In the present study medicinal plants available in the Pachamalai hills were listed and in vitro studies were carried out in an endangered medicinal plant *G. superba*. Literatures pertaining to above said aspects were reviewed here under;

The nature has provided a complete store house of remedies to cure all ailments of mankind. The higher plants are solar-powered biochemical factories which manufacture both primary and secondary metabolites from air, water, minerals and their energy from sunlight. Today, there is a vast knowledge on the medicinal uses of different plants. In recent years it has become difficult to maintain ample supply of medicinal plants due to ruthless exploitation, lack of conservation of the environment, increasing labour costs, economic and technical problems associated with the cultivation of medicinal plants. These problems are also associated with the species selected for the present investigation viz. *Gloriosa superba* L.

Pandey and Shukla (2003), analysed the sal (*Shorea robusta*) forest vegetation of Sohagbarawa wild life sanctuary, Gorakhpur, India, to asess plant diversity, regeneration pattern and status of species conservation. A total of 208 plants species representing 165 genera and 72 families were recorded. Species richness, mean density and basal area of individuals in the observed forests were compared with those of other sal dominated forests of India. The sal forests was
rich in Papilionaceae (23 species) which contributed maximally to the total number of individuals of <30 cm girth. After sal, density was maximum for leguminous shrubs, *Moghania chapper*. The plants regenerating as sprouts from under ground stems or storage organs contributed significantly to the sum total of individuals/ha. 45.5 % of the total individuals were of ramet origin and shared 10.6% of the total species richness of the forest. The low disturbance areas, thickets of dense entangled mass of vegetation, predominantly composed of thorny lianas were identified that usually contain less common and rare species like *Rauwolfia serpentina*, *Desmodium latifolium*, *Crotalaria alata*, and *Gloriosa superba* in addition to the frequent ones.

Soosairaj *et al.*, (2005), reported the Sorenson similarity index and coles association coefficient to analyse the similarity between forest types and association between individual plant species in the Pachamalai hills of Eastern Ghats of Tamil Nadu, South India. Sorenson index shows that the higher similarity existed between the ever green and semi ever green, the semi evergreen and dry mixed deciduous, the dry mixed deciduous and the dry savannah with the dry deciduous scrub, southern thorn scrub and southern thorn forest. Chi-square values are found to be significant between *Santalum album* with *Dodonaea viscosa*, *Cycas circinalis* with *Cleistanthus collinus*, *Combretum albidum*, *Pongamia pinnata* and *Symphorema involucratum*, *Nothopegia colebrookiana* with *Tarenna asiatica*, *Pterospermum xylocarpum* with *Doryopteris concolor*, *Smilax zeylanica* with *Acacia torta*, *Canarium strictum* with *Aglaia lawii* and *Diplcitra cuneapa*, *Cymbopogon citrates* with *Grewia hirsuta* and *Grewia tiliifolia*.

Ethnobotanical survey on the use of medicinal plants in Southern Western Ghats of India (Madurai district, Tamil Nadu) has been carried out. Information was collected from the palayar tribes using an integrated approach of botanical
collections, group discussions and interviews. Totally, 60 ethnomedicinal plant species distributed in 32 families are documented in that study. The medicinal plants used by paliyar are listed with latin name, family, local name, parts used, mode of preparation and medicinal uses (Ignacimuthu et al., 2006).

Soosairaj et al., (2007), reported that zonation of conservation sites has been modeled by overlaying different layers such as vegetation types, species richness, endangered, endemic, economic status maps and socio cultural value map using remote sensing and GIS in the Pachamalai hills, a part of Eastern Ghats in India, that is spread to an area of 527.61 sq. km. These hills are situated at the mid region of Tamil Nadu. They harbour eight vegetation types of which tropical dry deciduous forests are wide spread with rich diversity. The study reported that 0.93% (4.95 sq.km) of the total area of the hills for immediate conservation.

Albert et al., (2008), carried ethanobotanical investigation in the north Cachar Hills of Assam. The study revealed that there were 34 threatened species in several parts of the country and in the district itself, in which 6 species are included in the Red Data Book of Indian Plants, 5 are in the Red Data list of IUCN.

Sukumaran and Raj (2008), reported rare and endemic plants in the sacred groves of Kanyakumari District in Tamil Nadu. The study reveals that 201 sacred groves are present in Kanyakumari District and reported 329 plant species, 12 species of shrubs, herbs and climbers were listed as rare, endemic and threatened, belonging to 12 genera under eleven families. Species like Alpinia galanga, Gloriosa superba, Hemidesmus indicus, Kaempföra galangal and Rauvolfia serpentina are endangered and threatened, Justicia beddodi, Leea indica and Petiveria alliaceae are rare, Indigofera uniflora, Naregamia alata,
Ochlandra scriptoria and Osbeckia aspera var. wightiana are endemic to sacred groves of Kanyakumari District.

Selvamony Sukumaran and Solomon Jeeva (2008), carried out a floristic study on miniature sacred forests of Agathesehwaram, Southern Peninsular India. The area covers 2.6 ha. Altogether, 98 angiospermic species from 87 genera and 43 families were enumerated from the sacred grove. Out of these species 80.6% are used as medicine. 6.12% are timber value and 10.20% are used as minor forest product by the local inhabitants of the study area. Some of them are endangered and endemic plants confined to that grove. The attendant cultural rites and religious rituals have perpetuated the status of the sacred grove, which has ensured the protection of the grove vegetation.

Ethnobotanical survey in the district of North Cachar hills, Assam, North East India was investigated. The results revealed that 34 species of plants to be threatened in several parts of the country and in the red data book of Indian plants and 5 numbers of species have already been included in the Red Data list of the IUCN. 13 species reported in that paper have not been reported about its threatened status earlier, but are threatened in the district. These plants are used in various ways such as, medicinal, wild edibles, ornamentals. Building materials and other miscellaneous uses in their daily life. The declining populations like Taxus baccata, Renanthera inschootiana, Swertia chirata etc., the area warrants conservation in order to preserve them from extinction. (Sajem et al., 2008).
2.1. Tissue Culture

I. Indirect Organogenesis

Callus formation from different explants

Haberlandt in 1902 predicted the possibility of the production of artificial embryos from vegetative cells. He developed the concept of *in vitro* cell culture (Tabata, 1977). He is known as the father of plant tissue culture.

A callus is an amorphous mass of loosely arranged thin walled parenchymatous cell developing from proliferating cells of the parent tissue (Dodds and Roberts, 1985). In nature, callus develops by infection of microorganisms from wounds due to stimulation by endogenous growth hormones known as the auxins and cytokinins. However, it has been artificially developed by adopting tissue culture techniques.

Simola (1985), reported callus induction on new medium (N7) fortified with casein hydrolysate (1.0 mg/l) in *Betula pendula* when young leaf blades were used as explants.

Vasil, (1991), has reported that the application of plant tissue methods in crop-improvement depends upon the induction of viable callus culture and its maintenance in *in vitro* conditions. Natarajan *et al.*, (1991), reported that callus mediated shoot regeneration from stem explants of *Hybanthus enneaspermus*. They described that nodular calli were proliferated on MS medium containing 2 mg/l 2,4-D and 0.5 mg/l BAP. Shoot buds were induced and elongated on MS basal medium containing 5.0 mg/l BAP. The *in vitro* regenerated shoots were rooted on MS medium with 2.0 mg/l IBA. The rooted plants were then successfully hardened and transferred to the field.
Jaideep Mathur (1992), produced callus cultures of *Nadoslachys jatamansi* DC. from petiole explants on MS medium with 3.0 mg/l of NAA and 0.25 mg/l BAP. Philip *et al.*, (1992), initiated multiple shoots from the shoots tip explants of black pepper (*Piper nigrum*). The highest number of multiple shoots was initiated when the MS medium was fortified with 1.0 mg/l BAP.

Callus formation was seen in *Emblica officinalis* when MS medium with 1.0 - 4.0 mg/l, 2,4-D and 25.0 mg/l ascorbic acid was used for the culture (Umarani and Narmatha Bai, 1993). Suresh and Rao (1994), obtained callus in *Ricinus communis* when the terminal bud explants were used on MS medium with 0.4 mg/l BAP and 4.0 mg/l NAA.

Callus induction and proliferation was observed from immature leaf explants of *Aegle marmelos* (L) Carr. when treated with MS medium supplemented with B5 and different hormones concentrations of 2,4-D (0.5 mg/l), NAA (0.1 mg/l), Kn (0.1 mg/l) and IAA (0.5 mg/l) (Arumugam and Rao, 1998).

Arumugham and Rao (1998), developed a protocol for callus initiation and proliferation from leaflet explants of *Aegle marmelos* when cultured in MS medium with B5 vitamins and 0.1 mg/l NAA, 0.01 mg/l kinetin.

Anju John and Prathapasenan (1999), were succeeded in the regeneration of shoots from callus cultures in *Oryza sativa*, when L8 medium was used with 0.25 mg/l BAP and 0.1 mg/l IAA. Bidwell *et al.*, (2001), established a protocol for callus formation using stem and leaf explants of the nickel hyperaccumulator *Hybanthus floribundus* on a medium with 5 μM BA and 0.5 μM NAA.
The callus was induced from the meristem tips of *Ananas comosus* when the explants were cultured on MS supplemented with 1.5 mg/l NAA and 1.0 mg/l Kn. The callus when subcultured on MS with 1.5 mg/l Kn and 0.5 mg/l NAA produced large number of shoots within 3 weeks. After 4 weeks, shoots were excised and implanted on shoot elongation medium consisting of MS basal salts fortified with 15% coconut water. After harvesting shoots the old callus was transferred to fresh medium of the same constituents, where the old callus expanded and many new buds also emerged (Akbar *et al.*, 2003).

Callus and plantlet regeneration was induced from leaf explants of *Solanum nigrum*, an important plant on MS medium supplemented with different concentrations of IAA and NAA (0.5-3.0 mg/l) with or without BAP (1.0 mg/l) and GA₃ (0.01 mg/l). The highest frequency of green compact callus and multiple shoots were obtained on the MS medium containing 2.0 mg/l IAA, 1.0 mg/l BAP and 0.01 mg/l GA₃ (Jawahar *et al.*, 2003).

Sivakumar *et al.*, (2003), obtained callus when the nodes were inoculated on SH medium supplemented with 2,4-D combined with 2iP in the range of 4.52-9.84 µM and kept in darkness. The calli were yellowish in colour and compact.

Jawhar *et al.*, (2003), obtained the highest frequency of green compact callus and multiple shoots on MS medium containing 2 mg/l IAA, 1 mg/l BAP and 0.01 mg/l GA₃ in *Solanum nigrum* L.

Mohan *et al.*, (2004), reported highest frequency of callus induction in *Solanum nigrum* L, in MS medium containing 2.0 mg/l IAA (88.0%) and NAA (82.0%). It was observed that whitish and brownish friable callus was induced. The highest percentage of green compact calluses (73.0%) and shoot bud
induction (67.0%) as well as the highest number of multiple shoots (8.6 shoots/stem explant) were observed in medium supplemented with 2.0 mg/l IAA, 1.0 mg/l BA and 0.01 mg/l GA$_3$. All the regenerated shoots were rooted in the same medium.

Sivakumar and Krishnamurthy (2004), achieved 98% of callus induction in non dormant corm bud explants of *Gloriosa superba* L. The greatest number of multiple shoots (57) was observed in corm derived calluses. Vigorous root formation occurred in all the cases when multiple shoots were derived.

The leaf explants of *Allium sativum* L. was used to induce callus, proliferation and organ formation. Different combinations and concentrations of growth regulators like 2,4-D, NAA and BA were used in the study. The highest callusing was recorded at the best concentration of 2,4-D (1.0 mg/l) with 71.42 %. Highest regeneration and highest number of shoots per callus were found in combinations and concentrations of 1.0 mg/l NAA and 1.0 mg/l BA with 71.42 % (Salam *et al*, 2008).

**Regeneration of shoots from callus**

Anjana Baruah and Bordoloi (1991), observed shoot proliferation from leaf callus of *Cymbopogon marinii* in basal medium and B$_5$ medium with BA, Kinetin, Calcium pantothenate and biotin. It was found that 0.5 mg/l BAP and 0.5 mg/l kinetin was ideal for differentiation of about 20-25 shoots form a single explants in *Aegle marmelos*. (Rekha Bhatia *et al*., 1992).

Bhat *et al*. (1992), obtained shoot regeneration from the callus of *Piper longum*. When cultured on MS medium fortified with 0.5 mg/l IAA and 1.5 mg/l BA. Roy *et al*., (1992), obtained regeneration of shoots from root callus of...
Lathyrus sativus when the callus was cultured on MS medium containing 10.7 µM NAA and 0.9 µM – 1.4 µM kinetin.

Aminuddin et al., (1993) developed good number of shoots in Piper betel when the soot tip callus was cultured on MS medium supplemented with 0.9 mg/l Kinetin and 0.5 mg/l IAA. Umarani and Narmatha Bai (1993), reported shoot bud formation in Sesbania speciola when the callus was cultured on MS medium with 2.0 mg/l BAP and 0.5 mg/l NAA. Simanthi Chaudhuri and Nair (1994), produced shoot regeneration of Phaseolus vulgaris from root callus on Blayde’s medium with 3% sucrose.

Arulmozhi and Ramanujam (1997), reported that 10.0 mg/l BA and 2.0 mg/l IAA produced best shooting per explants of Solanum trilobatum. Baburaj et al., (2000) developed a protocol for the regeneration of shoot buds form leaf callus of Cleodendrum inerme on MS medium supplemented with 0.5 mg/l NAA and 2.0 mg/l BA. Arokiasamy et al., (2000) obtained best shoot differentiation in Cicer arietium. L through callus derived from the leaf explants on MS medium containing 2.21 µM BA and 1.44 µM GA3.

John Britto et al. (2002), developed a protocol for the regeneration of shoot buds from callus of Anisomelos indica on MS medium fortified with 13.32 µM BAP and 2.69 µM NAA.

Saravanan et al. (2007), reported that 2,4-D was the best hormone to develop callus in Pedalium murex (Linn.). Callus initiation was successful when the internodal segments of P. murex were cultured on MS medium suplemented with 3.0 mg/l 2,4-D was used. The callus produced the highest rate of shoot buds when cultured on MS medium fortified with 3.0 mg/l BAP.
Kolar et al. (2008), reported that the highest frequency of green compact callus and multiple shoots were obtained on MS containing 2.0 mg/l IAA and 0.5 mg/l BAP. The callus when cultured on MS basal medium fortified with different concentrations of BAP (3.0 - 8.0 mg/l) and IAA (0.5 mg/l) showed multiple shoot formation. The highest frequency of multiple shoots was obtained on MS containing 6.0 mg/l BAP and 0.5 mg/l IAA. The best rooting was obtained on MS containing 0.5 mg/l IBA. The well-rooted plants were hardened and finally planted in the garden.

II. Direct organogenesis

Multiple shoot induction from different explants

Pranayolov et al., (1987), obtained successful regeneration through callus formation from leaf, stem, leaf stalk, root and seeds of Valereina officinalis when MS medium fortified with 2,4-D, NAA and Kinetin.

Conchou et al., (1991), reported multiple shoots formation from shoot tip of Arnica montana when MS and B5 medium containing 1.0 mg/l BAP and 0.1 mg/l NAA was used. Baburaj and Tamilzhchelvan (1991), observed callus proliferation from the cut margin of leaf explants of Solanum surattense cultured on MS medium supplemented with 2.0 mg/l NAA and 1.0 mg/l BAP. Joshi et al. (1991), reported multiple shoots from cotyledonary and epicotyledonary nodes of Anogeissus pendula when they were cultured on MS medium having 10 mg/l BAP and 0.1 mg/l IAA.

Philip et al., (1992), reported multiple shoot production from Piper nigrum when cultured on MS medium fortified with 1.5 mg/l BAP. Mercier et al., (1992), achieved regeneration of shoots from stem callus of Gomphrena officinalis on MS medium with combination of 5.0 or 10.0 mg/l of BAP with 0.1 mg/l NAA. Rout et al., (1992), observed that the calli of Rosa hybrida produced
shoots when they were cultured on MS medium fortified with 0.2 mg/l BAP, 0.01 mg/l NAA, 0.01 mg/l GA₃ and 20.0 mg/l adenine sulphate. The same workers (1999) have succeeded in shoots regeneration from the callus of Plumbago zeylanica on MS medium supplemented with 4.44 μM BA and 1.42 μM IAA.

Gurudeep Kaur et al., (1992), worked on multiple shoot development in Angeissus serica on MS medium having 0.1 mg/l IAA and 4.0 mg/l BAP, 50.0 mg/l ascorbic acid, 25.0 mg/l citric acid and 25.0 mg/l adenine sulphate. Roy et al., (1992) regenerated shoots from root callus of Lathyrus sativus on MS medium supplemented with 10.7 μM NAA and different concentrations of kinetin (0.9 μM - 1.4 μM).

Taylor et al., (1993), achieved multiple shoots from the apical buds of Saccharum when they cultured on a MS medium with a concentration of 6.0 mg/l BAP. Umarani et al., (1993) produced multiple shoots from the shoot tips of Sesbania species when the shoot tips were treated with MS medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BAP.

Satya Kala et al., (1995) established multiple shoot formation from nodal explants of Pelargonium graveolens on MS medium with a concentration of BAP and IAA at 1.0 mg/l each. A maximum of 56 shoots per explants were observed in 30 days after inoculation.

Mirza and Narkhede (1996), were successful in producing multiple shoots in Capsicum annum on MS medium having a concentration of 5.0 mg/l BAP. Sudha and Seeni (1996), obtained in vitro shoot formation with high frequency (77%) in Rauwolfia micrantha on MS medium fortified with 13.2 μM BA and 2.68 μM NAA. Sitakanta Patnaik et al., (1996), reported the production of
multiple shoots in *Ocimum sanctum* on MS medium with 0.25 mg/l and 1.0 mg/l BA respectively.

Purohit and Ashish Dava. (1996) obtained multiple shooting in *Sterculia urens* from cotyledonary node segments on MS medium supplemented with 2.0 mg/l BAP. Sanchez Graz and Calvo (1996) reported micropropagation of *Lavandula latifolia* through nodal bud culture in the presence of 5 μM of BA.

Manjula *et al.*, (1997), established multiple shoots from shoot tip in *Aristolochia indica* L. When they were cultured on MS medium with 0.5 μM NAA and 13.3 μM BA.

Pinaki Sinha *et al.* (1997), established multiple shooting in *Bougenvillea buttiana* on MS medium with 1.0 mg/l BAP and 0.2 mg/l NAA.

Adolfina *et al.*, (1997) established multiple shoots in *Hedeoma multiflorum* by culturing the nodal explants on half strength MS medium with BA or NAA. Pramod Kumar and Dubey (1997), developed seedlings of *Amaranthus hypochondriacus in vitro* and shoots tips of 7 days old seedlings grown on MS medium supplemented with 4 concentrations of IAA (0.5, 1.0, 2.0, 3.0 mg/l) plus fixed concentration of Kinetin (0.5 mg/l). Maximum differentiation of shoot tips into shoots with slight callus at the base and bushy roots was obtained on MS medium fortified with 2.0 mg/l IAA plus 0.5 mg/l kinetin followed by 1.0 mg/l IAA plus 0.5 mg/l kinetin. Further regenerated plants were transferred to soil and their survivality was also noted.

explants of *Sesamum indicum* L. on MS medium containing 8.0 mg/l BAP and 0.5 mg/l NAA.

Anwar Shahzad *et al.*, (1999) developed callus in *Solanum nigrum* when leaf explants were cultured on MS medium containing 2.0 mg/L, 2,4-d and 0.05 mg/l BAP. Jagadish Chandra *et al.* (1999), reported the development of multiple shoots from axillary buds from a threatened medicinal tree, *Pisonia alba* on MS medium containing 1.0 concentration of Kinetin. Nagaraju *et al.* (1999), reported multiple shoots from meristem tips of *Ficus religiosa* on MS medium fortified with 0.5 mg/l BA. Arokiasamy *et al.*, (1999) used MS medium supplemented with 2.0 mg/l BAP to develop callus in *Datura metel*. Bhanu Verma and Kant,(1999) developed shoots from root callus of *Emblica officinalis* on MS medium containing 2.0mg/l BA, 2.0mg/l kinetin, 25.0mg/l citric acid, 25.0mg/l ascorbic acid and 25.0mg/l PVP.

Kamalam and Jegadeesan (1999) have obtained multiple shoots from nodal explants of *Eclipta prostrata* on MS medium supplemented with 2.0 mg/l IBA and 1.0 mg/l BA. Kathiravan and Ignacimuthu (1999) developed a protocol for the production of multiple shoots from nodes of *Canavalia virosa* on MS medium with BAP, GA3 and kinetin.

Ramanujam *et al.*, (1999) obtained a maximum of 8 shoots in *Amaranthus tristis* L. from nodal explants with MS medium fortified with 0.1 mg/l NAA and 10 mg/l BAP. Sen and Sharma (1999) reported shoot multiplication from shoot tips of seedlings of *Withania somnifera* L. when they were subcultured on MS medium supplemented with 2.3 μM 2,4- D.

Sumana et al., (1999) have successfully regenerated shoot tips of *Holarrhena pubescens* Wallich ex G. Don by culturing them on MS medium.
supplemented 3.0% Sucrose, 2.0 g/l glycine and 2.0 mg/l BA. Madhuri and Chandramati (1999) induced multiple shooting from the shoot tips of date palm (*Phoenix dactylifera*) in MS medium supplemented with 5.0 mg/l BAP and 0.1 mg/l NAA. Geetha *et al.*, (2000) established a micropropagation protocol for *Vanilla planifolia* when nodal explants were cultured on MS medium with 1.0 mg/l BAP.

Sivakumar *et al.*, (2000), reported shoot formation from the shoot tips of *Gloriosa superba* L. when cultured on MS medium supplemented with BAP in the range of 0.444μM-4.44 μM and 2iP in the range of 4.92 μM-9.84 μM with or without kinetin.

Manisha Thakur *et al.*, (2001), developed an multiple shoots in *Alnus nepalensis* using terminal bud on WPM fortified with 15 g/l glucose and 0.5 mg/l BA. Sanchita Chaudhuri and Usha Mukundan (2001) reported maximal shoot formation in *Aloe vera* L. using shoot tip explants. The ideal concentration for shoot formation was MS medium with a concentration of 10 mg/l BA, 160 mg/l Ads and 0.1 mg/l IBA. Sudharsan *et al.*, (2001), established multiple shoots from shoot tips of *Ziziphus maceritiana* on MS medium supplemented with 100 mg/l glutamine, 125 mg/l myo-inositol and 0.1 mg/l BA.

Fracoro (2001) developed a protocol for multiple shoot production in shoot tips of *Cunila galioides*. The highest multiplication rate was observed on culture medium having MS basal salts with 8.8 μM of BA. Repeated subcultures for eight months enabled mass multiplication of shoots.

Arockiasamy *et al.*, (2002), induced multiple shoots from nodal explants of *Solanum trilobatum* L. in LS medium supplemented with a combination of 5 mg/l BAP and 0.05 mg/l IAA.
Hassan and Roy (2005), obtained rapid shoot multiplication with 8 shoots per culture in *Gloriosa superba* L. when the apical and axillary buds of young sprouts were cultured on MS basal medium fortified with 1.5 mg/l BA and 0.5 mg/l NAA.

A protocol for reproducible *in vitro* mass multiplication of plants through root cultures established for *Datura metel*. Maximum number of shoots was formed on MS medium supplemented with BAP 4.0 mg/l after 15 days. Shoot elongation was achieved on MS medium containing BAP 2.0 mg/l and GA₃ 1.0 mg/l. Rooting was favoured in MS medium with IBA 1.0 mg/l. The well-rooted plantlets in plastic cups containing sterilized vermiculite, garden soil and farmyard soil in the ratio 1:1:1. The plantlets were successfully transferred and grow in soil mixture after a period of two weeks, with an impressive survival rate of 60% (Nithiya and Arockiasamy, 2006).

Chaplot *et al.* (2006) obtained maximum number of multiple shoots (12) from nodal explants of *Plumbago zylanica* L. with MS medium supplemented with 4.4 mg/l BA and 1.4 mg/l IAA.

**Rooting of regenerated shoots**

Anjana Baruah and Bordoloi (1991), found that the individual shoots of *Cymbopogon martini* when transferred to half strength liquid basal medium, B5 or full strength white medium with 2.0 mg/l IBA or NAA, produced profuse root formations in shoots after 2-3 weeks.

Rekha Bhatia *et al.* (1992), found that about 80% of the shoots of *Aegle marmelos* were rooted when they were subcultured on 1/4th strength MS medium fortified with 0.5 mg/l NAA or IAA.
Adventitious and axillary shoots developed in cultures in the presence of a cytokinin generally lack roots. To obtain full plants, the shoots must be transferred to a rooting medium which is different from the shoot multiplication medium, especially in its hormonal composition, half strength MS medium with 1 µM NAA (Upreti and Dhar, 1996).

Rout et al. (1999), has reported that there were 95.2% rooting of shoots in *Plumbago zeylanica* when cultured on half strength MS medium with 0.57 µM IAA and 2% glucose.

Sivakumar and Krishnamurthy (2000), reported that roots were formed in *Gloriosa superba* L. by any of the auxins except IAA in MS medium. When the medium containing IBA in the range of 0.492-4.92 µM and NAA in the range of 0.537-5.37 µM, the root formation was found to be the best.

Roxana ahmed et al., (2005), induced roots in *Annona squamosa* when half-strength MS media supplemented with IBA. The best concentration with high percentage of rooting was observed when 2.5 mg/l IBA was used in the MS medium. Poornima and Shivamurthy (2005), observed root formation in *Quisqualis indica* L. when WPM (Woody Plant Medium) fortified with IBA or IAA. The best rooting was observed when the media was supplemented with 1 mg/l IBA.

2.2. Phytochemical analysis of plant extracts

I. Gas Chromatography-Mass spectroscopy (GC-MS)

Literature survey pertaining to phytochemical analysis of medicinal plants revealed that the crude plants and their active principles have been effectively exploited against selective microorganisms *in vitro*. The phytochemical investigations of the plant extracts have revealed the presence of biologically
active compounds such as flavanoids, tannins, phenols, terpenoids, sesquiterpenes, glycosides, saponins, alkaloids etc., (Odebiyi and Sofowora, 1978; Ieven et al., 1979; Reinhold et al., 1981; Harbone, 1982; Bever, 1986; Barnabas and Nagarajan, 1988).

Sadyojatha and Vaidya (1996) reported two steroidal glycosides from chloroform extract of root powder of *Momordica dioica* Roxb. Ex. Wild and also an alkaloid.

Luo Lei et al., (1998) isolated three triterpenes and two steroidal compounds from the roots of *Momordica dioica* Roxb. Ex. Wild the compounds are \( \alpha \)-Spinasterol Octadecanonate; \( \alpha \)-Spinasterol-3-o-\( \beta \)-D-glucopyranoside; 3-o-\( \beta \) -D-glucopyranosyl gypsogenin (a new compound); 3-o- \( \beta \) -D-glucopyranosyl gypsogenin; and 3-o- \( \beta \) -D-glucopyranosyl hederagenin.

Presence of five anti-cancer compounds isolated from the dried roots of *Mimordica dioica* and identified them as bryonolic acid, 23, 24- dihydro cucurbitacin-F-25-acetate, \( \alpha \)-spinasterol-3-o-6-D-glucopyranosides, gypsoogenin and hederagenin (Li Zuquiang et al., 1999).

Edeoga et al., (2005) reported the presence of Alkaloids, Tannins, Saponins, Steroids, Terpenoids, Flavonoids, Phlobatanin and Cardic glycoside distribution in ten medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Cleome nutidosperma, Emilia coccinea, Euphorbia heterophylla, Physalis angulata, Richardia brasitensis, Scopania dulcis, Sida acuta, Spingelia anthelhnia, Stachytarpheta cayennensis, and Tridax procumbens*. All the plants were found to contain alkaloids, tannins and flavonoids in *S. cayennensis* respectively. The significance of the plants in traditional medicinal medicine and the importance of the
distribution of these chemical constituents were discussed with respect to the role of these plants in ethanomedicine in Nigeria.

Ogunkunle and Tonia A. Lade jobi (2006) established on ethnobotanical search on five species of *Senna* with in and around ogbomosa, Oyeo state, Nigeria, showed their relevance in the local herbal medicine. These plants include *Senna tora, Senna occidentalis, Senna alata, Senna podocarpa,* and *Senna siamea.* The phytochemical screening of their leaves revealed some major groups of pharmacological importance including alkaloids, flavonoids, tannins, phlobatannins, saponins, and anthraquinones. The study establishes concordance between the local medicinal applications of the plants, investigated and their constituent phytochemical groups which are relevant to the pharmaceutical industries. Lastly it confirms ethnobotany as viable tool in search for plant genetic resources for use in the industries.

II.  **High Performance Thin Layer Chromatography**

Gupta *et al.,* (2001) performed a simple, precise and rapid high performance thin layer chromatography method for the simultaneous quantitative determination of five olean e derivatives namely arjunic acid, arjunolic acid, arjungenin, arjunetin and arjunglucoside I from stem bark extract of *Terminalia arjuna.*

Nagasenkar and Tayade (2006) performed a simple, sensitive precise and rapid HPTLC method of analysis of artemether both as bulk and in pharmaceutical formulations and validated.

Motwani *et al.* (2006) performed a simple, sensitive, selective, precise and stable HPTLC method for determination of gatifloxacin both as a bulk drug
and from polymeric nanoparticles and was validated as per the International Conference on Harmonization (ICH) guidelines.

Shah et al., (2006) performed a rapid and sensitive high performance thin-layer chromatography (HPTLC) method and validated for the quantitative estimation of boswellic acid in formulations containing *Boswellia serrata* extract (BSE) and 11-keto-boswellic acid in human plasma.

Chaudhari et al., (2006), developed and validated a HPTLC method for the estimation of Atorvastatin Calcium and Ezetimibe. The mobile phase was the mixture of Chloroform:Benzene:Methanol:Acetic acid (6.0:3.0:1.0:0.1 v/v/v/v).

Esters et al., (2006), performed a quantitative densitometric high performance thin layer chromatography (HPTLC) for determination of glucosamine in a dietary supplement containing dried extracts of the main plants traditionally used for rheumatic disorders.

Bharathi et al., carried out HPLC for the quantification of Colchicine in six different species of *Gloriosa*. High levels of Colchicine was found in *Gloriosa planti* among the other species.

Bazylko et al., (2007) performed a method for separation and quantitative determination of oenothein B (OeB) and quercetin glucuronide (QG) in aqueous extract of *Epilobii angustifolium* by HPTLC-densitometry. The method was found to be relatively simple, precise and accurate for the quality control of *Epilobii angustifolium* extracts.

Phytochemical investigation of the rhizomes of *Curcuma longa* that lead to the isolation of pharmacologically active curcuminoids viz. curcumin,
demethoxycurcumin and bisdemethoxycurcumin. The purity of curcuminoids was analyzed by HPTLC by using a solvent system of chloroform and methanol (48:2 v/v) was reported by Paramasivam et al., (2008).

Arumugham et al., (2008) performed HPTLC for the chemical fingerprinting of S.anacardium plant parts and some commercial polyherbal formulations and quantification of tetrahydroamentoflavone. The method was able to identify and quantify tetrahydroamentoflavone from complex mixtures of phytochemicals and could be extended to the marker-based standardization of polyherbal formulations, containing S.anacardium.

Krauze-Baranowska and Poblocka-Olech, (2008), performed a HPTLC method for the qualitative and quantitative analysis of procyanidin B1 in willow barks. The content of procyanidin B1 varied from 0.26mg/g in the extract of Salix purpurea clone 1067-2.24 mg/g in the extract of Salix alba alone 1100 mg/g.

Divya et al., (2008), described a simple, rapid and sensitive HPTLC method to identify and quantify sesamin oil and the method was applied to polyherbal formulations containing the oil for their quantitative estimation.

Agrawal et al., (2008), performed a new, simple, sensitive, precise and robust HPTLC method for the estimation of connessine in herbal extracts and pharmaceutical dosage forms.

2.3. Protein profiling

Castillo et al., (1987) studied banding patterns of two isoenzymes: phosphoglutamase (PGM) and peroxidase (PX) of two Euramerican poplar

Przybylska (1986), carried out electrophoretic protein analysis for the systematic description of *Pisum* genetic resources. The electrophoretic patterns of the legumin fraction obtained by means of Urea-Poly Acrylamide Gel Electrophoresis and Isoelectrofocussing were helpful in discriminating the genetic resources samples.

Sammour (1991) reported that polyacrylamide gel techniques are a valuable tool to identify species and cultivars. This identification is very important for plant breeders, certification authorities and also in genetic resources management. Polyacrylamide gel techniques allow us to identify variation among the taxa of each species, screen the purity of the ever expanding number of cultivars, valify whether or not two or more morphologically identical accession in the collection were also electrophoretically identical and exploit the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield.

Sammour (1999), used SDS-PAGE for the extraction and characterization of six proteins with molecular weights of 55 kDa, 50 kDa, 47 kDa, 45 kDa, 43 kDa and 41 kDa in *Linum usitatissimum* L.

Sinha *et al.*, (2001) reported, soluble proteins from the tuberous roots of *Momordica dioica* the roots were analysed by SDS – PAGE to compare the protein profiles of the sex forms. Twenty eight bands with molecular masses ranging from approximately 15 kDa protein to more than 94 kDa, proteins was found to be common in both staminate and pistillate plants. The pistillate plant only had 22 kDa polypeptide which was not detected in staminate ones.
Immunoblot assay demonstrated that antibody raised against p-22 not only cross-reacted at 22 kDa antigen of the pistillate plant but also with 29 kDa and 32 kDa polypeptides of the staminate and pistillate plants. These indicate that the 3 polypeptide are electrophoretically distinct but antigenically similar. 22 kDa protein found in pistillate is sex linked. Variation in the intensity of 29 kDa and 32 kDa polypeptides of staminate and pistillate plants suggests that the interplay of the above 2 sex – linked polypeptides may contribute to the dioecism in *Momordica dioica*.

Alvaro Julio Pereira *et al.* (2002), investigated proteins from young unexpanded leaves of seven cassava cultivates *Manihot esculenta* Crantz (Euphorbiaceae) through Sodium dodecyl sulfate, Polyacrylamide gel electrophoresis (SDS – PAGE). The comparison was made through the protein patterns obtained, and their relative amounts determined in plants leaves infected or not with bacteria *Xanthomonas axonopodis* pv. manihotis. The electrophoretic protein pattern obtained from the investigated cultivars, showed a polypeptide subunit present extensively in the “Fecula Branca” cultivars, with a molecular weight of 93.5 kDa. The protein fractions were more intensely stained in young leaves of *M. esculenta* plants infected with bacteria. The 93.5 KDa protein fraction can be used as a molecular marker for the “Fecula Branca” cultivars.

Mohamed Bottag (2005), has applied SDS – PAGE technique to study the protein profile after phytotoxicity of lead in the root nodules in *Vicia faba* L. The investigation revealed that there was a great inhibition in the band intensity of large subunit (240 KDa) of nitrogenase enzyme after lead oxide treatment. The soil application of lead oxide also resulted in the complete inhibition of small subunits (52 to 73 kDa) of nitrogenase. The foliar application of PbO greatly stimulated a 23 KDa protein.
Petrova et al. (2006), studied the isoenzyme and protein patterns of invitro micropropagated plantlets of Gentiana lutea L. through electrophoretic investigations of reduced proteins and ADH, ACPH, EST and GOT. The variants, produced by applying different types and concentrations of growth regulations in MS medium showed qualitative isoenzyme variations in ADH, ACPH Aand Est while quantitative variations were seen in GOT isoenzymes.

Amandeep Kaur et al. (2006) utilized SDS-PAGE technique to study a new lectin isolated from the bulb of Crinum latifolium L. with carbohydrate specificity towards methyl – α-D – Manno pyranoside. In SDS-PAGE, lectin positive ion exchange fractions gave single bond with a subunit molecular mass of 12 KDa. Gel filtration with purified lectin gave a molecular mass of 24 kDa indicating it to be a dimer.

Shidesh Montasser Kouhsari et al., (2006), compared the electrophoretic patterns of six enzyme systems viz. lactate dehydrogenase (LDH), malate dehydrogenase (MDH), esterase (EST), superoxide dismutase (SOD), peroxidase (PRX) and polyphenoloxidase (PPO) from 15 seed samples of 7 species of Hyoscyamus L. from Iran to clarify taxonomic relationships within the genus. The results indicated that H.tenuicaulus is completely distinct from other species while H.arachnoides and H.niger are very heterogenous. H.kurdis showed a close relationship to H.niger and H.turcomanicus is very close to H.arachnoides.

Syed Dilnawaz Ahmad et al, (2007) have studied the eight ecotypes of Elaeagnus Umbellata (Thunb.) for comparison of their relationship and evolution based on SDS-PAGE of total seed proteins that showed each autmn olive population could be distinguished by their own specific protein bands with reference to a molecular marker. The dendrogram, so generated, indicated that
populations, having the same base of origin fall under and simultaneous groups p6 and p8 in one group and p1, p2, p3, p4, p5, and p7 in the second group.

Zubaida Yousaf et al. (2008) investigated the protein profile of 42 accessions belonging to 7 species of 4 different genera – Datura, Hyoscyamus, Withania, and Atropa. A dendrogram based on UPGMA revealed close association between Withania / Datura and Atropa / Hyoscyamus. It also showed the difference in the morphology and protein profile of specimens of W. somnifera collected from panjgu in the form of allelic variation at band 66, 64, 50, 42, 22, and 16 kDa. The study provides useful information for the identification of the taxa, their relationship and the delimitation of their taxonomic status.

2.4. Random Amplified Polymorphic DNA (RAPD)

Rajaseger et al., (1997) analysed genetic diversity among Ixora cultivars (Rubiaceae) using RAPD analysis. The DNA from fresh laminas of 22 cultivars of Ixora was subjected to RAPD analysis individual taxa could be identified using specific DNA markers from the RAPD profiles. Cluster analysis of data from six primers grouped all 22 cultivars distinctly under two cultivar groups viz., Ixora coccinea and Ixora Javanica.

Rout, et al., (1998) used RAPD markers to evaluate genetic stability of micropropagated plants of Zingiber officinalis cv. V3S18. 15 arbitrary primers were used to amplify DNA from in vivo and in vitro plants to assess genetic fidelity. All RAPD profiles from micropropagated plants were monomorphic and similar to those of field grown control plants. No variation was detected within the micropropagated plants.
Padmesh et al., (1998) analysed intraspecific variability in *Andrographis paniculata* by RAPD which revealed moderate variation within the species. Similarity measurement using UPGMA followed by cluster analysis resulted in 5 major groups based on geographical distribution that generally reflected expected trends between the genotypes. The results indicated that RAPD could be effectively used for genetic diversity analysis in wild species of prospective value as it is reliable, rapid and superior to those based on pedigree information.

Hollingsworth et al., (1998) explained the reliability of RAPD to amplify polymorphisms in the asparagus (*Asparagus officinalis* L.) genome. The six *Asparagus* cultivars used in the study were distinguished by unique banding patterns generated by each primer.

Thai et al., (1998) evaluated the genetic relationship between three biotypes of water spinach (*Ipomea aquatica*) collected from Florida using forty-eight RAPD primers in which eighteen were informative that yielded 188 resolvable bands, of which 58 (31%) were polymorphic. 5 primers produced unique DNA fingerprints useful for identification of the biotypes.

Nkongolo et al., (1998) has used seedlings as an alternative for embryogenic cultures to isolate DNA and RAPD analyses. Efficient and consistent amplification was achieved using primer concentration ranging from 1.6 to 6.0 μM with 0.1 to 1.0 μg of template DNA.

Helena Persson et al (1998), studied genetic variation within populations and relatedness among populations of *Lilium martagon* from Sweden, Denmark, Norway and Lithuania, and four native populations from Lithuania and Switzerland using RAPD markers. The analysis revealed the domesticated populations did not appear to be less variable than the native populations.
Cluster analysis and multidimensional scaling showed that one of the native Lithuanian populations were remarkably dissimilar to the remaining populations. The native Italian populations was quite similar to some of the Scandinavian populations.

Chia-Szu Wen *et al.*, (1999) examined five populations of *Lilium longifolium* Thunb. from different parts of Taiwan using 140 primers. The investigation revealed that except one population, the closer the geographical locations of the populations, the closer were their genetic relationships and also that of the total variations in the species, 14.08% was attributable to population differences and 85.92% to individual differences within populations.


Pedro Jordano and Jose A. Godey (2000) examined the RAPD variation among seven *Prunus mahaleb* populations extending over 100 km² to examine local differentiation in relation to spatial isolation due to both geographical distance and differences in elevation. No less than 51.4% of the RAPD loci were polymorphic, but very few were fixed and among population variation accounted for 16.46% of variation in RAPD patterns.

Rout *et al.*, (2001) evaluated genetic fidelity in micropropagated *Paulownia tomentosa* through through RAPD. Out of the twenty different
primers tested, three primers (OPN-08, OPA-10 and OPA-09) produced good amplification products that were monomorphic across all the micropropagated plants. The RAPD analysis indicated that the plants raised directly from the meristems were genetically similar with the mother plants.

Lalitha Sunil Kumar et al., (2001) studied genetic variation between 28 Indian populations of the rice pest *Scirpophaga incertulas* using inter-simple sequence repeats (ISSR)-PCR assay. A dendrogram constructed from 79 amplification product using nine primers suggested that there was no geographical bias to the clustering and the gene flow between populations appeared to be relatively unrestricted.

Katia Diadema et al., (2003) suggested a protocol for the isolation of high quality and quantity DNA suitable for AFLP in *Carpobrotus sps.* which gave an average yield of 1800 ng/g of DNA from callus and high reproducible profiles in AFLP. This technique was useful for studying genomic polymorphism in succulent plants having high amounts of polysaccharides and polyphenols.

Rajan Kumar Mishra and Swati Sen-Mandi (2004) studied molecular profiling and development of DNA marker associated with drought tolerance in Tea clones growing in Darjeeling. RAPD analysis of DNA of ten short-listed (on the basis of field performance for drought tolerance) clones using 11 primers, revealed 180 PCR products of which 131 were polymorphic bands. The activity of drought-specific superoxide dismutase (SOD) and ascorbate peroxidase (APX) isozymes was found to be appreciably high in RR17/144, CP1, TV26 and AV2.

Eshgrahti et al., (2005) studied genetic stability in micropropagated plantlets in *Phenix dactylifera* L. by extracting DNA from mother plant as well
as regenerated plants and amplifying the DNA using decamer oligonucleotide primers. The genetic similarity between the mother plant and callus-derived plantlets ranged between 94% (for R1, R2) and 83% for R5. Cluster analysis by UPGMA showed a single large cluster at an estimated similarity coefficient (90.2%).

Asnita Abu Harirah and Norzulaani Khalid (2006), determined the clonal fidelity on *in vitro* *Musa acuminata* cv. Berangan micropropagated from male inflorescence derived from the mother plant. Eighteen arbitrary decamer primers were used to amplify DNA from *in vitro* plants. All RAPD profiles from regenerated plants were monomorphic and no somaclonal variation was detected.

Gaafar and Saker (2006), screened seven varieties of the cultivated Strawberry grown in Egypt, using RAPD marker. Only four primers produced polymorphic DNA bands differentiating the cultivars. Based on those markers, the genetic distances between varieties were determined and their genetic relationships were estimated. The phylogenetic tree revealed that the seven cultivars showed close similarity within the group. Although minor morphological variations were recorded in the leaves of some clones, the RAPD profiles of different micropropagated clones were typical to that of the donor mother plant.

Padmalatha and Prasad (2006), optimized DNA isolation and PCR conditions for RAPD analysis of selected medicinal and aromatic plants from Peninsular India containing high levels of polysaccharides, polyphenols and secondary metabolites. The DNA isolation method involved a modified CTAB extraction employing polyvinyl pyrrolidone while grinding, successive long-term Chloroform: Isoamyl alcohol extractions, an over night RNase treatment
with all steps carried out at room temperature. The RAPD protocol was optimized based on the use of higher concentration of MgCl$_2$ (3 mM), lower concentrations of primer (0.5 µM) and Taq polymerase (0.2 units), 50 ng of template DNA and an annealing temperature of 37°C resulted in optimal amplification.

Hong Chen and Yong-Qing Wang (2006), carried out molecular analyses to investigate the interaction between cells in graft union of tomato and eggplant. RAPD analysis of calluses in the homo- and hetero-graft union revealed the presence of graft union specific bands, while donor specific bands were found absent in the hetero-graft union.

Islam et al., (2007), used RAPD to study genetic diversity in different populations of *Curcuma zedoria* (Christm.) Roscoe. The RAPD based estimations showed that hilly populations maintain higher genetic diversity which was also found to be distinct from plainland and plateuland populations.

The effects of high levels in cytokinins on micropropagation of banana and the genetic stability of plantlets using RAPD and ISSR markers. A total of 50 RAPD and 12 ISSR primers generated 17,400 bands whose intensity histogram confirmed their monomorphic nature with no genetic variations in all the plantlets were reported by Lakshmanan Venkatachalam et al., (2007)

El-Dougdoug et al., (2007) detected somaclonal variations in tissue culture-derived banana plants BBTV and CMV. 15 arbitrary base primers were used to amplify DNA extracted from banana plants propagated in *in vivo* and *in vitro* conditions. The DNA fingerprint revealed 25% polymorphisms between total plants and approximately 10% of the analysed plantlets (8 regenerates).
Asit Mandal et al., (2007) studied the RAPD pattern of *Costus speciosus*, an important medicinal plant of Andaman and Nicobar Islands using 12 decamer random primers of which 4 showed appreciable intra-species variation. UPGMA analysis revealed 35% variation in the collections, which was useful in formulating sound conservation strategies for the plant.

Manoj Kumar Panda et al., (2007) has performed RAPD analyses in order to confirm the genetic integrity of micropropagated *Curcuma longa* L. by using twenty selected RAPD primers. The study gave rise to a 86 scorable bands ranging from 300 bp to 2700 bp in size. The banding pattern showed no RAPD polymorphism in the micropropagated plants. The study suggested that *in vitro* shoot multiplication using axillary buds of the rhizomes may be used for rapid clonal propagation with a low risk of generating somoclonal variations in *Curcuma longa* L.

Abdin et al., (2007) developed a simple and efficient protocol for isolating genomic DNA from fresh and dry roots of medicinal plants involving a modified CTAB method with 3% CTAB, 4% β-mercaptoethanol, 2M NaCl and 5% PVP. The DNA yield ranged from 33 to 68 μg per g of root samples and was 1.47 times greater in dried than fresh samples.

Asit et al., (2007), carried out RAPD-PCR analysis to assess the quantum of genetic variations in *Costus speciosus* Koen ex. Retz. an important medicinal plant of Andaman and Nicobar Islands. The study revealed the great deal of polymorphism among the 14 accessions collected from different parts of the islands.

Kumar et al., (2007) carried out RAPD analysis to assess the authentic identification as well as to solve the taxonomic problems between *Senna*
surattensis and Senna sulfurea. Amplification with 10-mer primers was performed under pre-standardize condition of 38 accessions along with Senna occidentalis, Cassia fistula, Senna tora and Senna siamea collected from different mixed populations. Out of sixty primers utilized fifty four were successful in amplification and among them one was species-specific. The results demonstrate the ability of RAPD markers to reliably differentiate between Senna surattensis and Senna sulfurea.

Siddharth Kumar Palai et al., (2007) investigated genetic variation within eight high yielding varieties of ginger through RAPD markers. A total of 55 distinct DNA fragments were analysis indicated that the eight varieties formed two major clusters. The first major cluster had only one variety ‘S-558’ with 43% similarity with other seven varieties. Second major cluster had seven varieties and was divided into two minor clusters. One minor cluster had six varieties and the other having only one variety. The second minor cluster was further divided into two sub-minor clusters. The study showed the distant variation within the varieties.

Harini et al., (2008) developed a protocol for high quality DNA isolation from the bulb tissues of Urginea indica and optimization of conditions for RAPD-PCR analysis that involved cell lyses using extraction buffer supplemented with cetyltrimethylammonium bromide (CTAB) and sodium chloride. The emulsification involved washes with phenol: chloroform : isoamyl alcohol followed by re-precipitation with salt to remove high protein and polysaccharide contaminations. RAPD profiling from the isolated DNA was optimized to produce scorable, clear amplicons in all populations studied.

Seemanti Ghosh et al., (2008) has employed molecular data to screen polymorphism in five different populations of Gloriosa superba L. Random
Amplified Polymorphic DNA (RAPD) analysis revealed 76% polymorphisms among the populations. Cluster analysis using RAPD resulted in three main cluster groups.

Ananda Kumar et al., (2008) investigated the genetic relationship between Coffea and Indian Psilanthus species through RAPD and ISSR markers. The two marker systems showed significant difference in the genome of Indian Psilanthus species from Coffea. Clustering of Psilanthus species correlated with their geographical distribution showing diversity of peninsular Indian species from the Northeast Indian species. In case of Coffea, analysis of species relationship using RAPD and ISSR markers indicated correlation between the similarity of the genomes and the geographical distribution. The true east African species showed close relationship with each other but having high level of diversity from west and central African species.

Sathyanarayana et al., (2008) employed RAPD analyses in order to assess the genetic integrity of tissue culture clones. RAPD pattern of 10 randomly selected micropropagules obtained from auxillary bud explants were compared with the mother plant using 14 primers. All the tested primers produced monomorphomic pattern across all the shoots, confirming the genetic uniformity of the micropropagated plant material.

Hasibe Cingilli Vural (2009) developed four modified new methods that produced good quality DNA from aromatic and medicinal plants growing in Turkey. Furthermore, the aim is to assess the available genetic diversity for each species to provide more accurate and detailed information than is available using classical phenotypic data in this subject various types of plant materials and a number of different protocols for the isolation of DNA were tested in order to obtain good quality DNA for PCR reactions. Ten populations of different
aromatic and medicinal plants from Turkey were tested in the study the number of plants examined for each population varied from two to five. When fresh (or) frozen leaves collected in autumn were used for the isolation of DNA, no positive result in PCR reaction was obtained regarding the isolation protocol being used. Four different DNA methods were compared for the isolation of DNA from the different plant homogenates, namely the CTAB, plant Genomic DNA purification kit, and EZ1 Nucleic acid isolation methods and DNA extraction with phenol purification and liquid nitrogen method.

2.5. **Antimicrobial Studies**

Perumalsamy and Ignacimuthu (2000), studied the antibacterial properties of 30 Indian folklore medicinal plants used by the tribal healers. The bacteria used in the experimental study were *Bacillus subtilis, E.coli, Klebsiella aerogenes, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Among the 30 plant species the extracts of *Cassia occidentalis* and *Cassia auriculata* exhibited significant broad spectrum of activity against *Bacillus subtilis* and *Staphylococcus aureus*.

The antibacterial effects of leaf extracts of 23 medicinal plants on an anthrachose fungi infecting *Capsicum annuum* was studied. It was observed that extracts of *Solanum torvum, Datura metel* and *Prosopis juliflora* were most effective in curbing the growth of the fungi (Gomathi and Kannabiran, 2000).

Bansal and Rajeshkumar Gupta (2000), studied the effects of leaf extracts of *Azadirachta indica, Atropa belladonna, Calotropis procera, Ocimum bacillicum, Eucalyptus amydaslina, Ailanthus excelsa* and *Lantana camera* on *Fusarium oxysporum*. Among the seven plant extracts, *Azadirachta indica* was effective against the growth of *Fusarium oxysporum*. 
Fabiola Barbieri Holetz et al. (2002), screened extracts of 13 Brazilian medicinal plants for their antimicrobial activity against bacteria and yeasts. Of these, 10 plant extracts showed varied levels of antibacterial activity. *Piper regnellii* presented a good activity against *Staphylococcus aureus* and *Bacillus subtilis*, a moderate activity on *Pseudomonas aeruginosa*, and a weak activity against *Escherichia coli*. *Punica granatum* showed good activity on *Staphylococcus aureus* and was inactive against the other standard strains. *Eugenia uniflora* presented moderate activity on both *Staphylococcus aureus* and *E. coli*. *Psidium guajava*, *Tanacetum vulgare*, *Arctium lappa*, *Mikania glomerata*, *Sambucus canadensis*, *Plantago major* and *Erythrina speciosa* presented some degree of antibacterial activity. *Spilanthes acmella*, *Lippia alba*, and *Achillea millefolium* were considered inactive.

The antimicrobial activity of the hexane, dichloromethane and methanol extracts of *Lippia multiflora* and carvacrol isolated from the hexane extract was examined. The result showed the hexane extract to be the most active, while the methanol extract exhibited no antimicrobial activity. The isolated carvacrol from the hexane fraction showed tremendous antimicrobial activity. The results confirmed the traditional uses of *Lippia multiflora* in the treatment of disease conditions due to microbes (Okunle et al, 2003).

Martin et al, (2003) investigated the antimicrobial effect of olive leaves against bacteria and fungi. The microorganisms tested were inoculated in various concentrations of olive leaf water extract. Olive leaf 0.6% (w/v) water extract killed almost all test bacteria within 3 hours. Dermatophytes were inhibited by 1.25% (w/v) plant extract following a 3 day exposure whereas *Candida albicans* was killed following a 24 hours incubation in the presence of 15% (w/v) plant extract. Olive leaf extract fractions, obtained by dialysis that showed antimicrobial activity consisted of particles smaller than 1000 molecular rate
cutoffs. Scanning electron microscopic observations of *C. albicans*, exposed to 40% (w/v) olive leaf extract showed complete destruction. These findings suggested an antimicrobial potential of olive leaves.

Singh and Singh (2003), yielded mixture of terpenoids: betsitosterol, alpha-amyрин, lupeol, hexacosanic acid, ceryl alcohol and hexacosane. The sequential extracts isolated terpenoids and reference antibiotics were screened for their possible bioactivities against selected pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus* and fungi, *Aspergillus flavus* and *Penicillium chrysogenum*. Out of the sequential extracts, the benzene extract was more potent against *Staphylococcus aureus* and *Rhizobium phaseoli*.

The antibacterial activity against human pathogenic strains such as *E. coli*, *Klebsiella pneumoniae*, *Streptococcus biogens*, *Staphylococcus aureus*, *Pseudomonas aerogenosa* and *Salmonella typhi* using methanol extracts of *Thespesia populnea*, *Centella asiatica* and *Solanum trilobatum* was examined. The observation revealed that methanolic extract of *Centella asiatica* inhibited the growth of all the bacterial strains tested (Sheela and Kannan, 2003).

Khan *et al.* (2003), checked the antimicrobial activity of the methanolic extract of bark of *Eupomatia laurina* against 13 gram positive bacteria, 12 gram negative bacteria, 1 protozoan and 9 fungi. The bark extract were found to be containing alkaloids that had the property of antibacterial activity.

The *in vitro* antimicrobial activity of the essential oil and various extracts obtained from the aerial parts of *Thymus eigii*. It was observed that the oil had strong antimicrobial activity (Tepe *et al.*, 2004).
Zaidan et al. (2005), determined the antibacterial activity in the crude extracts of some of the commonly used medicinal plants like *Andrographis paniculata*, *Vitex negundo*, *Morinda citrifolia*, *Piper sarmentosum* and *Centella asiatica* against bacterial species like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. None of the five plant extracts showed antibacterial activities to *E.coli* and *K.pneumoniae* except for *Andrographis paniculata* and *P.sarmentosum* which showed activity towards *P.aeruginosa*.

Jigna Parekh and Sumitra Chanda (2006), evaluated the antibacterial effects of *Abrus precatorius*, *Cardiospermum halicabum*, *Gmelina asiatica* and *Caesalpinia pulcherimma* on certain bacterial species like *Staphylococcus aureus*, *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella pneumoniae*. The most active antibacterial plant was found to be *Caesalpinia pulcherimma*.

Seenivasan Prabhuseenivasan et al. (2006), carried out antibacterial assay of 21 plant essential oils on 6 bacterial strains namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*. The result showed that cinnamon oil had inhibitory activity even at low concentrations, whereas aniseed, eucalyptus and camphor were least active against the bacteria which confirmed the antibacterial nature of cinnamon oil.

Nalina and Rahim (2007), investigated the antimicrobial influence of crude extract of *Piper beetle* L. on *Streptococcus mutans*. The study was further carried on to determine the chemical components of the extract using Thin Layer Chromatography (TLC) and Gas Chromatography Mass spectroscopy (GCMS). Transmission electron microscopy (TEM) was used to determine the effect of the extract on the ultrastructure of *S.mutans*.
Murugan et al. (2008), screened five plant species namely *Bauhinia purpurea* L., *Cardiospermum halicabum* L., *Cissampelos pariera* L., *Rhinacanthes nastus* (L.) Kurz. Var nostus and swertia against different bacterial species *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* by using different solvents benzene, petroleum ether, chloroform, methanol and the aqueous extract of the plant. It was observed that 44 plant extracts were found to be active against *Bacillus subtilis*, 40 against *Streptococcus lactis*, 39 against *Staphylococcus aureus*. It was observed that the aqueous and organic extracts from the same plant showed different activities.

Doughari et al. (2008), investigated the antimicrobial properties of leaf extracts of *Senna obtusifolia* L. against both clinical and laboratory isolates of both bacteria and fungi. The acetone leaf extract showed the highest activity followed by dichloromethane. The studies suggests that *Senna obtusifolia* L. can be used to source antibiotic substances for possible treatment of bacterial and fungal infections including gonorrhea, pneumonia, urinary trait and some mycotic infections.