Chapter – IV

Results and Discussion
4. Results and Discussion

4.1. Germplasm collection

The plants of *W. obtusifolia* and *W. somnifera* were collected from Karudamangalam in Tiruchirappalli and Pulavarnatham in Thanjavur districts of Tamil Nadu (Plate 1).

4.2. Habitat

The exploration survey was conducted at similar parts of Tiruchirappalli, Perambalur, Ariyalur and Tanjore districts of Tamil Nadu. It was found that the *W. obtusifolia* and *W. somnifera* plants were distributed in wild lands and it is mixed with cotton field and on the sides of the river. These species are mostly found to occur in the red soils and black soils. Flowering was seen throughout the year.

4.3. Seed dormancy and germination

Seed dormancy and its subsequent germination are complex adaptive traits in higher plants that are influenced by a large number of genes and environmental factors and the temperature is one of the important factors affecting seed germination in nature (Berrie 1966). When the seeds are exposed to high temperature conditions, both germination (Reynolds and Thompson 1971, Carter and Stevens 1998) and seedling growth (Ungar 1974, Sattelmacher *et al.*, 1990) are generally inhibited. Increase in temperature cause enhanced transpiration and evaporation and so, there is deficiency of water in plants. Decrease in moisture level in the germinating medium deeply affects the seed
performance. Studies of genetics and physiology have shown the important roles of the plant hormones, abscisic acid and gibberellins in the regulation of dormancy and germination (Gates, 1968). Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells (Aleel, 2006).

In *W. obtusifolia*, the highest (65%) seed germination was recorded in GA$_3$ at 500ppm, which was significantly greater than all other treatments (Table 3). The next best (60%) was recorded at 400ppm which was on (55%) par with GA$_3$ level at 600ppm. Among the IBA treatments, maximum germination (55%) was recorded in IBA at 400ppm. The next best (50%) was recorded in IBA at 500ppm which was on par with IBA at 600ppm (45%). The lowest (25%) seed germination was recorded in control. Among the GA$_3$ treatments, at 500ppm (65%) and among IBA treatments, 400ppm (55%) recorded higher germination percentage (Plate 2).

Whereas in *W. somnifera*, the highest (57%) seed germination was recorded in GA$_3$ at 500ppm, which was significantly superior over all other treatments. The next best (50%) germination was recorded in GA$_3$ at 400ppm, which was on par with GA$_3$ at 600ppm (45%) (Table 3). Among the IBA treatments, maximum (45%) germination was recorded in IBA at 400ppm and minimum (30%) germination at 600ppm. The lowest (23%) seed germination was recorded in control. Among the GA$_3$ treatments, 500ppm (57%) and among IBA treatments, 400ppm (45%) recorded higher germination percentage (Plate 2).
### Table 3. Effect of seed treatment on germination of *Withania* species

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Hormone</th>
<th>Concentration</th>
<th>Germination (%) (out of 100 seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>GA₃</td>
<td>200ppm</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300ppm</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400ppm</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500ppm</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600ppm</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>200ppm</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300ppm</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400ppm</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500ppm</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600ppm</td>
<td>30</td>
</tr>
<tr>
<td><em>W. obtusifolia</em></td>
<td>Control</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>GA₃</td>
<td>200ppm</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300ppm</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>500ppm</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600ppm</td>
<td>55</td>
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<tr>
<td></td>
<td>IBA</td>
<td>200ppm</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600ppm</td>
<td>45</td>
</tr>
</tbody>
</table>

*Time duration of treatments – 5hrs*
Plate 2
Seed germination - hormone treated and hormone without treated plants

Hormone Treated - $GA_3$

Hormone Treated - IBA

Hormone without treatment
Verma, *et al.*, (2001) reported similar result in *W. coagulans* and *W. somnifera* where treatment of both seeds with 500ppm gibberellic acid resulted in improved percentage of germination, emergence and coefficient of velocity of germination and a reduced germination time compared with control seeds. Barathkumar *et al.*, (2003) observed seed germination of *Ocimum sanctum* in GA₃ and IBA. Among the seed treatments, *Ocimum* seeds treated with GA₃ (500 ppm) recorded higher rate of germination and vigour than IBA.

The results of the present study revealed that the seed germination of *W. obtusifolia* showed higher response than *W. somnifera* in GA₃ and IBA treatments. Seeds of both *Withania* species showed similar responses in control.

### 4.4. Morphological character

The present study reveals that these two species (*W. obtusifolia* and *W. somnifera*) can be distinguished from each other on the basis of their morphological characters. There are some morphological differences among the populations of *W. somnifera*. For example, in Pakistan *W. somnifera*, teeth of fruiting calyx were small and triangular whereas the Turkish *W. somnifera* was having longer, filiform teeth (Hawkes and Edmon, 1972). According to Baytop (1978), the morphological characters of the leaf, flower, fruit and seed characters of *W. coagulans* are different from those of *W. somnifera*.

Recently, Kumar *et al.*, (2011) compared the cultivated and wild species of *W. somnifera*. Cultivated *W. somnifera* is a perennial shrub, 120-130 cm tall having sharply acute, slightly haired membranous leaves with entire margin and
Results and Discussion

Comparative studies of *Withania somnifera* (L.) Dunal, and *Withania obtusifolia* - M. SENTHIL KUMAR

six to seven shoots arise from the crown. The flowers are pentamerous, actinomorphic, hypogynous, and hermaphroditic. The fruiting calyx is globular, very faintly ribbed the flower bears five epipetalous stamens and an exserted pistil. The ovary is bilocular with axile placentation of ovule. The berry colour is always red and the seeds are oily to touch. Wild *W. somnifera* is an annual, rarely more than 40cm tall having small ovate, sub acute, stellately pubescent, veins inconspicuous, subcoriacious leaves with undulate margin. The flowers are similar to those of wild accessions but the fruiting calyx is elongated, prominently ribbed and berry colour is invariably yellow/orange with non-oily/dry seeds. They suggested to relegate the rank of wild accessions of *W. somnifera* to the separate species, *W. ashwagantha*.

*W. obtusifolia* is a perennial shrub, leaves are alternate, less pubescent, elliptic ovate to broadly ovate and obtuse characters, the fruit is a berry, 100-115cm height, slow growing, scattered branching pattern, whereas *W. somnifera* is perennial shrub, herbaceous, evergreen, 115-125cm height, branching is extensive, leaves are alternate elliptic ovate to broadly ovate, densely pubescent. Stems are erect branched in both *Withania* (Plate 3).

Floral results of these two species studied were showing the attributes of flower type, floral symmetry, pedicle, calyx and corolla colour. The colour of flower is whitish yellow in both *Withania* species (Table 4 and Plate 4). The fruits are berry in both species but cherry red in colour of *W. obtusifolia* whereas in *W. somnifera*, yellowish red in colour. *W. obtusifolia* seeds are reddish brown and bigger in size where as *W. somnifera* seeds are yellow and small (Plate 5).
Plate 3
Morphological Parts

W. obtusifolia

W. somnifera

W. obtusifolia

W. somnifera
Table 4. Morphological characters of the *Withania* species studied

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>W. somnifera</em></th>
<th><em>W. obtusifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Habit</em></td>
<td>Perennial shrub</td>
<td>Perennial shrub</td>
</tr>
<tr>
<td><em>Colour</em></td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td><em>Leaf shape</em></td>
<td>Elliptic ovate to broadly ovate</td>
<td>Elliptic obtuse</td>
</tr>
<tr>
<td><em>Leaf type</em></td>
<td>Alternate</td>
<td>Alternate</td>
</tr>
<tr>
<td><em>Leaf</em></td>
<td>Simple</td>
<td>Simple</td>
</tr>
<tr>
<td><em>Flower</em></td>
<td>Whitish yellow</td>
<td>Whitish yellow</td>
</tr>
<tr>
<td><em>Fruits</em></td>
<td>Berry</td>
<td>Berry</td>
</tr>
<tr>
<td><em>Seeds</em></td>
<td>Cherry red</td>
<td>Yellowish red</td>
</tr>
<tr>
<td><em>Stem</em></td>
<td>Erect branched</td>
<td>Erect branched</td>
</tr>
<tr>
<td><em>Root</em></td>
<td>Tuberous root</td>
<td>Tuberous root</td>
</tr>
</tbody>
</table>
Plate 4
Reproductive Parts

W. obtusifolia

W. somnifera

W. obtusifolia

W. somnifera

W. obtusifolia

W. somnifera
Plate 5
Fruits and Seeds

W. obtusifolia

W. somnifera

[Images of fruits and seeds for W. obtusifolia and W. somnifera]
The root morphology of *W. obtusifolia* is completely different from that of *W. somnifera*. Root of *W. somnifera* is highly branched and fibrous, not easily fragmented because of hard pith formation. Whereas in *W. obtusifolia* hard wood formation is absent and easily breakable due to powdered pith formation, it is fleshy long cylindrical, high tuber yield, Tapioca tuber like structure, full of powdered formation of thick fleshy root portions (Plate 6).

Morphological variation between *W. obtusifolia* and *W. somnifera* found in the present study is of the same type as that reported by previous workers (Sundari *et al.*, 1999, Pannerselvam *et al.*, 2003).

**4.5. Anatomic features of *Withania* species**

Morphological differences are not necessarily reflections of genetic events. The clear cut distinction between *W. obtusifolia* and *W. somnifera* in anatomical level is one of the major differences between previous and present study.

**4.5.1. Anatomy of leaf**

The shape of the epidermal cells of the upper and lower surfaces is irregular and sinous in both species. The two *Withania* species are amphistomatic having stomata at both the adaxial (upper) and abaxial (lower) surfaces of the leaf (Plate 7). The distribution of stomata on both the upper and lower surfaces of the leaves of the species reveals anisocytic or paracytic type (Plate 7). The stomatal type varies from paracytic (two subsidiary cells for each stoma) to anisocytic (three or more subsidiary cells around a stoma). The guard
Plate 6
Comparison of root morphology and physical properties

*W. obtusifolia*  
*W. somnifera*
cells are elliptical, the stomatal pore remain tightly closed. In *W. obtusifolia* only anisocytic type of stomata are present but in the case of *W. somnifera* possess two types of stomata namely anisocytic and paracytic. The stomatal index results are valuable and very reliable in distinguishing between the leaves of medicinal species of *Ocimum* from non medicinal ones (Olowokudejo (1990).

In *W. obtusifolia*, the leaf exhibits dorsiventral symmetry with reference to the midrib and palisade-spongy mesophyll differentiation. The leaf is dorsiventral with prominent midrib and bilaterally symmetrical lamina in *W. somnifera*. The midrib has broad, dome shaped adaxial part and tangentially extended semi circular abaxial part. It is 1.3mm in vertical plane and 1.2mm in horizontal plane in *W. obtusifolia*. The vascular system consists of a broad boat shaped bicollateral strand of xylem band and small groups of phloem along the outer and inner passages of the xylem arc. The size of vascular is 800mm wide and 150mm thick in *W. obtusifolia* species but in *W. somnifera* the midrib has broad adaxial hump and still broader, semicircular abaxial part and the midrib was 1.15mm thick in vertical axis; the adaxial hump is 650mm wide and the adaxial part is 1.7mm wide (Plate 7).

In *W. obtusifolia*, the venation pattern has lateral veins form dense reticulate, their islets are distinct in shape with various size and mostly rectangular in outline. In *W. somnifera*, the venation pattern has lateral veins which are prominent and form distinct reticulate venation pattern. The islets are polyhedral in outline and random in orientation. The vein terminations are thick comprising of three or four rows of tracheids. They may be short or long.
Plate 7
Anatomy of the Leaf

*W. obtusifolia*  
*W. somnifera*

Epidermal Morphology

*W. obtusifolia*  
*W. somnifera*

unbranched or branched and dendroid usually only one vein termination occurs in a vein islet (Plate 8).

The trichomes are different in both the species of *Withania*. Glandular and non-glandular trichomes are observed in the leaves of *W. obtusifolia*. But in the case of *W. somnifera*, only non-glandular hairs are present. The trichomes are predominantly branched in the form of “Y” having a stalk cell and two lateral arms. The basal cell of the trichome is 30µm thick the lateral branches are 20µm thick. The height of the trichome varies from 50-100µm in *W. obtusifolia* but in the case of *W. somnifera*, the glandular trichome are club shaped and less in number. They have a short stalk and ovoid head, the terminal head-cell has dark inclusions. These trichomes are 60-80µm in height and 25µm thick. The trichomes are predominantly branched and dendroid. The branching is dichotomous or trichotomous. The cells of the trichomes are wide rectangular in vertical plane and thin walled. The terminal cells are pined. The pits are not prominent and the trichomes range in height from 100-450 µm to 30 µm wide (Plate 8 and Plate 9).

### 4.5.2. Anatomy of stem

Stem section of *W. obtusifolia*, is circular in view with shallow ridges and furrows. It consists of thin epidermis, fairly wide cortex, a hollow thick vascular cylinder and wide pith. The cortex consists of an outer zone which has four or five layers of collenchyma and 2 or 3 layers of inner parenchyma. The vascular cylinder is 700 µm thick and the vascular cylinder is hollow and thick. It consists of well developed secondary xylem and secondary phloem. The stem powder
Plate 8
Venation Pattern

W. obtusifolia

W. somnifera

Trichome Distribution

W. obtusifolia

W. somnifera

VI– Vein islets, VT– Vein termination, LV– Lateral vein, MR – Mid rib,
EP– Epidermal cells, Tr– Trichome
Plate 9
Glandular and Non Glandular Trichome

*W. obtusifolia*

Anatomy of Stem

*W. obtusifolia*  *W. somnifera*

and maceration shows xylem fibers are abundant. The fibers are wide and thin walled or narrow and thick walled. The wider fibers are spindle shaped with tapering ends and are 350-400 µm long and 30 µm thick along the middle. The narrow fibers are more than 550 µm long and 20 µm thick and lateral wall pits are not evident. The vessel elements are narrow and cylindrical with short tails. The vessel elements range from 290 µm to 420 µm in length and 50-70 µm in breadth. The tracheids are sparsely seen in the powder. They are larger than vessel elements which are narrow. The lateral wall pits are well developed, equal to the vessels. The pits are circular, multiseriate and alternate. The tracheids are about 800 µm long and 30 µm wide.

Stem section of *W. somnifera* is circular in view and the vascular cylinder has outer and inner phloem. The outer phloem consists of small masses of sieve elements forming a ring. The inner phloem, medullary phloem or intraxylary phloem occurs adjacent with inner boundary of the secondary xylem. It consists of discrete strands of narrow sieve elements. Secondary xylem consists of wide and thick walled. The vessel elements are 30-50 µm wide. The vascular cylinder including outer and inner phloem is 500 µm thick and the stem powder and maceration show xylem fibres in abundant. The range of fibres is 500-600 µm long and 40 µm wide. The narrow fibres are upto 700 µm long and 20 µm thick. The vessel elements are narrowing long and cylindrical vessel elements are very frequent in the stem powder. They have simple, wide, oblique perforation plat. The vessel elements are taillers. The lateral wall pits are either scalariform or circular and dense. The vessel elements are 400 - 450 µm long and 50-60 µm wide (Plate 9) and (Plate 10).
Plate 10
Stem Material

\textit{W. obtusifolia} \hspace{1cm} \textit{W. somnifera}

Trachid and Vessels elements

NFi- Narrow fibre, St– Secondary tube, WFi– Wide fibre, Fi– Fibres,
VE– Vessels elements, Fi– Fibres, Pa– Parenchma cells, VE– Vessels elements,
LWP– Lateral wall pits, Fi– Fibre, Fi– Fibre, PP– Perforation plate, VE– Vessel elements
4.5.2. Anatomy of root

Regarding the root section of *W. obtusifolia*, the tuberous root is circular in view with fissured surface. It is more than 6mm thick. The cortex is narrow and has tangentially stretched parenchyma cells. The periderm is narrow and well defined. It has a cambial zone which produces a thin continuous cylinder of secondary xylem. The secondary xylem includes short radial multiples of wide, angular thin walled vessels, ensheathed by thick walled fibers. The intervening tissue between the radial segments of xylem is parenchymatous. The xylem nests are in radial lines they do not have phloem components. Phloem is found in thin radial file along the periphery of the outer xylem cylinder. The vessels are wide and thick walled measuring 120mm in diameter. The vessels are surrounded by thick walled fibers. Small groups of three or four phloem elements are seen scattered in the ground parenchyma. The distorted vascular elements (xylem and phloem) are due to the proliferation of the ground parenchyma during enlargement of the root.

But in the case of *W. somnifera* the tuberous root is circular in sectional view with rough surfaced periderm. The periderm is narrow and not well defined. The cortex is wide circular even cylindrical – comprising of outer dense vascular cylinder, middle scattered vascular strand and ground parenchyma cells and central, small core of dense xylem elements. The outer vascular cylinder is produced by a cambial zone. The cylinder is 400µm thick. It consists of fairly wide, angular, thick walled, xylem rays and xylem fibres on the outer part of the xylem cylinder. There are small groups of phloem elements which form the outer secondary phloem. The vessel elements of the xylem
Plate 11
Anatomy of the root

W. obtusifolia

W. somnifera

Enlarge view of the root

strands are thick walled, angular and 40µm wide. The central part of the root has a solid core of vessels and fibrous. The xylem core is irregular which are up to 50µm wide. There are scattered nests of phloem around the xylem mass and the presence of calcium oxalate crystals are seen filling the entire lumen of the parenchyma cells (Plate 11).

Metcalfe and Chalk (1965) and Tămas (2004) were observed the main characteristic element is the presence of sand cells in the cortical region, as well as in the secondary phloem and xylem that are characteristic for the Solanaceae. Vessels are solitary, partly in multiples of 2-4, very small clusters while in Caragana korshiskii and Hedysarum scoparium, vessels are in multiples or clusters with solitary, the outline of vessels is round, oval or angular, with 20-44µm tangential diameter; vessel elements are 126-16µm in length (Yang et al., 2007).

In W. obtusifolia, the presences of calcium-oxalate crystals are seen filling the entire lumen of the parenchyma cells. The starch grains are abundant in the central part of the root. The circular starch grains are concentric with central hilum. The starch grains are 10-15mm wide. Whereas in W. somnifera the presence of calcium oxalates crystal are the predominant inclusions in the ground parenchyma. Starch grains are not evident. The crystals are minute and granular and these crystals are called sand crystals, which fill up the cells. The crystal bearing cells are more in the outer portion than in the central portion of the W. somnifera (Plate 12).
Plate 12

Distribution of crystal and starch grains in the root

W. obtusifolia

W. somnifera

Scr – Sand Crystals, Pi – Pith cells, PM – Palisade mesophyll, SM – Spongy mesophyll, Dr – Druse
The presence of calcium oxalate crystals in the root is an interesting feature in solving taxonomic problem (Kausch and Horner 1982). These crystal production is partly genetically controlled in plant species and that the shape and location of crystals within specific tissues may have taxonomic significance (Horner and Wagner 1995; Webb 1999; Lersten and Horner 2000, 2008; Bouropoulos et al., 2001; Cervantes et al., 2005; Hartl et al., 2007). Morphometric studies of individual crystals are limited and have mainly been undertaken on druse type of calcium crystal within the Solanaceae (e.g. Jones and Bryant 1992; Monje and Baran 2002). Studies have showed that druses produced by different cactus genera may be differentiated based on size and shape, with the latter being the more significant variable (Jones and Bryant 1992; Monje and Baran 2002). Monje and Baran (2002) demonstrated that morphologies are determined by crystal size and druse type of the other hand tends to vary more within a single plant, depending on the function of the cell or tissue in which it formed as well as the amount of calcium available during crystal formation (Franceschi and Nakata 2005). There are three types of crystals such as, solitary crystals and druses that are the most common crystal types amongst dicotyledons, and the other crystal types, such as spherocrystals are rarely observable (Metcalf and Chalk, 1983).

In the present results, *W. obtusifolia* alone has calcium oxalate crystals in their roots and druses types are present. The application of anatomical study has proven to be of immense assistance in interpreting problems related to identification of differences between the two species.
4.6. Antimicrobial activity of *Withania* plant species

The results of antimicrobial activity of methanolic extract of *Withania* species from leaf and root samples against microorganisms using agar well diffusion method (Table 5). Methanolic extract was active against bacteria (*Escherichia coli, Bacillus subtilis* and *Shigella* sp) and fungi (*Aspergillus niger* and *Trichophyton rubrum*) strains. As general *Withania* is considered highly active against both fungi and bacteria when the zone of inhibition is greater than 6 mm (Muhammed and Muhammed 2005).

In leaf sample of *W. somnifera*, a maximum inhibition zone was found to be 7.16 mm in the concentration of 100µl, against an isolated human pathogen *Bacillus subtilis* and the moderate inhibition zone 6.68 mm found in same 100µl concentrations against *E.coli*. The minimum inhibition zone 2.8mm was found in concentration of 25µl *Shigella* sp. The fungal isolate of the pathogen *Trichophyton rubrum*, has the least inhibition zone found. The minimum inhibition zone in concentration of 25µl was observed in *T. rubrum* with 2.9 mm and maximum inhibition zone from100µl was observed in *Aspergillus niger* with 6.1 mm. In root sample of *W. somnifera*, a maximum inhibition zone was found to be 5.08 mm in the concentration of 100µl against *E.coli* and the moderate inhibition zone 4.98 mm found in same 100µl concentrations against pathogen *B. subtilis*. The minimum inhibition zone 2.76mm was found in concentration of 25µl *Shigella* sp. The fungal isolate of *T. rubrum*, has the least inhibition zone. The minimum inhibition zone in concentration of 25µl was observed in *T.ru brum* with 4.4mm and maximum inhibition zone from100µl was observed in *A. niger* with 5.74mm (Plate 13 to 17) and (Table 5 and Plate 18).
Table 5. Leaf and root extracts against fungal and bacterial isolates in *W. somnifera*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone formation (mm) at volume of leaf sample loaded (µl)</th>
<th>Zone formation (mm) at volume of root sample loaded (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4.56±0.34</td>
<td>4.78±0.30</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>5.6±0.28</td>
<td>5.84±0.27</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>2.84±0.32</td>
<td>3.34±0.34</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>4.75±0.22</td>
<td>5.06±0.35</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>2.96±0.41</td>
<td>3.44±0.34</td>
</tr>
</tbody>
</table>
In *W. obtusifolia*, the maximum inhibition zone was found to be 4.8 mm in 100μl, for the leaf samples against an isolated human pathogen *Shigella* species and the moderate inhibition zone 4.4mm was found in the concentration of 100μl, against *B. subtilis*. The minimum inhibition zone 2.8mm was found in the concentration of 25μl against *E. coli*. The least inhibition zone was found in fungal isolate of the pathogen *T. rubrum*. The minimum inhibition zone in concentration of 25μl was observed in *T. rubrum* with 2.7mm and maximum inhibition zone from100μl was observed in *A.niger* with 5.18mm. In root sample of *W. obtusifolia*, the maximum inhibition zone was found to be 6.78mm in 100μl, against *E. coli* and the moderate inhibition zone 6.6mm was found in the concentration of 100μl, against *B. subtilis*. The minimum inhibition zone 4.2 mm was found in the concentration of 25μl, against *Shigella* species. The fungal isolate of the pathogen *T. rubrum*, had found the least inhibition zone. The minimum inhibition zone in concentration of 25μl was observed in *T. rubrum* with 2.8mm and maximum inhibition zone from100μl was observed in *A.niger* with 6.34mm (Plate 13 to 17) and (Table 6 and Plate 19).

The present study showed enhanced activity in the leaf when compared to root in *W. somnifera*. Whereas in *W.obtusifolia* maximum microbial activity was found in the root when compared to leaf.

4.7. Phytochemical analysis of *Withania* species

The GC-MS analysis of the root extract obtained with absolute alcohol of *W. obtusifolia* and *W. somnifera* was carried out individually. In GC-MS
Table 6. Leaf and root extracts against fungal and bacterial isolates in *W. obtusifolia*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone formation (mm) at volume of leaf sample loaded (µl)</th>
<th>Zone formation (mm) at volume of root sample loaded (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.8±0.28</td>
<td>3.4±0.21</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>3.28±0.31</td>
<td>3.98±0.47</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>3.22±0.38</td>
<td>3.4±0.33</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>3.6±0.28</td>
<td>4.18±0.41</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>2.72±0.21</td>
<td>3.2±0.32</td>
</tr>
</tbody>
</table>
Plate 13

Leaf and root extracts an antibacterial (Escherichia coli) activity in Withania species

a) *W. somnifera* leaf extract
b) *W. obtusifolia* leaf extract
c) *W. somnifera* root extract
d) *W. obtusifolia* root extract
Plate 14
Leaf and root extracts an antibacterial (*Bacillus subtilis*) activity in *Withania* species

a) *W. somnifera* leaf extract  
b) *W. obtusifolia* leaf extract  
c) *W. somnifera* root extract  
d) *W. obtusifolia* root extract
Plate 15
Leaf and root extracts as an antibacterial (*Shigella Sp*) activity in *Withania* species

a) *W. somnifera* leaf extract  
b) *W. obtusifolia* leaf extract  
c) *W. somnifera* root extract  
d) *W. obtusifolia* root extract
Plate 16

Leaf and root extracts an antifungal (*Aspergillus niger*) activity in *Withania* species

a) *W. somnifera* leaf extract

b) *W. obtusifolia* leaf extract

c) *W. somnifera* root extract

d) *W. obtusifolia* root extract
Plate 17

Leaf and root extracts as an antifungal (Trichophyton rubrum) activity in Withania species

a) *W. somnifera* leaf extract
b) *W. obtusifolia* leaf extract

c) *W. somnifera* root extract
d) *W. obtusifolia* root extract
Plate 18
Leaf and root methanolic extracts in *W. somnifera*

a. Bacterial isolates

b. Fungal Isolates
Plate 19
Leaf and root methanolic extracts in *W. obtusifolia*

a. Bacterial isolates

b. Fungal Isolates
Results and Discussion

Comparative studies of Withania somnifera (L.) Dunal, and Withania obtusifolia - M. SENTHIL KUMAR

Analysis, 24 bioactive compounds were identified in the absolute alcohol extract of *W. obtusifolia*, such as compounds Galactose, Cyclopentane, 1-methyl–3-(2methylpropyl), Propane, 11,3-tiethoxy, Octanoic Acid, 8-Azabicyclo (3,2,1) octan-3-8-methyl, endo-, Undecanoic acid, 1,3-Propanediol, 2-ethyl-2, 3-Hexenoic acid, butyl ester, Dodecanoid acid, 1H-Indole, 2-Pyrrolidin-2-yl, Oxirane, 6βBicyclo (4.3.0) nonane, 5β-iodomethyl, 1β–isopropenyl - 4α, 5α–dimethyl, Phenol, 4(1-Phenylethyl, Tetradecanoic acid, Didodecyl phthalate, 1H cyclopenta (b) quinoline, 2,3-dihydro-9-amino, n-Hexadecanoic acid, Pentadecanoic acid, 2,6,10,14-tetramethyl-methyl ester, 7,11-Epoxymegastigma-5(6) en-9-one, 1E-11,z-13- octadecatriene, 9,12-Octadecadienoic acid, Oleic acid, Phenol,2,4-bis(1-phenylethyl), 1,2- Benzenedicarboxylic acid disooctyl ester were studied and results are presented in (Table 7 and Plate 20, fig 1a to 1k).

Whereas in *W. somnifera* only 21 bioactive phytochemical compounds were identified in the same extract and analysed such as compounds are Propane, 1,1 diethoxy -2 – methyl, 2,3,4,5-Tetrahydropyridazine, Butane, 1,1-diethoxy-2-methyl, Phenylethyl Alcohol, 3-Hexenoic acid, butyl ester, (Z), 8-Azabicyclo (3,2,1) octan-3-, 8-methyl, endo- Tropanol, Sucrose, Amyl Nitrite, Dodecanoid acid, 3-tert-Butyl-4-hydroxynisole, 1H-Indole, 2-Pyrrolidin, Tetradecanoid acid, Decanoic acid, 2- methyl, n-Hexadecanoic acid, decanoic acid, ethyl ester, 1, E-11, Z-13- Octadecatriene, 9-Octadecenal, 1-Tridecyne and Oleic Acid and the results are presented (Table 8 and Plate 21, fig 2a to 2n).
Table 7. Phytocomponents identified in the ethanolic extract of the *W. somnifera* (GC MS study)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.71</td>
<td>Propane, 1,1-diethoxy-2-methyl-</td>
<td>C₆H₁₈O₂</td>
<td>146</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>3.18</td>
<td>2,3,4,5-Tetrahydropyridazine</td>
<td>C₄H₈N₂</td>
<td>84</td>
<td>11.07</td>
</tr>
<tr>
<td>3</td>
<td>3.64</td>
<td>Butane, 1,1-diethoxy-2-methyl-</td>
<td>C₆H₂₀O₂</td>
<td>160</td>
<td>11.60</td>
</tr>
<tr>
<td>4</td>
<td>4.85</td>
<td>2-Nonanone</td>
<td>C₆H₁₈O</td>
<td>142</td>
<td>0.84</td>
</tr>
<tr>
<td>5</td>
<td>5.04</td>
<td>Propane, 1,1,3-triethoxy</td>
<td>C₆H₂₀O₃</td>
<td>176</td>
<td>1.45</td>
</tr>
<tr>
<td>6</td>
<td>5.74</td>
<td>PhenylethylAlcohol</td>
<td>C₉H₁₀O</td>
<td>122</td>
<td>1.30</td>
</tr>
<tr>
<td>7</td>
<td>6.71</td>
<td>3-Hexenoic acid, butyl ester, (Z)</td>
<td>C₁₀H₁₈O₂</td>
<td>170</td>
<td>0.97</td>
</tr>
<tr>
<td>8</td>
<td>7.40</td>
<td>8-Azabicyclo[3.2.1]octan-3-ol,8-methyl-endo</td>
<td>C₈H₁₅NO</td>
<td>141</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>10.03</td>
<td>Sucrose</td>
<td>C₁₂H₂₂O₁₁</td>
<td>342</td>
<td>0.47</td>
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<tr>
<td>10</td>
<td>10.68</td>
<td>Amyl Nitrite</td>
<td>C₅H₁₁NO₂</td>
<td>117</td>
<td>23.64</td>
</tr>
<tr>
<td>11</td>
<td>11.63</td>
<td>Dodecanoic acid</td>
<td>C₁₂H₂₄O₂</td>
<td>200</td>
<td>0.55</td>
</tr>
<tr>
<td>12</td>
<td>11.68</td>
<td>3-tert-Butyl-4-hydroxyanisole</td>
<td>C₁₁H₁₆O₂</td>
<td>180</td>
<td>4.42</td>
</tr>
<tr>
<td>13</td>
<td>12.60</td>
<td>IH-Indole, 2 pyrrolidin-2-yl-</td>
<td>C₁₂H₁₄N₂</td>
<td>186</td>
<td>12.32</td>
</tr>
<tr>
<td>14</td>
<td>14.33</td>
<td>Tetradecanoic acid</td>
<td>C₁₄H₂₈O₂</td>
<td>228</td>
<td>0.81</td>
</tr>
<tr>
<td>15</td>
<td>16.70</td>
<td>Decanoic acid, 2-methyl</td>
<td>C₁₁H₂₂O₂</td>
<td>186</td>
<td>2.81</td>
</tr>
<tr>
<td>16</td>
<td>17.40</td>
<td>n-Hexadecanoic acid</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>0.36</td>
</tr>
<tr>
<td>17</td>
<td>17.78</td>
<td>Decanoic acid, ethyl ester</td>
<td>C₁₂H₂₄O₂</td>
<td>200</td>
<td>11.07</td>
</tr>
<tr>
<td>18</td>
<td>20.13</td>
<td>1,E-11,Z-13-Octadecadiene</td>
<td>C₁₈H₃₂</td>
<td>248</td>
<td>11.60</td>
</tr>
<tr>
<td>19</td>
<td>20.23</td>
<td>9-Octadecenal</td>
<td>C₁₈H₃₄O</td>
<td>266</td>
<td>0.84</td>
</tr>
<tr>
<td>20</td>
<td>20.45</td>
<td>1-Tridecyne</td>
<td>C₁₃H₂₄</td>
<td>180</td>
<td>1.45</td>
</tr>
<tr>
<td>21</td>
<td>20.57</td>
<td>Oleic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>1.30</td>
</tr>
</tbody>
</table>
Fig 1a: D-Galactose

Fig 1b: Propane, 1,1,3-triethoxy-
Fig 1c: octanoic acid

Name: Octanoic Acid
Formula: C\textsubscript{8}H\textsubscript{16}O\textsubscript{2}
MW: 144 CAS#: 124-07-2 NIST#: 150168 ID#: 6701 DB: replib
10 largest peaks:
60 999 | 73 591 | 43 498 | 41 489 | 27 405 | 29 382 | 55 303 | 39 226 | 45 193 | 85 157 |

Fig 1d: 8-Azabicyclo[3.2.1]octan-3-ol,5-methyl-, endo-

Name: 8-Azabicyclo[3.2.1]octan-3-ol, 5-methyl-, endo-
Formula: C\textsubscript{9}H\textsubscript{15}NO
MW: 141 CAS#: 120-29-6 NIST#: 221174 ID#: 40683 DB: mainlib
10 largest peaks:
82 999 | 83 789 | 96 780 | 42 513 | 124 341 | 141 298 | 57 233 | 97 224 | 113 181 | 55 177 |
Fig 1e: Undecanoic acid

Name: Undecanoic acid
Formula: C_{11}H_{22}O_{2}
MW: 186 CAS#: 112-37-8 NIST#: 113003 ID#: 6698 DB: replib
10 largest peaks:
60 989 |  73 939 | 41 807 | 43 680 |  55 603 | 29 413 | 57 363 | 71 322 | 27 298 | 129 276 |

Fig 1f: Dodecanoid acid

Name: Dodecanoid acid
Formula: C_{12}H_{24}O_{2}
MW: 200 CAS#: 143-07-7 NIST#: 290533 ID#: 6704 DB: replib
10 largest peaks:
60 996 |  73 978 | 43 697 | 41 675 |  55 657 | 57 528 | 129 398 | 157 290 | 85 284 | 71 276 |
Fig 1g: 1H-Indole,2-pyrrolidin-2-yl-

Name: 1H-Indole, 2-pyrrolidin-2-yl-
Formula: C_{12}H_{14}N_{2}
MW: 186 CAS#: N/A NIST#: 303415 ID#: 81722 DB: mainlib
10 largest peaks:
120 999 | 185 990 | 186 954 | 157 687 | 156 615 | 131 399 | 117 388 | 143 352 | 80 331 | 129 297 |

Fig 1h: Tetradecanoic acid

Name: Tetradecanoic acid
Formula: C_{14}H_{28}O_{2}
MW: 228 CAS#: 544-63-8 NIST#: 113060 ID#: 8360 DB: replib
10 largest peaks:
73 999 | 43 967 | 60 924 | 41 911 | 55 748 | 57 651 | 29 446 | 129 379 | 69 340 | 71 331
Fig 1i: 9,12-octadecadienoic acid (Z,Z)-

Fig 1j: Oleic acid
Fig 1k: 1,2-Benzene dicarboxylic acid, diisooctyl ester

Name: 1,2-Benzenedicarboxylic acid, diisooctyl ester
Formula: C_{36}H_{32}O_4
MW: 390 CAS#: 27554-26-3 NIST#: 113206 ID#: 19804 DB: replib
10 largest peaks:
149 999 | 167 350 | 57 341 | 70 254 | 41 225 | 71 224 | 55 218 | 43 200 | 150 107 | 83 100 |

Fig 1L: 1H-Indole, 2-pyrrolidin-2-yl-

Name: 1H-Indole, 2-pyrrolidin-2-yl-
Formula: C_{11}H_{14}N_2
MW: 186 CAS#: N/A NIST#: 303415 ID#: 81722 DB: mainlib
10 largest peaks:
130 999 | 185 990 | 186 954 | 157 687 | 158 615 | 131 399 | 117 388 | 143 352 | 89 331 | 129 297 |
Table 8. Phytocomponents identified in the ethanolic extract of the *W. obtusifolia* (GC MS study)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular Formula</th>
<th>Molecular weight</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.73</td>
<td>D. Galactose</td>
<td>C₆H₁₂O₆</td>
<td>180</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>4.24</td>
<td>Cyclopentane, 1-methyl-3- (2-methylpropyl)-</td>
<td>C₁₉H₂₀</td>
<td>140</td>
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<tr>
<td>3</td>
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<td>Propane, 1,1,3-triethoxy</td>
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<td>0.19</td>
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<tr>
<td>4</td>
<td>6.42</td>
<td>Octanoic Acid</td>
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<td>144</td>
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<tr>
<td>5</td>
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<td>8-Azabicyclo[3.2.1]octan-3-ol,8-methyl-endo</td>
<td>C₈H₁₃NO</td>
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<td>4.06</td>
</tr>
<tr>
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<td>9.11</td>
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<td>C₁₁H₂₂O₂</td>
<td>186</td>
<td>0.65</td>
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<tr>
<td>7</td>
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<td>1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)</td>
<td>C₆H₁₄O₃</td>
<td>134</td>
<td>19.7</td>
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<tr>
<td>8</td>
<td>10.64</td>
<td>3-Hexenoic acid, butyl ester, (Z)</td>
<td>C₁₀H₁₈O₂</td>
<td>170</td>
<td>1.14</td>
</tr>
<tr>
<td>9</td>
<td>11.63</td>
<td>Dodecanoic acid</td>
<td>C₁₂H₂₄O₂</td>
<td>200</td>
<td>5.64</td>
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<tr>
<td>10</td>
<td>12.58</td>
<td>IH-Indole, 2-pyrrolidin-2-yl</td>
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<td>12</td>
<td>13.88</td>
<td>β-Bicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-</td>
<td>C₁₅H₂₅</td>
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<tr>
<td>13</td>
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<td>C₁₄H₁₄O</td>
<td>198</td>
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<tr>
<td>14</td>
<td>14.30</td>
<td>Tetradecanoic acid</td>
<td>C₁₄H₂₈O₂</td>
<td>228</td>
<td>1.81</td>
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<tr>
<td>15</td>
<td>15.75</td>
<td>Didodecylphthalate</td>
<td>C₁₂H₂₄O₄</td>
<td>502</td>
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<tr>
<td>16</td>
<td>16.15</td>
<td>IH-Cyclopenta (b)quinoline, 2,3-dihydro-9-amino-</td>
<td>C₁₃H₂₂N₂</td>
<td>184</td>
<td>0.14</td>
</tr>
<tr>
<td>17</td>
<td>17.31</td>
<td>n-Hexadecanoic acid</td>
<td>C₁₄H₂₈O₂</td>
<td>256</td>
<td>6.89</td>
</tr>
<tr>
<td>18</td>
<td>17.70</td>
<td>Pentadecanoic acid, 2,6,10,14-tetramethyl-methyl ester</td>
<td>C₂₀H₃₆O₂</td>
<td>312</td>
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<td>19</td>
<td>18.66</td>
<td>7,11-Epoxymegastigma- 5(6)-en-9-one</td>
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<td>208</td>
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<td>1,E-11,Z-13-Octadecatriene</td>
<td>C₁₃H₃₂</td>
<td>248</td>
<td>1.29</td>
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<tr>
<td>21</td>
<td>20.09</td>
<td>9,12-Octa decadienoic acid (Z,Z)-</td>
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<td>280</td>
<td>3.78</td>
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<tr>
<td>22</td>
<td>20.43</td>
<td>Oleic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>0.88</td>
</tr>
<tr>
<td>23</td>
<td>25.50</td>
<td>Phenol, 2,4-bis (1-Phenylethyl)-</td>
<td>C₂₃H₇₆O</td>
<td>302</td>
<td>0.66</td>
</tr>
<tr>
<td>24</td>
<td>26.22</td>
<td>1,2-Benzenedicarboxylic acid, diisooctyl ester</td>
<td>C₂₃H₃₆O₄</td>
<td>390</td>
<td>48.56</td>
</tr>
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</table>
Fig 2a Propane, 1,1,3-triethoxy-

Name: Propane, 1,1,3-triethoxy-
Formula: CgH2yO3
MW: 176 CAS#: 7789-92-6 NIST#: 90826 ID#: 6491 DB: replib
10 largest peaks:
58 999 | 47 816 | 103 749 | 87 556 | 57 550 | 45 514 | 75 489 | 85 274 | 58 182 | 86 165 |

Fig 2b: 8-Azabicyclo[3,2,1]octan-3-ol, 8-methyl, endo-

Name: 8-Azabicyclo[3,2,1]octan-3-ol, 8-methyl, endo-
Formula: CgH2yKO
MW: 141 CAS#: 120-29-6 NIST#: 221174 ID#: 40863 DB: mainlib
10 largest peaks:
82 969 | 83 780 | 96 750 | 42 513 | 124 341 | 141 298 | 57 233 | 97 224 | 113 181 | 55 177 |
Fig 2c: Undecanoic acid

Name: Undecanoic acid
Formula: C₁₁H₂₂O₂
MW: 186 CAS#: 112-37-8 NIST#: 113003 ID#: 6698 DB: replib
10 largest peaks:
60 999 | 73 939 | 41 807 | 43 660 | 55 603 | 29 413 | 57 363 | 71 322 | 27 298 | 129 276 |

Fig 2d: Dodecanoic acid

Name: Dodecanoic acid
Formula: C₁₂H₂₄O₂
MW: 200 CAS#: 143-07-7 NIST#: 290933 ID#: 6704 DB: replib
10 largest peaks:
60 999 | 73 978 | 43 697 | 41 676 | 55 657 | 57 526 | 129 398 | 157 290 | 85 284 | 71 276 |
Fig 2c: 1H-Indole, 2-pyrrolidin-2-yl-

Name: 1H-indole, 2-pyrrolidin-2-yl-
Formula: C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>
MW: 266 CAS#: N/A NIST#: 303415 ID#: 81722 DB: mainlib
10 largest peaks:
130 899 | 185 960 | 166 954 | 157 687 | 156 615 | 131 399 | 117 388 | 143 352 | 89 331 | 129 297 |

Fig 2f: Tetradecanoic acid

Name: Tetradecanoic acid
Formula: C<sub>14</sub>H<sub>28</sub>O<sub>2</sub>
MW: 228 CAS#: 544-83-8 NIST#: 113085 ID#: 8390 DB: reptlib
10 largest peaks:
73 999 | 43 967 | 60 924 | 41 911 | 55 748 | 57 651 | 29 446 | 129 379 | 69 340 | 71 331 |
Fig 2g: 2,3,4,5-Tetrahydropyridazine

Fig 2h: Butane, 1,1-diethoxy-2-methyl-
Fig 2i: Phenylethyl Alcohol

Name: Phenylethyl Alcohol
Formula: C9H13O
MW: 132 CAS#: 60-12-5 NIST#: 155643 ID#: 11319 DB: replib
10 largest peaks:
91 990 | 92 535 | 122 246 | 65 157 | 39 66 | 51 46 | 93 43 | 77 40 | 31 36 | 63 29 |

Fig 2j: Sucrose

Name: Sucrose
Formula: C12H22O11
MW: 342 CAS#: 57-50-1 NIST#: 25030 ID#: 32526 DB: mainlib
10 largest peaks:
73 999 | 57 946 | 31 745 | 45 573 | 90 528 | 61 357 | 44 278 | 71 227 | 69 212 | 45 191 |
Fig 2 k: Amyl Nitrite

Name: Amyl Nitrite
Formula: C₇H₁₅NO₂
MW: 117 CAS#: 110-48-3 NIST#: 233778 ID#: 20276 DB: mainlib
10 largest peaks:
57 998 | 41 997 | 60 871 | 29 869 | 43 702 | 30 523 | 71 308 | 39 302 | 27 294 | 42 232 |

Fig 2 L: 3-tert-Butyl-4-hydroxyanisole

Name: 3-tert-Butyl-4-hydroxyanisole
Formula: C₁₄H₁₄O₂
MW: 180 CAS#: 121-00-8 NIST#: 250108 ID#: 21309 DB: replib
10 largest peaks:
165 999 | 137 621 | 160 443 | 39 225 | 41 211 | 77 179 | 107 155 | 91 147 | 53 146 | 68 141 |
Fig 2m: 9,12-Octadecadienoic acid (Z,Z)-

Name: 9,12-Octadecadienoic acid (Z,Z)-
Formula: C\textsubscript{18}H\textsubscript{32}O\textsubscript{2}
MW: 280 CAS#: 60-33-3 NIST#: 27829 ID#: 7156 DB: replib
10 largest peaks:
67 999 | 55 954 | 81 793 | 41 708 | 69 560 | 95 545 | 68 530 | 54 486 | 43 480 | 82 479 |

Fig 2n: Oleic acid

Name: Oleic Acid
Formula: C\textsubscript{18}H\textsubscript{34}O\textsubscript{2}
MW: 282 CAS#: 112-80-1 NIST#: 154664 ID#: 4240 DB: replib
10 largest peaks:
55 990 | 41 607 | 69 560 | 57 471 | 43 356 | 73 332 | 83 321 | 29 259 | 56 224 | 60 192 |
The chromatographic profile was similar for both species, differing only in some compounds. This similarity had been observed in GC-MS analysis. Most of the extracted compounds had retention times between 30 and 36 min and were identified as being different phytochemical. For the characterization of the compounds detected in GC-MS, a process which increased the confidence of the identification of compounds by the mass spectrometry was applied. The fragmentogram obtained for each compound was compared with the fragmentation data base of the mass spectrometer, for obtaining a list of the different probable substances.

The MS data of the compounds found in *Withania* samples were studied. *W. somnifera* showed an alkaloid and nitrogen group of compounds which were more in abundance whereas in *W. obtusifolia* plant only alkaloid group of compounds were present in rich quantities. This method was checked by the confirmation of the identity of some proposed structures through the injection of authentic standards. The retention times and molecular ion were obtained for those standards.

Freire *et al.* (2002) also used GCMS analysis in various *Eucalyptus* species. They observed that the dodecanoic and tricosanoic acids are specific to *E. urograndis* and *E. camaldulensis*. 
4.8. Molecular characterization of Withania species

4.8.1. SDS-PAGE

SDS-PAGE is one of the most important chromatographic methods of bio-systematic and comparative analysis of plants such as relationships and germplasm management (Zubaida et al. 2008). In our study, SDS-PAGE has been effectively used for the comparative study of proteins between the seed, root and leaf of W. obtusifolia and W. somnifera. In lane 2, 4 and 6 seeds root and leaf of W. somnifera and in lane 3, 5 and 7 seed, root and leaf of W. obtusifolia were electrophoresed.

In second lane, seed, showed five bands in the range between 20 and 10kda, exactly one band was observed which was prominent and equal in size in both seed (lane 2) and leaf (lane 6) sample. In the results analogous to the standard 20 band of protein marker, second and third bands were observed in the range between 15 and 10Kda, fourth and fifth bands were seen in 10 and 5Kda. It was observed that in lane fourth band does not appear in the root. In the lane six of the leaf four bands were observed between 25 and 10Kda, first and second band were observed in between 25 and 20Kda, second band showed and a prominent band in 20, whereas third band was not clear but observed in the range between 15 and 10Kda, fourth band is observed in the range between 10 and 5Kda in W. somnifera.

Whereas in W. obtusifolia, seed sample observed in lane three, five bands were seen in the range between 25 and 10Kda, first and second band were observed in between 25 and 19Kda whereas third and fourth band were observed
in the range between 15 and 10 Kda, fifth band was observed in between 10 and 5Kda, root sample observed in lane five and only one band was observed in 25, the leaf sample of lane seven, among the two bands situated between 25 and 5Kda one band was observed in 25 was prominent and another band was observed in the range between 10 and 5Kda.

From the results obtained in *W. obtusifolia* and *W. somnifera*, it can be differentiated only based on the protein profiling of leaf and seed. Also there was a presence of unique marker in the plant *W. somnifera* with a molecular weight of 20Kda present in the lane second and sixth. While comparing both samples, it can be inferred that some of the proteins are common for both and some proteins are restricted only to certain parts like seeds, root and leaf (Plate 22). A similar study was done by Zubaida, *et al.* (2008) in different *Withania* species and exhibited differences in their protein profile.

**4.8.2. RAPD analysis**

Characterization of the genetic variation is an essential step towards executing any organized plant conservation or improvement program. Considerable morphogenetic diversity in Indian populations of *W. somnifera* has been studied extensively, Atal and Schwarting (1962) documented five morphological forms within the Indian populations. Only 15 primers were selected to use in this study, because wide range of available RAPD primers have been used to study the intra species population variability in *W. somnifera*. Recently, Aslam *et al.*, (2010) had reported the considerable genetic variations among the 15 wild accessions of Tamil Nadu using RAPD markers (OPA 03, 06,
Plate 22
Protein profiling by SDS-PAGE in *W. somnifera* and *W. obtusifolia*

Lane 1: Protein marker
Lane 2: *W. somnifera* seed
Lane 3: *W. obtusifolia* seed
Lane 4: *W. somnifera* root
Lane 5: *W. obtusifolia* root
Lane 6: *W. somnifera* leaf
Lane 7: *W. obtusifolia* leaf
12 and 16), but not used in those primers, which are suitable to study the intraspecies population variability, therefore the first priority was given for choosing the RAPD primers, which have been resulted in the monomorphic bands among the reported intra species variant. A thorough literature perusal and our earlier works on studying intra species variation in *W. somnifera* helped to select 15 primers for this study. All the *W. somnifera* marked samples (*WS* 1, 2, 3, 4 and 5) irrespective of their location have been used to test the ability and reproducibility of producing monomorphic bands with the selected primers. The same experiment was also conducted separately in *W. obtusifolia* marked samples (*WO* 1, 2, 3, 4 and 5), surprisingly 7 primers out of 15 primers failed to give any amplification in *W. obtusifolia* and 4 primers remained out 8 produced polymorphic bands among the *W. obtusifolia* samples. These results showed that there is most important evidence for genetic variation between these two species. Remaining 4 primers were tested for the polymorphic bands between the *W. somnifera* and *W. obtusifolia*. All the 4 primers produced totally 52 bands among this 34 bands were polymorphic for these tested samples (Plate 23). The outcome of the study is essential for eliminating the problems related to the identification of the species and can be exploited for their correct utilization by pharmaceutical industries. A similar study was done in *W. somnifera* and *W. coagulans* from the districts of Kohat and Karak in Pakistan by Syed (2009).
Plate 23

RAPD analysis of *W. somnifera* and *W. obtusifolia*