine’, present in roots of ashwagandha, posses pharmacological activities.
2.3. *Withania obtusifolia*

*W. obtusifolia* forms the natural population. Aswagandha is commonly called Nattu ammukkaran. It is a shrub, stems are erect, evergreen, branched, glabrous and leaves are less pubescent, elliptic ovate and obtuse. Flowers are light yellow, stigma capitates, fruits are green when unripe, deep red when matured. Seeds are smooth, discoid, 18-23 seeds per fruit. The roots differ from *W. somnifera* and show the presence of hard wood at the central portion of the root. It is a weed of drier warm parts of the world. Its frequency may be due to the hand of man owing to its minor use as a medicinal herb in folk remedies. It is a very rare plant natural to population of Tamil nadu. The root portion is a recognized folk remedy for a number of diseases in traditional and indigenous medicine of Tamil nadu. The root is prescribed in medicines for a number of diseases viz., aphrodisiac, arthritis, asthma bronchitis, cancer, cold, seminal debility, dropsy, erysipelas, fever, gynecopathy, hiccups, back pain, hypertension, psoriasis, infertility, skin diseases, swelling obesity and general immunity (Pannerselvam *et al.*, 2003).

Recently Kumar *et al.*, (2011) analysed the morphological, cytogenetical and chemical profilings among wild and cultivated species of *W. somnifera* they reported that the chloroplast DNA diversity and somatic chromosome number (2n=48) were not helpful in identifying the differences among them.

2.4. **Seed Germination**

Stanislaw and Elbieta (1982) reported that triacontanols were ineffective in germination of lettuce seeds under high temperature conditions. Wilen *et al.*,
Comparative studies of *Withania somnifera* (L.) Dunal, and *Withania obtusifolia* tuckh - M. SENTHIL KUMAR

(1995) and Dhaubhadel *et al.*, (1999) observed that the brassinosteroids increased the tolerance of cabbage and tomato seedlings against high temperature stress. In addition, it was found that in many plant species where exposed to high temperature, there was an increase in the endogenous polyamine contents (Roy and Gosh 1996, Bouchereau *et al.*, 1999). The medium temperature cause enhances in transpiration and evaporation and so, deficiency of water in plants (Gates 1968). Decrease in moisture level in the germinating medium severely affects seed germination and growth. Various studies indicated that the changes in endogenous level of the growth regulators caused by high temperature stress, increases the inhibitors (Gonai *et al.*, 2004) and decreases in the stimulation of (Corbineau *et al.*, 1993) seed germination. The optimum temperature for germination of *Vinca rosea* was reported to be 20-27°C (Mastalerz, 1976; Atwater, 1980) by different workers. The optimum temperature for other medicinal plants was 20 -30°C for *Atropa belladonna* and *Solanum laciniatum*, 25°C for *Solanum nigrum* (Horowitz and Givelberg, 1982) and 30°C for *Solanum viarum* (Tyagi and Sharma, 1982).

Wilens *et al.*, (1995) and Dhaubhadel *et al.*, (1999) observed that the brassinosteroids increased the tolerance in cabbage and tomato seedlings against high temperature stress. In addition, it was found that in many plant species exposed to high temperature, there was an increase in the endogenous polyamine contents (Roy and Gosh 1996, Bouchereau *et al.*, 1999). The temperature range of 25-30°C for *Hibiscus subdariffa* and 20 – 30°C for *Vinca rosea* were best suited for germination (Ellis *et al.*, 1985). It was 25°C for *Plantago ovate* (Verma *et al.*, 1989), Optimum temperature for germination of *Andrographis*
paniculata was 20°C, 30°C for Thespesia populnea (Gagre et al., 1996), 20 – 30°C for Mikania glomerata (Scheffer et al., 1999), 30°C for Smilax campestris (Rosa and Ferreiva, 1999), 25°C for Origanum majorana (Thanos, 2000). The role of substrate for seed germination is considered to be vital study of the characters for an ideal medium for germination, density and retension of moisture and porus. Sand was the best medium for germination of Gymnema sylvestre (Harakumar et al., 1999).

2.5. Seed treatment

Breaking the dormancy of freshly harvested seeds is necessary to induce the germination. The seed may require different types of treatment to break dormancy and to make the seed ready to germinate in the forthcoming seasons. Geetha (2001) has reported that the germination was improved by 12% when the seeds were soaked in water for 24 h. Water soaking might have leached out some of the water soluble inhibitors present in the glumes resulting in germination improvement of seeds in Cenchrus spp.

Usberti et al., (2000) quoted that the germination through acid scarification was tried but again it was not a complete success. Seeds scarified for 6 min exhibited a moderate increase in germination (36%) with high vigour index. The result was conformed in many plant species, such as, Brachiaria brizantha, B.decombuns and Panicum antidotale. Previero et al., (1996) pointed out that the seeds scarified with sulfuric acid for 6 min and soaked in KNO₃ increased the germination percentage to a higher extent (94%). The study
revealed that soaking of seeds in 0.5, 1.0 and 1.5% KNO₃ for 2 hrs increased the germination to 94%. Similar results were reported in *Panicum maximum*.

Ajmal Khan *et al.*, (2004) have reported that the seeds had no dormancy, and about 90% of the seeds germinated in nonsaline control. Seed germination decreased with the increased salinity, and more than 10% of the seeds germinated at 900 mmol/L NaCl. Almost all the seeds germinated within 24 hours, and no seed germination was observed after this time. Gibberellic acid has no effect in alleviating salinity effects, however, kinetin and fusicoccin substantially alleviated the effect of salinity on germination, while ethephon almost completely reverted the effect of salinity.

Baskin and Baskin (1998) have reported that the physiological dormancy is the main cause for the delay of seed germination in halophytes. Germination-regulating compounds such as gibberellic acid (GA₃) and kinetin (Ungar 1977, 1982, 1984) are useful to alleviate the effect of high salinity on the germination of halophytes. Effects of salinity on germination of *C. lanata* seeds are completely alleviated in the presence of ethephon in the medium. Applications of ethephon break the seed dormancy in several species (Ketring 1977; Bewley and Black 1994; Abeles and Lonski 1969; Adkins and Ross 1981; Whitehead and Nelson 1992) and reverse the inhibitory effect of abscisic acid during osmotic stress (Karssen 1976; Schonbeck and Egley 1981).

Ethylene may act as stimulator for seed germination of non dormant seeds or by breaking dormancy in dormant seeds (Ketring and Morgan 1969; Egley
GA\textsubscript{3} failed to alleviate the effect of salinity on the germination of \textit{C.lanata} seeds, under both saline and non-saline conditions. GA\textsubscript{3} was reported to cause a differential response to the germination of halophytes. Seed germination under saline conditions was almost completely alleviated in \textit{Atriplex stocksii} and \textit{Zygophyllum simplex} (Khan and Rizvi 1994; Khan and Ungar 1997). Kinetin substantially alleviates seed germination of \textit{C.lanata} in salinity tested. Kinetin also caused a similar response in \textit{Atriplex triangularis}, \textit{Atriplex stocksii}, and \textit{Zygophyllum simplex} (Khan and Ungar 1985, 1998).

Santapan (1956) suggested that poor germination in \textit{Rauvolfia serpentina} seed was due to the fact that the embryo of freshly harvested seeds were not fully developed and that required some more time for full development. Singh and Motial (1970) reported that seed treatment with GA\textsubscript{3} (500ppm) for 12hrs, significantly increased seed germination and seedling survival in \textit{Rauvolfia serpentina}. Soaking the seeds in 5 X 10\textsuperscript{-4}M concentration of GA\textsubscript{3} improved the germination of \textit{Solanum nigrum} both in dark and light condition (Horowiz and Givelberg, 1982). \textit{Gloriosa superba} seed germination was improved by soaking in thiourea and an early germination was achieved by soaking in GA\textsubscript{3} (Superna \textit{et al.}, 1993). Singh \textit{et al.}, (1995) reported that maximum germination of \textit{Quercus lecctrichophora} seeds was obtained by treating the seed with 500ppm GA\textsubscript{3} for 24 hrs.

Pappiah and Ananthan (2001) noticed that \textit{Bixa orellana} seeds were soaked in water with GA\textsubscript{3} and KNO\textsubscript{3} (0.1%). The results were indicated that the higher percentage of germination (62.7\%) was recorded in seeds treated with
water soaking for 24 hrs. The same treatment had also produced taller plants, more number of leaves, maximum growth of stem and higher number of branches and length of roots were observed than other treatments. Gupta et al., (2002) found that *Asparagus racemosus* with seed treatments of physical and chemical scarification treatments were more effective in breaking of seed dormancy. Gibberellic acid also accelerated germination but to a lesser extent. Sulphuric acid (up to 20%) increased the germination rate (84 - 85%) as compared to the control (56 – 60%) and resulted in a seedling survival rate of 80% in the field.

Kuberreddi et al., (2003) recommended that Daheya (*Streblus asper*) is an important dioecious evergreen, medicinal tree listed under endangered species because of the problem of natural regeneration. Among the various growth regulator treatments, the maximum seed germination was recorded in combination of GA$_3$ and thiourea at 200ppm. Anbukamaraj et al., (2003) studied the effect of thiourea, KH$_2$PO$_4$ and GA$_3$ on germination of henna (*Lawsonia inermis*) a dye yielding plant. KH$_2$PO$_4$ (2% recorded the highest percentage of germination of (94%) followed by GA$_3$ (200ppm) with 88% whereas the control recorded 54% germination.

Seeds of *Ocimum sanctum* were soaked for 24h in GA$_3$, NAA, potassium nitrate and thiourea. Among the seed treatments, *Ocimum* seeds treated with GA$_3$ (250 ppm) placed in sand recorded higher rate of germination and vigour than the other. (Sunitha and Manivannan, 2003) It was observed that the seeds soaked
Comparative studies of *Withania somnifera* (L.) Dunal, and *Withania obtusifolia* tackh - M. Senthil Kumar

in 250ppm IAA for 12hrs gave the highest percentage of germination of (59%) compared with other treatments (Barathkumar and Vijayakumar, 2003).

2.6. **Physico – chemical treatments**

The highest rate of germination in *Tephrosia purpurea* was achieved by scarification with sand followed by pre-soaking in hot water at 50°C for five min (Sundararaj *et al.*, 1971), while treatment with concentrated sulphuric acid for 15min was suggested by Dharmalingam *et al.*, (1973) for improving germination. Mitra and Kushari (1987) reported that poor germination of *Solanum viarum* seed was due to hard seed coat and suggested scarification with 10% nitric acid for 15min for removal of seed coat gives better seed germination.

Tansi (2003) reported that the highest percentage of germination of *Capparis spinosa* seeds after being treated with 400ppm GA₃ for five hours or in sulphuric acid for 20 min followed by GA₃ 400ppm soaking for two hours. Seeds were scarified by using an emery board shows a significant increase in germination. Kumar and Subramanyam (1985) found improvement in germination due to pre-soaking for different periods in *C. roseus* seeds. Krause (1988) reported that the germination of *Catharanthus roseus* seed could be accelerated by soaking the seeds for 24 - 48 hrs in water at 30°C. Vakeswaran (2001) reported that mechanical scarification with sand for six minutes followed by soaking in GA₃ 500 ppm solution for five hours significantly improved the germination of seeds.
2.7. **Morphological studies**

Morphological variation may be related to genetic variation; these two do not correlate perfectly. Morphological variation depends on seasonal or developmental changes that affect many individuals in a population regardless of genotype. For example, domesticated population of black caraway (*Bunium persicum*) showed a large heterozygocity in the population for seed yield and tuber weight per plant apparently due to the differential performance of plants belonging to different age groups (Kapila et al., 1997). Interestingly, there are ‘plastic’ phenotypes in which growth form depends on the environment (Stearns, 1989; Schmid, 1992). On the other hand, the extensive variation exhibits by many *Piper* species in their leaves, flowering habit and fruiting spikes are not formed by environmental influence but through genetic divergence (Ravindran et al., 1990).

Ball and Getliffe (1976) collected Davis plant (*Satureja parnassica* Heldr. *Sipylea*) samples from two different localities on Marmara Island and Spil Mountain, and the morphological, anatomical and chemical characteristics of the species were investigated. *S. pilosa* was similar to *S. parnassica et al.*, described in Flora Europaea. *Solanum* species are mostly herbs with branched tap root. The stem is herbaceous erect and hairy, leaves are alternate, opposite in floral region, simple and estipulate. Tindall, (1986) the placentation is axile, ovules are numerous, stylle single terminating in a bilobed stigma. The fruit is berry and the seeds are minute endospermic with a straight or curved embryo (Esua, 1977). The use of morphological and leaf epidermal features have been found to be of immense interest in systematics. An excellent review of the application of

Genetically determined morphological diversity can be continuous or discrete; and may be coded by many or few genes together with some input from the environment (Mallet, 1996). Genes affecting morphology are most interesting because morphology is often under strong selection. In many cases of adaptation, small number of loci have major effects on traits such as resistance to chemicals, diseases, parasites etc. (Macnair, 1991), although there are good theoretical reasons for expecting the most adaptation of gradual and polygenic (Fisher 1958; Barton and Turelli, 1989).

Nwachukwu and Mbagwu (2009) have reported that stem type, colour and bark showed the two taxa are erect-branched from base, green and smooth respectively. The floral morphology further strengthens the intraspecific relationship among these two taxa. The floral result revealed that the flower type, symmetry, arrangement pedicel, calyx and corolla colour and all are shapes the same in the two taxa studied.

*W. coagulans* and *W. somnifera* are two medicinally important species of *Withania* in Pakistan. *W. coagulans* is used to cure ailments relating to digestive systems and *W. somnifera* as aphrodisiac tonic or to cure rheumatic pains. This genus is found in the areas, which come under the category of Saharo-Sindian and Sino-Japanese. Leaf, flower, fruit and seed characters played a key role to
differentiate \textit{W. coagulans} from \textit{W. somnifera}. Baytop, (1978) gave importance to these characters to distinguish the Turkish species of \textit{W. somnifera}. However this species is somewhat different from the Indo-Pak \textit{W. somnifera}. The difference lies in fruiting calyx. In Pakistan, the teeth of \textit{W. somnifera} calyx are small and triangular whereas, the Turkish \textit{W. somnifera} has longer, filiform teeth. Hawkes and Edmond, (1972) misapplied the name of \textit{W. frutescence} for \textit{W. coagulans}. All the morphological characteristics were mentioned for \textit{W. frutescens} exactly matched with the characteristics of \textit{W. coagulans}.

Senthil Kumar et al., (2010) have reported that the morphological (vegetative and floral) and leaf-epidermal features of the species are studied to confirm the interspecific relationships of \textit{W. somnifera} and \textit{W. obtusifolia}. The leaf, flower arrangements and fruit characters are morphologically distinct and leaf epidermal features observed in these studies has systematic value because they are reasonably constant in the studied species.

2.8. Anatomical studies

Keck (1936) has reported that the occurrence of pectic channels in leaves of \textit{Argyroxyphium} appear which is secreted in pith regions and transpoted to the outer regions of “pectic warts”. But secretion of pectic compounds in other plants has been observed of microtechniques which tend to destroy them the pectic type in mucilaginous substances. Ayensu, (1970) studied the similarities in root anatomical structures of two different \textit{Solanum} species such as size and number of vascular bundles were observed that are small and few, in one species but in another taxa the vascular bundles are small and the number is more than
previous taxa and appeared together the cortex and well differentiated epidermal cells in both taxa were showed strong interspecific relationships and concluded that both the species belonged to the same genus. The differences of root anatomy also distinguished the two taxa and suggested that the two taxa are different species of same genus. These anatomical variations strengthen the reliability of anatomical characters in systematic botany.

Metcalfe and Chalk (1972) while stating the existence of a well-developed peridermis layer on woody stem, have pointed out that intervacular fibres form intervacular cambium and they separate the continuous xylem at woody stem. In the woody stem, in addition to a sclerenchyma groups on phloem, there is also a sclerenchymatous ring. However, on the herbaceous stem, there are sclerenchyma groups but there is only one sclerenchyma ring.

Metcalfe and Chalk (1972) observed that mesophyll tissues of Salvia species are completely parenchymatous and collenchyma was observed in both under and over the median vein. It has been observed that in S. hypargeia, mesophyll is parenchymatous and the median vein is surrounded by collenchyma cells. And the characteristic feature of Lamiaceae family is a quadrangular stem and well developed collenchymas, supporting tissue at the corners of stem and the arrangement of vascular bundles in the petiole of this family is important in the point of taxonomy.

Passioura (1976) has reported that the qualitative and quantitative anatomical leaf characters are used to discuss that ecological phenomenon of
leaves of 11 species. The stomatal frequency is generally higher at the apex than at the base and higher at the margin than in the vicinity of the midrib. Roth (1977) has described the presence of a hypodermis, mainly constituted by collenchyma, it is constant in all the studied species; however, there is a specific variation in the number of layers and the degree of lignification. The collenchymatous hypodermis is common in fruits with a thick outer skin, which is observed in many berries and drupes, such as some species of *Ribes, Berberis* and *Paris*.

The *Solanum* species are mostly herbs with branched tap roots. The stem is herbaceous, erect and hairy; leaves are alternate, opposite in flora region, simple and estipulate. The placentation is axile, ovules are numerous, style single terminating in a bilabel stigma Esua (1977). Metcalfe and Chalk (1979) pointed out that there were both anomocytic and anisocytic stomata present in the family Boraginaceae, mainly anisocytic stomata were observed. Anisocytic stomata were also described in some *Onosma* species (Boraginaceae) Olowokudejo, (1990). The plant species are amphistomatic with more stomata on the lower surface (abaxial) than the upper surface (adaxial). The percentage of stomatal index of the two taxa was the highest in the lower epidermis 44% – 60% compared to the upper epidermis 18% – 21%.

The same workers have reported that there are glandular and non glandular hairs on the epidermis of stem, leaf, petiole and calyx. They are unicellular or multicellular. Non glandular hairs are more common and abundant than glandular hairs. It is determined that the family Boraginaceae had glandular
and Non glandular hairs. Nwachukwu and Mbagwu (2006) have reported that the absence of trichome in the two plant species studied may not be of taxonomic importance and hence not strange since insisted that much reliability is not always accorded to trichomes alone for taxonomic conclusion due to their similarities in different species.

Metcalfe and Chalk (1979) have reported dense glandular and eglandular hairs on the surfaces of both epidermis. The covering eglandular trichomes are 1-5 cellular. Unicellular hairs are usually on the lateral side of leaf. There are two types of glandular trichomes labiatae type and head and stalk unicellular type, they are embedded in the surfaces of both epidermal layers. Walter, (1983) has reported the morpho-anatomical characters of leaves and stems in plants of the studied populations showed numerous common features indicating that the species *T. arduini* belongs to the adaptive type of xerophytic ecotype of the same species.

Jellings and Leech (1984) studied the effect of ploidy level on leaf anatomy, studied the variation in over 20 leaf characters including morphological, anatomical and cellular features in nine *Triticum* genotypes at three levels of ploidy, where the characters measured most strongly influenced by ploidy level was cell size. Trease and Evans (1984) have explained that the genus *Datura* is characterized by anisocytic stomatas, glandular trichomes, non glandular trichomes, non – glandular and druse type of calcium oxalate crystals differentiating *D. inoxia* from *D. stramonium* the arrangement of drusa types in the form of “U” shaped in *D.inoxia*, while in *D. stramonium* were distributed in
dispersed form, besides, the glandular trichomes in *D. inoxia* possess stalk and unicellular head; in *D. stramonium* the stalk is bicellular and the head is multicellular.

Werker *et al.*, (1985) showed that the morphological characters such as the number of fertile stamen, type of stamen, characteristics of glandular and non glandular trichomes, shape of corolla and calyx, structure of bract are the important taxonomic value. Classification of glandular and non glandular trichomes of *S. hypargeia* have been made according to the previous classification, in addition to that the number of basal cells of glandular and non glandular hairs also have been used in this study. *S. hypargeia* has an important feature is brown-green bracts with their thick and broadly-ovate forms like the normal leaves.

Wills (1985) has reported that the genus *Solanum* belongs to the family of Solanaceae. Members of this family are mostly herbs and twiners with about 70 genera and 2,000 species. (Ahmed, 1964 and Patel 1969), have recorded about 85 genera and 2,200 species. Schippers, (2001) had reported that the *Solanum macrocarpom* and *Solanum nigrum* are edible. They serve as foliage for feeding livestock but excess intake of *Solanum* plants, especially those with the bitter taste, may lead to fruit toxicity and spinal bifida i.e. non joining of spinal bones due to ingestion of two much solanine.

Myers *et al.*, (1987) observed that osmoregulation by the cell size is not as effective as regulation by changes in solute concentration, the latter process
accounted for much of the difference in tissue osmotic potential of leaves of *Castanospermum austral* (Bazzez, 1979). Messier and Bellefleur (1988) have reported that the plants grown under high light intensities also show a high stomatal frequency. The difference in stomatal frequency is always small between the stream and non-stream habitats, as well as between the margin and the interior of the forest, the higher stomatal frequencies may primarily be a reaction to the favourable photosynthetic conditions and the radiation is many times higher under pioneer vegetation than within the forest.

Nakipoglu and Oguz (1990) have mentioned that the separation of vascular bundles of seven *Salvia* species into two groups as those in the species with basal leaves and those in the species without basal leaves and to this separation, the central vascular bundles of the species with basal leaves were divided, while those of the species without basal leaves were single, large and undivided. *Salvia argentea* L., a plant with basal leaves, had 4-7 central vascular bundles and 3-5 small bundles at each end of petiole.

Pyke *et al.*, (1990) has pointed out the comparison of anatomical features of leaves between diploid and natural tetraploid species of *Triticum*. They reported anatomical variation in first leaves of *Triticum*. Barboza and Hunziker (1994) have mentioned that annual herbaceous plants are often viscid, prostrate, and adaptated to dry environments with stony soil. The genus has been traditionally placed in subfamily Solanoideae. This position is supported by phytochemical analysis that demonstrates that *Exodeconus* shares the presence of *Withanolids* in the 13 others members of its subfamily.
Edeoga and Okoli (1995) have reported that the presences of one or two types of calcium oxalate crystals in the root of *S. nigrum* are also interesting features. The type of crystals are an important characters used to solving taxonomic problems. This is not a new thing since long back most researchers have applied it in several families including *Curcurbitaceae* and *Dioscoreaceae*.

D’arcy *et al.*, (1996) showed that the presence of calcium oxalate crystals is disposed in a band of cells just inside the epidermis at the stomium level. When pollen is matured, the anthers will break down, releasing their crystals. Crystals are found appearing in the early stages of flower development in most Solanaceous anthers. Oxalate crystals are disposed in a band of cells just inside the epidermis at the stomium level.

Krause and Arduin (1997) said that free-hand cross sections, using a microtome, were made in the median portion between stem internodes. They were clarified using 50% sodium hypochlorite solution. Mayo, *et al.*, (1997) and LAWG, (1999) had quoted the term “midrib” preferred to “midvein” because the midrib among monocots usually contains several to many discrete and scattered veins arising independently within the vascular bundles in midribs enter the lamina at intervals particular or in groups to form the secondary veins. In many *Araceae* aroid genera to term lateral veins refers to “Lateral primaries”. The primary vein is dominant in pinnate leaves and normally synonymous with the midrib. Okwulehi and Okoli, (1999) reported that the morphological and leaf epidermal features of *Capsicum* plants are of immense interest in taxonomy.
Stern (2000) has reported that the *Capsicum annum* and *Capsicum frutescens* are third among the cultivated vegetables being utilized in the dry state as spice, *Capsicum* content, an alkaloid that is a digestive stimulant, is used as ointment for arthritis and neuropathic pains. Edeoga (2002) studied the morphology of the leaf epidermis in *Annona* and suggested the utilization of this character in the identification of the species. Davis, (1959) showed the most extensive investigated family where anatomical features provided the very useful taxonomic characters in *Gramineae* and several authors have constructed keys for the identification of some taxa within the family based on leaf epidermal characters. Edeoga and Ikem (2001), Metcalfe and Chalk, (1960) observed that the anomocytic type of stomata which are characteristic features of the two taxa such as *Boeheavia* species and *Vigna* species. Silva – Lima *et al.*, (2005) have reported that the knowledge of anatomy is essential when vegetative propagation is used to identify important structural features necessary for propagation success. The stake process, in perennials xylem, adventitious roots is formed from meristematic cells in the secondary phloem, radial parenchyma or vascular cambium. Tetraploid type has secondary vascular tissues, phloem and xylem, more developed than in the diploid type, it is possible that the numbers of adventitious roots are greater in tetraploid than diploid one.

Mbagwu and Edeoga (2006) mentioned the differences in vegetative anatomy and suggested that a separate specific status for *C. afer* and *C. lucanusianus* as opposed to the conspecific treatment given to them by previous researchers in Leguminosae-Caesalpinoideae, where the nature of
unicellular and multicellular trichomes are described in certain species of *S. hirsuta* and the diagnostic features of these two type of trichomes. Mbagwu and Edeoga, (2006) studied the root anatomy of *S. macrocarpon* and *S. nigrum*. The anatomical features of the roots of these two taxa have not been documented. The objective of these investigations was to describe the root anatomical characters of the two *Solanum* species and to assess the relevance, the root anatomical features could be utilized in biosystematic consideration of similarities in structural and reproductive biology.

Senthil Kumar *et al.*, (2010) have reported that in the anatomical studies of *W. somnifera*, the leaves are dorsiventral with prominent midrib and bilaterally symmetrical lamina. The midrib has broad abaxial hump and semicircular abaxial. The midrib consists of a wide, arch shaped shallow bicollateral vascular bundle and heterogeneous growing tissue. The xylems elements are narrow, thick walled and are disposed in short radial files. The lamina is 270 μm thick. Non glandular trichomes are abundant especially on the abaxial side of the lamina. The lateral veins are prominent and form distinct reticulate venation pattern and the periderm is narrow and not well defined.

2.9. **Phytochemical analysis – GCMS**

Le Thanh *et al.*, (1997) have reported the presence of more than 40 constituents in the rhizome oil of *Piper betle* through GC-MS study. The major constituents are α – cadinol (26.2%) δ cadinon (11.7%) and about equal amounts of T-cadinal and T. mucrols (unseparated, together 20.7%). Meei-Jen Liou *et al.*, (2002) has reported the isolation and structural elucidation of four new
naphthothydroquinones, rubinaphthins A-D, together with 11 known compounds from the root of *Rubia yannanenbis* plant and the presence of 15 biologically active compounds through GC-MS in two fatty oil fractions from leaves of *Artabotrys odorantissimus*.

Edeoga *et al.*, (2005) carried out phytochemical analysis in the medicinal plants like *Cleome nutidosperma*, *Emilia collinea*, *Euphorbia heterophylla*, *Physalis angulata*, *Richardia bransitensis*, *Scoparia dulcis*, *Sida aceta*, *Spigelia anthelmia*, *Stachytarpheta cuyennesis* and *Tridax procumbens*. All the plants contained alkaloids, tannins and flavonoids, except the absence of tannins in *Sida acuta* and flavonoids in *Stachytarpheta cayemnessis* respectively. Owoyale *et al.*, (2005) have carried out phytochemical analysis in the leaves of *Senna alata* to reveal that the most active chromatographic component was a flavonoid glycoside.

Theeshan *et al.*, (2005) reported that the primary and secondary metabolite composition of vegetative and reproductive parts of *Cassia fistula* emphasise on potent phenolic antioxidants such as anthraquinones, flavonoides and flavan-3-ol derivatives. Ogunkunle *et al.*, (2006) have the established a concordance between the local medicinal applications of the plants and the phytochemical groups which are relevant to the pharmaceutical industry. His study represented the plants as potential sources of raw materials in the chemical and pharmaceutical industry. Areej *et al.*, (2007) have reported a comparative study of the fatty acid contents of three *Clematis* species growing in Saudi
Arabia namely \textit{Clematis hirsuta}, \textit{C.sinensis}, \textit{C.wighti} by GC-MS technique. Palmitic acid was reported to be the major fatty acid in the three species.

Nwaogu \textit{et al.}, (2007) have carried out phytochemical analysis in the leaves of \textit{Landolphia Owariensis} to reveal the presence of alkaloids, flavanoids, tannis and saponins. They have carried out this analysis in twelve species of Indian medicinal plants to reveal the presence of alkaloids (positive test for Wagnor’s) and flavonoid, though the later was in lesser amount than the other secondary metabolites of tannins, saponins, steroids and cardiac glycosides. Sapana \textit{et al.}, (2008) have investigated the chemical constituents in the aerial roots of \textit{Ficus benghalensis} by means of GC-MS, which showed the existence of hydrocarbons ranging from 16-36 carbon atoms.

2.10. \textbf{Antimicrobial screening}

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Cowan, 1999). Plant derived medicines have been part of traditional healthcare in many parts of the world for thousands of years and increases interest in plants as sources of agents to fight microbial diseases (Chariandy \textit{et al.}, 1999). There is an alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000) and there is a constant need for discovering new and effective therapeutic agents (Bharnani and Ballow, 2000).

Atalay \textit{et al.}, (1999) reported the antimicrobial screening of 16 crude extracts of 8 plants, tradionally used in Turkey for treatment of many diseases.
The plant species were found to possess an activity against at least one or more test microorganisms. The water-insoluble parts of the methanolic extracts were found to be more effective than the water soluble ones against test microorganisms. The n-hexanic extract from *Tymus fallax*, methanolic extracts from *Nigella sativa, Hypericum scabrum* and *Allium scorodoprasum* showed marked activity against gram-positive bacteria whereas seven were active against *Bacillus cereus* and eight against *Salmonella aureus*.

The bioactive amides affinin and Capsaincin isolated respectively from roots of *Heliopsis longipes* and fruits of *Capsicum sp.* were assayed for activity against *Escherichia coli, Pseudomonas solanacearum, Bacillus subtilis* and *Saccharomyces cerevisiae* suspension cultures. The alkamide affinin inhibited growth of *E.coli* and *S. cervisiae* at concentrations as low at 25 μg/ml. higher concentrations of affinin were necessary to inhibit the growth of *P. solanacearum* and *B. subtilis*. However, high concentrations of Capsaicin only recorded the growth of *E. coli* and *P. solanacearum*, whereas growth of *B. subtilis* was strongly inhibited and that of *S.cerevisiae* was initially enhanced (Jorge et al., 1999).

Kudi et al., (1999) screened eight Nigerien medicinal plants used traditionally in the treatment of infectious and septic diseases in both human and animals using hole-plate diffusion. The ethanol extracts were assessed for antibacterial activity against gram-positive and gram-negative bacterial species. The result showed that the ethanol extracts of *Anogeissus schimperi* and
Anacardium occidentale had good antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*, which are gram-negative.

Prashanth and John (1999) demonstrated the antibacterial activity of *Peganum harmala* using three solvents such as Petroleum ether, Chloroform and Methanol. The results showed that the methanol fraction was found to be most effective against all tested microorganisms followed by the Chloroform fraction. Balakrishna, *et al.*, (2000) demonstrated antibacterial activities of the chloroform soluble and insoluble portions of the alcoholic extract of the aerial parts of *Solanum trilobatum*. The study was done in eight bacteria at 20.0, 15.0, 10.0, 7.5, 5.0 and 2.5 mg/ml concentration levels. Streak method was employed. Activities were found only at higher concentration levels, (10-20 mg/ml) for bacteria. It was found that chloroform soluble fraction did not contain any phenol or alkaloid compounds except traces of solasodine.

Aqueous and alcoholic extracts of leaves of *Gloriosa superba* were subjected to antimicrobial activity against some bacteria such as *Staphylococcus sureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*. Filter paper disc agar diffusion method of Maruzzella and Henry was used for the *in vitro* evaluation of the antimicrobial activity. The antimicrobial results were found to be significant for both aqueous and alcoholic extracts against all strains (Subashini *et al.*, 2000). Thapliyal, *et al.*, (2000) analysed the antifungal activities of crude protein extract, isolated from the leaves of *Rauvolfia tetraphylla* and some other plants, were tested against *Trichosporium vesiculosum*, *Macrophomina phasenlina* and *Aspergillus flavus*. The spore
germination of all the pathogens was totally inhibited by the extracts of *R. tetraphylla, Andrographis paniculata* and *Piper longum* at the concentrations of 66, 48 and 30μg, respectively. Extracts of *Plumbago zeylanica* and *Terminalia arjuna* showed 90% inhibition of spore germination of *T.vesiculosum*.

Aida *et al.*, (2001) studied the antifungal activity of aqueous, dichloromethane and methanol extracts from 14 Paraguayan plants used in traditional medicine for the treatment of skin diseases. The dichloromethane extracts of *Trichophyton avellanedae, G.repens* and *Clostridium multiflorum* showed a broad spectrum activity. The bark of *Trichophyton avellanedae* was found to be active against most of the fungal strains tested. It showed maximum inhibition zones against *Aspergillus fumigatus, Clostridium neoformans, Microsporum gypseum, Penicillium purpurogens, Saccharomyces cerevisiae* and *Trichophyton mentagrophytes*.

Anburaji *et al.*, (2001) screened the effect of methanolic extracts of 19 Jordanian plants combined with 7 different antibiotics for the resistance of *Pseudomonas aeruginosa*. A resistant strain isolated from a patient and standard strains of the same were used in the study. The result showed that there were significant variations in the effects of some combinations used on the resistant and standard strains. Antibacterial activity of essential oils of *Anisomeles malabarica* and *Ocimum gratissimum* were studied. The essential oils were extracted by hydro distillation using Clevenger’s apparatus. The effect of the essential oils against four Gram positive two Gram negative bacterial strains was tested by filter paper disc diffusion technique, having Gentamycin a broad
spectrum antibiotic as control. *O. gratissimum* was found to be more effective with the six bacterial strains tested (Anne *et al.*, 2001)

John Britto (2001) analysed the comparative antibacterial activity of leaf and stem extracts of *Solanum incanum* against some mutant strains of *E. coli*. It was found that both the stem and leaf extracts of this plant were active against all strains of *E. coli*. However, the extent of inhibition varied among the strains. In KL16 the leaf extracts showed that they are relatively more active than the stem extracts.

The antifungal activity of *Belamcanda chinensis* was evaluated by a single-cell bioassay method. Tectorigenin (5,7-dihydroxy-3(4-hydroxy phenyl)-6-methyox-4H-1-benzophron-4-one), isolated from the active fraction, showed marked antifungal activity against dermatophytes in Trichophyton, the minimum inhibitory concentration (MIC) being in the range of 3.12-6.25 mg/ml (Oh KB *et al.*, 2001). The antimicrobial activity of *Allium sativum* was tested on three human pathogenic bacteria viz., *Staphylococcus aureus, Salmonella typhimurium* and *Yersinia enterocolitica* by disc diffusion method. The zone diameters observed were 30, 27 and 23 against $7 \times 10^8$ cells of *S. aureus, S. typhimurium* and *Y. enterocolitica* respectively at corresponding minimal bactericidal concentrations (MBCs) of *A. sativum*. The minimal inhibitory concentrations (MICs) and MBCs against *S. typhimurium, S. aureus* and *Y. enterocolitica* were 7.5, 8, 10 mg ml$^{-1}$ and 22.5, 24, 40 mg ml$^{-1}$ respectively. The results showed that *A. sativum* was also effective towards *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans* (Elizabeth, 2001).
The chemical composition of the volatile constituent from flowering parts of *Thymus revolutus*, an endemic plant of Turkey was analysed by GC/MS. Twenty-two components were identified and carvacrol was found as predominant in the oil. Using disc diffusion method the essential oil was tested against 11 bacteria. The results showed that *Thymus hymalis* displayed high level of antimicrobial activity (Karaman et al., 2001).

Pundarikakshudu *et al.*, (2001) tested alcoholic extracts of *Galega officinalis*, on gram-positive and gram-negative bacteria as the plant was claimed to hasten skin healing after surgery. Ethanolic (60%) extract exhibited significant inhibition on growth of both gram-positive and gram-negative bacteria.

The antimicrobial as well as anti-inflammatory activity of crude methanol extracts of leaves of *Mallotus peltatus* was evaluated. The MIC ranges 128 to 2000mg/ml for bacteria and 128 mg/ml for fungi, while the MBC was 2, 4 fold higher than MIC. The methanol extract at 200 and 400 mg/kg and the n-butanol fractions A and B at 25 mg/kg, exhibited significant anti-inflammatory activity in albino rates compared with indomethacin. Further study with fraction showed that the antibacterial and anti-inflammatory activity was due to either fraction A (ursolic acid) alone or the combination of fractions A and B (Beta sitosterol and fatty acids) of the extract (Chattopadhyah *et al.*, 2002).

Fyrquist *et al.* (2002) have reported the antimicrobial activity of the crude extracts of *combretum* and *Terminalia* species by the agar diffusion method. The
methanol extracts of the roots of *Terminalia sambesiaca, T. kaiserana, T. sericea, Combretum fragrans* and *C. padoides* showed marked inhibition against gram-positive bacteria and also on *Enterobacter aerogenes*. All extracts of the roots of *T. sericea* (methanol, ethanol acetone and hot water) had good antimicrobial activity. The methanolic leaf extract of *T. kaiserana* was found to have a bacteriocidic effect on *E.coli*. The crude ethanolic extracts of 16 Siberian medicinal plants were tested on bacterial organisms such as *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Of the 16 plants tested, 12 showed antimicrobial activity against one or more species of microorganisms. The most active antimicrobial plants were *Bergenia crassifolia, Cheliclonium mayus, Rhaponticum carthamoides, Sanguisorba officinalis* and *Tussilago farfara* (Kokoska et al., 2002).

MeGaw et al. (2002) studied the effect of roots of *Schotia brachypetala* used by traditional healers to treat dysentery and diarrhoea. Activity directed fractionation of the ethanol extract of the dried leaves yielded 9, 12, 15-octadecatrienoic acid and methyl-5, 11, 14, 17-eicostetraenoate. These fatty acids exhibited antimicrobial activity against the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and to a lesser extent against the gram-negative *Escherichia coli* and *Klebsiella pneumoniae*.

Panizzi et al. (2002) tested *Rubus ulmifolius*, for its antimicrobial activity on bacteria of increasing polarity extract and also isolated some constituents, such as quercetin-3-O-β-D-glucuronide; kaempferol-3-O-β-D-glucuronide gallic acid, ferulic acid and tiliroside. The phenolic and tannin fractions showed
high antimicrobial activity. Suri, *et al.*, (2002) analysed by disc-diffusion method methanolic and volatile oil extracts of *Citrus hystrix* and *in vitro* derived callus. The volatile oil obtained from leaf and fruit inhibited bacterial growth, which could be ascribed to the presence of citronellal. All gram-negative bacteria were weakly inhibited by the volatile oil obtained from callus treated with 0.25mm proline. Leaves were more potent against gram-positive bacteria. Yashodharan *et al.*, (2002) screened the seeds of 21 Scottish plant species from 14 different families. The n-hexane, dichloromethane and methanol extracts were assessed for antibacterial activity against 11 pathogenic bacterial species. The results of the testing showed that the methanol extracts of 11 plant species showed significant antibacterial activity.

Acetone, methanol and water extracts obtained from the shoots of *Arctotis arctotoides* through shaking and homogenization, were investigated for their antimicrobial activities. Growth inhibition using agar dilution assays was determined against ten-selected bacterial strains. Acetone and methanol extracts were very active against the gram-positive bacteria. The gram-negative bacteria were, however, more resistant to the extracts than the gram-positive ones. None of the extracts inhibited *Klebsiella pneumoniae* and *Pseudomonous aeruginosa* (Afolayan, 2003).

Extracts of *Zehneria scabra* tubers were studied for their antibacterial activities against *Eschericha coli, Pseudomonas aeruginosa, Salmonella typhi, Enterobacter faecalis* (gram-negative) *Bacillus subtilis* and *Shigella dysenteriae* (gram-postivie) using disc-diffusion method. Aqueous, chloroform and ethanolic
extracts were screened for the activity and the zones of inhibition were recorded. Results suggest that the ethanolic root extract had significant antibacterial activity against the test microorganisms. Present findings justify the claimed uses of *Zehneria scabra* in the indigenous system of medicine to treat various infectious diseases like fever, diarrhoea and skin rashes (Anand and Jayachandran, 2003). Antibacterial activity of aerial parts of the extract of *Achyranthes bidentata* was analysed by using four solvents, Petroleum ether, Chloroform, Methanol and Aqueous) and four bacterial organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*). The results from Methanol extract showed optimum antibacterial activity when compared to the other extracts (Balakrishnan *et al.*, 2003). Aqueous and methanol extracts of the leaves of *Juniperus oxycedrus* were investigated for their *in vitro* antimicrobial properties. The antimicrobial activity of the extracts against 143 laboratory strains was evaluated, based on the inhibition zone using the disc-diffusion assay. The aqueous extract of *J. oxycedrus* had no antimicrobial effect against the test microorganisms whereas the methanol extract had inhibitory effects on the growth of 57 strains of 24 bacterial species in the genera of *Bacillus*, *Brevundimonas*, *Brucella*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Xanthomonas* (Karaman *et al.*, 2003).

*Alangium salviifolium* is a valuable ethnomedicinal plant used in indigenous systems. The leaves were extracted with water, ethanol and chloroform and each extract was evaluated for antibacterial activity against pathogenic strains of *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*,

Comparative studies of *Withania somnifera* (L.) Dunal, and *Withania obtusifolia* tackh - M. SENTHIL KUMAR
Enterobacter faecalis, Serratia marcescens and Klebsieilla pneumoniae by using disc diffusion method. The zones of inhibition were recorded and compared with standard antibiotic drugs i.e Chloromphenicol. Ethanolic extract showed a high degree of inhibition when compared with chloroform and aqueous extracts (Natarajan et al., 2003).

Cocculus hirsutus, studied the antimicrobial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi using agar disc-diffusion method. Petroleum ether extract, ethanolic extract and crude alkaloidal fraction were screened for the activity in various concentrations and zone of inhibitions were recorded. Results suggested that the ethanolic extract and crude alkaloidal fraction were concentration dependent (Satish and Singhai, 2003).

Somchit et al. (2003) investigated antimicrobial activity of crude ethanol and water extract of Cassia alata. The leaves and barks were studied against bacteria Staphylococcus aureus and Escherichia coli by using disc-diffusion method. The result of the testing showed that the water extract exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zones of 11-14 and 9-11mm, respectively). Sunayana, et al., (2003) have worked on the antibacterial activity of the aqueous, ethanol and chloroform extracts from the leaves of Plectranthus amboinicus by using disc-diffusion method against Escherichia coli, Proteus vulgaris, Serratia marcescens, Shigella dysenteriae, Salmonella typhi and Pseudomonas aeruginosa. Among various extracts, the ethanol leaf extracts showed high degree of inhibition than the other solvents.
used. It exhibited the greatest activity against *E. coli* and *proteus vulgaris*, moderate activity against *Serratia marcescens*, less inhibition against *Salmonella typhi* and no activity against *Pseudomonas aeruginosa*.

Babayi and Kolo (2004) reported the inhibitory activity of methanolic extract of *W. somnifera* root powder against *Staphylococcus aureus* and *E. coli* whereas the results of methanolic root extract of *W. somnifera* are better than the results against *Streptococcus mutans*. Negative control showed no formation of zone of inhibition. The encouraging results indicate the aqueous and methanolic root extracts of *Withania somnifera*. Jain, *et al.*, (2004) reported that *Mimosa hamessa* pronounced bio efficacy against the bacteria *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Alanis *et al.* (2005) screened 26 medicinal plants from Mexico for antibacterial properties against eight different species of Enteropathogens: *Escherichia coli*, *Shigella sonnei*, *Shigella flexneri* and *Salmonella* species. The extracts from *Caesalpinia pulcherrima*, *Chiranthodendron pentadachtylon*, *Cocos nucifera*, *Geranium mexicanum* (aerial parts and roots), *Hippocratea excelsa* and *Punica granatum* possessed strong antibacterial activity against most of the pathogens tested. The results of *Shigella sonnei* showed the highest susceptibility to both extracts. Asghari, *et al.*, (2006) has reported that the methanolic extract was active against *S. aureus*, *S. epidermidis*, *E. fecalis*, and *L. monocytogenes*. Chloroform and hexane extracts were active against *S. aureus*, *S. epidermidis* and *E. Fecalis*. No activity was observed against five Gram-
negative microorganisms (E.coli, Klebsiella, Proteus, Salmonella and P. aeruginosa) and B. subtilis as a Gram-positive microorganism. Raghavendra et al., (2006) have reported that the methanol extract showed highly significant activity when compared with K-cycline and Bact-805 against plant pathogenic bacteria, the human pathogenic bacteria methanol extract showed moderately significant antibacterial activity when compared with streptomycin. Among plant pathogens X a.p. was highly sensitive. Among human pathogens S. Flexneri, Salmonella paratyphi Streptococcus faecalis and S. aureus were highly sensitive to methanol and ethanol extracts.

Abdulmoneim (2007) has reported the methanol and chloroform, the maximum inhibition zone was found in 75 mg mL\(^{-1}\) extract concentration and it was 16.18 – 1.65 mm in the fungal isolate of the pathogen Trichophyton rubrum, while the least inhibition zone in the same concentration was 15.11-0.48 mm for the pathogen Candida albicans. The maximum inhibitory effect for methanol extract at 25 mg mL\(^{-1}\) was observed also against Candida albicans where the zone of inhibition was 14.00 – 0.11mm against standard antibiotic (16.00-0.1mm) and a minimum activity was observed against Trichophyton rubrum (10.51-0.45mm).

Kambizi and Afolayan (2008) reported that Aloe ferox and W. somnifera have water extracts from W. somnifera, however, exhibited complete inhibition of N. gonorrhoea at 10 mg/ml. The ability of water extract from this plant to inhibit the growth of N. gonorrhoea may be the reason for its use by the herbalists for the treatment of N. gonorrhoea. Musyimi, et al., (2009) have
reported that the *A. mossambicensis* has confirmed antimicrobial activity against gram negative bacterium *S. typhi* and gram positive bacterium *S. pyogenes*, including the fungal pathogen *A. niger*. The leaf extracts showed greater growth inhibition than roots. *Streptococcus pyogenes* showed lesser growth inhibition with root extracts in comparison with the other two organisms, suggests that the growth inhibition was caused by different active compounds.

Farah *et al.* (2010) reported that the aqueous extract of *W. somnifera* shows maximum antimicrobial activity is against the test microbes with zone of inhibition in the range of 33 mm to 50 mm, but in the methanolic extract of *W. somnifera* also showed significant antimicrobial activity with the zone of inhibition in the range of 15mm to 38mm. Ciprofloxacin was used as positive control as an antibacterial antibiotic which produced the inhibition zones ranging between 10 and 35 mm whereas *C. albicans* was found to show a zone of inhibition of 22 mm to Ketoconazole which was used as positive antifungal antibiotic.

Senthil Kumar *et al.* (2010) has reported that the enhanced of maximum microbial activity found in the root when compared to leaf of *W. obtusifolia*. The phytochemical analyses showed the presence of nitrogen compound (8-Azabicyclo [3.2.1] octan-3-ol, 8-methyl-, endo-) as major constituents of the plant leaves and root are commonly known to posses antimicrobial activity. The *W. obtusifolia* acts as a potential source for biological antimicrobial activity against selective fungi and bacteria, which enable only human pathogenic fungi and bacteria to be controlled without any side effects.
Senthil Kumar et al. (2011) has reported that the fungal isolate of the pathogen *Trichophyton rubrum*, has the least inhibition zone found in leaf when compared to root for the same concentration. The minimum inhibition zone in 25μl concentrations was observed in *T. rubrum* with 1.2mm in leaf extract. Maximum inhibition zone was found in leaf extract 2.7mm in 100μl. There was an enhanced of maximum microbial activity found in the leaf when compared to root of *W. somnifera*.

### 2.11. Molecular characterization

In recent years seed proteins profile is used widely by SDS PAGE resolving systematic relationships and for the studies of inter and intra specific studies (Karihaloo et al., 2002). Khalifa et al., (1998) used protein profile to reassess the taxonomic relationships of 45 species belonging to 15 genera and 8 tribes of the Solanaceae. Based on the results he supported the conventional classification of this family. Two important species *W. somnifera* and *W.coagulans* of the same genus were also analyzed for seed protein (Karihaloo et al., 2002 and Anu and Peter, 2003).

Andre et al. (2005) has carried out SDS – PAGE analysis of the proteins extracted from *Glycine wighti* (Wight and Arn.) which revealed some variation in the banding pattern of seed protein. The protein patterns obtained from cotyledon calli powder electrophoresis was similar with proteins obtained from Soyabean and *G.wightii* seeds, predominance of bands with molecular mass characteristic of b-coglycinin and those of the glycinin, the main reserve proteins of soybean seeds.
Zubaida et al. (2006) have used SDS - PAGE as one of the tools for the evaluation of taxonomic status of species of Solanum. The seed protein profiles of 54 accessions belong to 11 species of Solanum genera. The worker has reported seed protein profiles of 54 accessions belong to 11 species of 2 different genera of the family Solanaceae, analyzed by SDS – PAGE. A dentogram based on UPGMA revealed the generic status of Solanum and Capsicum. S. surattense with white flowers showed variation based on protein profiles. Similarity index of S.villosum and S.americanum was 53% but both species showed of similarities 78% with S.nigrum the similarity was 41% between S.nigrum and S.americanum.

Zubaida et al. (2008) has reported that the W. somnifera acc 1097187 also exhibited differences in their protein profile. The band of 66 Kda and 64 Kda although with low intensities but present only in W. somnifera and absent from W. somnifera acc. 1097187. Similarly band at 50 Kda was part of W. somnifera profile only. Few bands in two profiles showed variation in their intensities. Band at 42 Kda was darker in W. somnifera acc. 1097187 whereas as band at 16 Kda was more intense in W. somnifera. Band at 22 Kda was absent from the profile of W. somnifera. This accession is also geographically different from the rest of specimens of W. somnifera. These differences are enough to give it the rank of sub species of W. somnifera.

Bhat et al. (2011) reported the protein band for highest molecular weight i.e., 56 KD generated in case of S.melongena while that of lowest molecular weight i.e., 10 KD was generated in D. alba. The similarity index calculated
after proteins of five selected experimental genera of Solanaceae was maximum i.e., 22.22% between *W. somnifera* and *S. xanthocarpum*, and next between *S. melongena* i.e., 20%, the lowest similarity index was found between *Lycopersicon esculentum* and *D. alba* i.e., 10% to find out intergeneric or interspecific correlation between members of family Solanaceae.

**RAPD**

Dellaporta *et al.* (1983) have reported that the polysaccharide co-precipitation is avoided by adding a selective precipitant of nucleic acids, cetyltrimethylammonium bromide (CTAB) to keep polysaccharides in solution through SDS. Katterman and Shattuck, (1983), have reported the presence of polyphenols, which are powerful oxidizing agents present in many plant species, can reduce the yield and purity by binding covalently with the extracted DNA making it useless for most research applications.

Dellaporta *et al.* (1983) have reported that the increasing use of DNA fingerprinting in plant and its potential use in herbal drug industry, the preparation of good quality and large quantity DNA has become a major concern. The extraction from tissue needed to be simple, rapid, efficient and inexpensive methods when many samples were used, such as in population studies, molecular breeding and screening of raw herbal drug materials. Several methods for extracting plant DNA from different plant parts including roots were developed (Keim *et al.*, 1989; Kumar *et al.*, 2003).
Dellaporta et al. (1983) have reported that the extraction of DNA is not always routine and simple, and conventional methods are not reproducible for all species, especially for dry material. Simple extraction protocols suggested by Wang and Taylor (1993) did not yield any DNA from dry materials of _Hesperis_ specimens, and the use of detergents such as CTAB (Doyle and Doyle, 1987), SDS (Dellaporta, 1983), and polyvinylpyrrolidone (PVP) (Lodhi et al., 1994).

Doyle and Doyle (1987) have reported that high quality DNA is required for molecular biological studies of plants. Several DNA extraction procedures for isolating genomic DNA from various plant sources have been described, including the salt extraction method and the cetyltrimethyl ammonium bromide (CTAB) and its modifications (Rogers and Bendich, 1985). The need for a rapid and simple procedure is urgent, especially when hundreds of samples need to be analyzed (Kang and Yang, 2004).

Doyle and Doyle (1987) have reported that the efficient protocol for isolation of DNA as well as the optimization of the PCR conditions is required. DNA extraction was improved by modifying some of the steps in the original CTAB DNA isolation protocol. Leaves are fresh and young. Samples frozen in liquid nitrogen and stored at –80°C for several weeks can also be used. The presented procedure resulted in extracting, high quality, low-polysaccharide genomic DNA from eight different plant species belonging to different genera including recalcitrant woody species. Various protocols for DNA extraction have been successfully applied to many plant species which were further modified to
provide DNA suitable for several kinds of analyses (Wang and Taylor, 1993; Ziegenhagen and Scholz, 1998).

Zehr et al. (1987) reported that the alteration of repeated DNA sequences induced genetic variation during the callus culture RAPDs were being used for detection of genetic polymorphism in cereals (Wang et al., 1994). Gene introgression studies (Durham et al., 1994) to characterize different wild and cultivated genomes, salt tolerant and resistant rice species/varieties and genome specific, species specific and cultivar specific RAPD markers can be identified (Farooq et al., 1995; Farooq et al., 1996).

Williams et al. (1990) reported that the RAPD (Random Amplified Polymorphic DNA) fingerprints showed several advantages over the conventional methods used previously, isozyme polymorphism and RFLP (restriction fragment length polymorphism). Isozymes are limited to a number of genes coding for soluble proteins, consequently they have limited power of discrimination among cultivars and varieties and also these methods are limited by environmental conditions. RFLP technology has been till now the most frequently used tool to evaluate DNA polymorphisms among cultivars. Its limitation is due to complex methodology and radioactive needs. In addition to that micrograms of genomic DNA are also required to perform RFLP hybridization.

Williams et al. (1990) reported that the Polymerase Chain Reaction (PCR) technology has made possible to study the genetic differences in crop
plants and animals. DNA fingerprinting, gene mapping and phylogenetic studies have tremendously benefited from PCR. Eight different RAPD primers have to be used to confirm the variations. Polymorphism thus detected was based largely on the presence and or the absence of characteristic band, intensity of these bands and in some cases variation in the size of the bands. Similar primers were used to detect polymorphism for identification of different genotypes (Farooq et al., 1995; Farooq et al., 1996).

The technical ease of RAPD markers and the facility of their application to new species have led their employment in many organisms including forest trees, crop as well as medicinal plants and lower plants for genetic linkage mapping (e.g., Carlson et al., 1991; Tulsieram et al., 1992; Nelson et al., 1993; Grattapaglia et al., 1994), phylogeny and systematics (Caetano, et al., 1992; Kaemmer et al., 1992; Wilde et al., 1992; Joshi and Nguyen, 1993; Samec, 1993; Caetano, 1994) and population genetics This technique is one of the best methods among available DNA-based tools for scoring variations between cultivars within species (Penner et al., 1993; Jones et al., 1997). This is due to the sensitivity of RAPD banding patterns to reaction conditions, and the difficulty in exactly replicating reaction conditions across laboratories, where different brands of thermocyclers may be used.

Clumps of Conospormum somatic embryos derived from single zygotic embryo underwent adventitious embryogenesis and differentiation from embryogenic callus. RAPD analysis was performed on 20 shoots and 20 embryos regenerated from these cultures to assess genetic stability Wang and
Taylor (1993). Castiglione et al., (1993) reported that the treatments of DNA with protease K gave sharp and clear amplification products, compared with untreated DNA. This may have resulted by inactivation of endogenous endonucleases. Annealing temperatures lower than 35°C led to the generation of very crowded RAPD patterns, while higher annealing temperatures gave insufficient amplification products. In this connection, Castiglione et al., (1993) reported that an annealing temperature of 30°C gave more polymorphic bands.

Shah et al. (1994) have reported that the potential application of RAPD marker technology reveals its importance as a powerful tool in genetics and breeding which can play an important role in palm molecular biology. In this connection, they reported the utility of RAPD markers for the determination of genetic variations in oil palm.

Ratnaparkhe et al. (1995) used RAPD for assessing the genetic diversity and relationships in many plants, in an attempt to identify pigeonpea (Cajanus cajan) cultivars and their wild relatives. In coffee (Coffea arabica), the differences in morphology and geographical origin of the genotypes were reflected in the RAPD patterns (Orozco, et al., 1994). The procedure was also used to establish genetic diversity of potato genotypes including siblings and genotypes with no immediate relationship (Demeke et al., 1996). The genetic relationships between members of Fagaceae were assessed by RAPD which ascertained the taxonomic studies of a particular population of Fagus sylvatica, the ‘tortuosa’ variety (Gallois et al., 1998) Rajasegar et al., (1997) had also demonstrated the usefulness of RAPD analysis for cultivar development in
Ixora. In another study, the clonal identification and fingerprint analysis were demonstrated for *Picea abies* by Scheepers *et al.*, (1997) and a single primer was used to distinguish all the 100 different clones. On the other hand, a study in *Eucalyptus* genotypes with RAPD markers revealed the technique in resolving ambiguities in sampling and genotype identification. And also this technique could be effectively used to develop strain-specific SCAR (sequence-characterised amplified region) marker (Hermosa *et al.*, 2001). The potential use of RAPD technique to study the genetic diversity in *Brassica juncea* and its relationship to heterosis was successfully demonstrated by Jain *et al.*, (1994). Their studies showed that the genetic diversity formed a very useful guide for the selection of parents for heterotic hybrid combinations.

Moursy and Saker (1996) have reported that the improvement of date palm is very difficult due to its long life cycle, strongly heterozygous nature and difficulties to determine at early stages of growth. Most of the published studies on genetic characterization, detection of genetic variations and gene mutation have concentrated on the variations in chromosome numbers, isozyme polymorphism and biochemical diversity, the utilization of RAPD markers are very useful for analysis of the genetic diversity among different cultivars and various populations (Skrotch *et al.*, 1992, Bagheri *et al.*, 1995).

Powell *et al.* (1996) have reported that the genetic similarities were measured on the basis of AFLP results more reliable than the other markers since they have been based on the analysis of a large number of unbiased genetic markers. These markers represented a random sample of genetic loci distributed
along the genome and thus reduced the variation of similarities. Ellis, et al., (1997) and Zhu et al., (1998) have proved that AFLP is extremely proficient in revealing genetic diversity at the species level and also effective to study the wide area of the genome in single assay. However, comparative analysis using several PCR-based markers showed that the multi-locus AFLP technology is one of the best methods available for evaluation of germplasms (Powell et al., 1996 and Russell et al., 1997) claimed that AFLP are more efficient than SSR markers even though SSRs are co-dominant and detect the highest level of polymorphism per locus.

Jones et al. (1997) have reported that several PCR-based markers had been used to provide information on genetic variation in plant species. Initially, RAPD (random amplified polymorphic DNA) markers were employed for genetic analyses, but problems regarding reproducibility were reported. AFLP (amplified fragment length polymorphism) technique was introduced as a reliable and reproducible marker system. It was preferred to other DNA-based markers mainly because of its high multiplex ratio and non requirement of prior sequence information.

Paul et al. (1997) reported that in W. coagulans, a low level of polymorphism was observed, and this was expected as only a few plants have recently been introduced in Barmer district of Rajasthan from Baluchistan. Similarly, it has been observed that the Cambod population exhibited a low level of variability, because a small population size was analyzed.
Nkongolo (1998) reported that the genetic analysis of plants relied on high yields of pure DNA samples. In conifers, such DNA samples were usually derived from tissue culture. RAPD profiles obtained from embryogenic culture and seedlings were similar and highly reproducible. Efficient and consistent amplification was achieved using primer concentrations ranging from 1.6µm and 6µm with 0.1 to 1.0 mg template DNA. The intensity and resolution of faint bands resulting from non-optimized conditions were easily improved through further rounds of synthesis in a fresh PCR with amplified DNA diluted 500-fold.

Perera et al. (1998) have reported that the analysis of the data matrix of Withania individuals employing UPGMA resulted in the differentiation of W. coagulans from W. somnifera. The two major clusters obtained in W. somnifera correlate well with those based on the morphotypes. Zhu, et al., (1998) demonstrated the efficiency of the AFLP marker system in date palm cultivar fingerprinting using a few number of primer combinations. This could be attributed to the high multiplex ratio of the AFLP technique. These results were in consistence with the findings of different authors on different plant species of Paran et al., (1998) in pepper; Pillay and Myers (1999) Hussein et al., (2002) in cotton; Negi et al., (2000) in Withania species.

Barker et al., (1999) had reported that the percentage of polymorphism detected differed from one primer to other, which did not necessarily correlate with the number of bands generated. Similarly, it had been shown in Salix that out of five primer combinations used four produced similar patterns of clustering whereas the fifth primer combination gave discordant results. Kaeppler, et al.,
(2000) had reported that frequency of this variation was largely affected by the length of incubation period and cultivar specificity. Many other factors including genetic and epigenetic elements and accumulation of genetic changes might also be involved. It appeared that the changes at genetic level were abrupt and could not be reproduced successfully with similar tissue culture conditions. It was already documented that the genome of a somaclone could be variant at different location and the changes accumulate in complex characters during callus culture. A single somaclone might be variant in several traits and in progeny analysis, appeared to assort independently (Skirvin et al., 1994). The phenomenon was also supported by a number of reports (Sun and Zheng, 1990; Kaeppler et al., 1998; Olhoft and Phillips, 1999; Kaeppler et al., 2000).

Negi et al. (2000) reported the genetic variation among 35 individuals of W. somnifera and 5 individuals of W. coagulans which were xerophytic in nature and grew naturally in Baluchistan and two distinct morphological types of W. somnifera, namely Kashmiri and Nagori and employed AFLP markers to analyze the genetic variation among W. somnifera individuals. Based on the AFLP data, an attempt has been made to estimate the genetic similarity and identify genetic relationship within and between Withania species. Paterson et al., (2000) reported that comparative genetic molecular mapping in plants reveals a high level of conservation of gene content and order within grasses, crucifers, and legumes species more than 80% of the genes annotated in Arabidopsis was also found in rice (Bennetzen 2002). Comparative organization of the chromosomes of pepper and tomato revealed that both species are highly conserved (Tanksley et al., 1988; Livingstone et al., 1999).
Kreader et al. (2001) reported that the RAPD markers were a modification of PCR contrived in the late 1980’s (Williams et al., 1990). PCR provides a means by which billions of copies of a particular target DNA fragment would be made from a complex mixture of genomic DNA. Now it is becoming more powerful with the introduction of user-friendly and fully automated techniques. Puchooa and Sookun, (2003) have analysed tissue culture response and the effects of induced mutation through gamma-ray irradiation on Anthurium, an economically important crop of Mauritius. However, these changes could not be detected through the RAPD-PCR analysis of the DNA extracted from the irradiated explants. These have similar fragments showing that some more analytical methods such as Southern blotting and AMD (amplification and mismatch detection) could be used for the analysis of the genomic sequence variation.

Sharma et al. (2003) have explained the use of liquid nitrogen (or freeze-drying (lyophilization) of tissue for the initial grinding, and these processes are unavailable in many laboratories. In the protocols provided by Maniatis et al., (1982), after grinding the tissues in various extraction buffers, DNA is extracted with phenolchloroform, or the extract is dialyzed against EDTA and a buffered Tris-HCl solution. After extraction, the aqueous phase is concentrated, either by ethanol or isopropanol precipitation (Aljanabi and Martinez, 1997; Fang et al., 1992), or with microconcentrators (e.g., the Wizard genomic DNA purifi-cation system; Promega, USA).
Anista and Norzulaani, (2006) carried out RAPD to determine the clonal fidelity on *invitro Musa acuminata* CW. Betangan micropropated from male inflorescence derived from the same mother plant. Eighteen arbitrary decamer primers were used to amplify DNA from *invitro* plant materials. The result implied that male inflorescence could be used as an alternative explant commercial planting. Mandal *et al.*, (2007) had reported RAPD in *Costus speciosus*, an important medicinal plant species collected from 14 localities of Andaman and Nicobar Island through recurrence survey for RAPD-PCR analysis involving 12 decamer random primers used to assess the quantum of genetic variation at genomic level. Four primers showed appreciable intraspecies variation or molecular polymorphism at amplicon levels. Despite morphological identity a great deal of polymorphism was observed among the accessions.

John De Britto and Dharmar (2011) reported that the genetic variability in *W. somnifera* among accessions of different geographical region in Tamil Nadu was assessed through Random Amplified Polymorphic DNA (RAPD) markers. Five accessions of *W. somnifera* were screened with ten primers of which six primers were found to be the most informative. These primers produced multiple band profiles with a number of amplified DNA fragments varying from 5 to 9. A total of 37 polymorphic bands were observed. The utility of RAPD markers in estimating genetic variability has been demonstrated in several studies. A similar study was done in *W. somnifera* by Bilal *et al.*, (2010). Seven populations of *W. coagulans* from the districts of Kohat and Karak in Pakistan were analyzed by Syed (2009). Molecular analysis in *Urginea indica* collected from different location of Karnataka was reported by Harini *et al.*, (2008). Ruan *et al.*, (2008)
analyzed the DNA molecular characters of *Centella asiatica* with RAPD technology. Rout (2006) reported the genetic variation within 15 clones of *Tinospora cordifolia* through RAPD markers. Genetic diversity analysis in *Rauvolfia serpentina* and *Rauvolfia tetraphylla*, using RAPD Markers was carried out by Padmalatha and Prasad (2006, 2007).