CHAPTER – II
REVIEW OF LITERATURE
CHAPTER-II

2.1. Medicinal plants

Tiwari et al., (2011), reported that plants were a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants were claimed to possess the antibiotic properties in the traditional system and were also used extensively by the tribal people worldwide. It is now believed that nature had given the cure of every disease in one way or another. Plants had been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents had become a challenging task for the researchers.

Babita et al., (2011), found that Haritaki (Terminalia chebula) was a common herbaceous plant, which was extensively used in the preparation of Ayurvedic medicines. It was found to be present throughout India, chiefly in deciduous forests and areas of little rainfall. It grows under a wide variety but thrives best in clay and sandy soil. The fruits ripe from November to March depending upon the locality. The extract obtained from Haritaki fruit contains a substance which showed antibacterial and antifungal properties. This substance inhibited the growth of bacteria and fungi such as E.coli. It was also used in the treatment of Leucorrhoea, Chronic ulcers, Pyorrhea and other type of fungal infections of the skin.

Bag et al., (2012), explained that medicinal plants were part and parcel of human society to combat diseases from the dawn of civilization. Terminalia chebula Retz. (Fam. Combretaceae) is called the ‘King of Medicine’ in Tibet and is always listed at the top of the list of ‘Ayurvedic Materia Medica’ because of its extraordinary power of healing. The whole plant possessed high medicinal value and traditionally used for the treatment of various ailments for human beings. Some of the folklore people used this plant in the treatment for Asthma, Sore throat, Vomiting, Hiccough, Diarrhea, Dysentery, Bleeding piles, Ulcers, Gout, Heart and Bladder diseases. The plant has been demonstrated to possess multiple pharmacological and medicinal activities, such as antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimitogenic, antiproliferative, radioprotective, cardioprotective,
antiarthritic, anticaries, gastrointestinal motility and wound healing activity. But no systematic updated information on the therapeutic effectiveness of *Terminalia chebula*, a popular herbal remedy in India and South-East Asia had so far been reported. That result provided an incentive for proper evaluation of the plant as medicinal agent against the human diseases and also to bridge the lacunae in the existing literature and future scope which might offer immense opportunity for researchers engaged in validation of the traditional claims and development of safe and effective botanical medicines.

Gupta, (2012b), said that the medicinal plants had been considered valuable and cheap source of unique phytoconstituents which were used extensively in the development of drugs against various diseases. A large proportion of the world population, especially in the developing countries relied mainly on the traditional system of medicine. The use of plants and plant products in medicines is getting popularized because the herbal medicines are cheap and have natural origin with higher safety margins and lesser or no side effects. *Terminalia chebula* Retz. belongs to the family Combretaceae and is one of the most important medicinal plants used in medicines of ayurveda, siddha, unani and homeopathy. It is called “the king of medicines” in Tibet and is listed first in the Ayurvedic material medica because of its extraordinary power of wound healing and a wide spectrum of medicinal properties. *Terminalia chebula* possesses antibacterial, antifungal, antiviral, antidiabetic, antimutagenic, antioxidant, antiulcer and wound healing properties. It also prevents cardiac damage and is used for the treatment of kidney disease. It is a mild, safe and effective laxative in traditional medicine. *Terminalia chebula* and its phytoconstituents have therapeutic effect with no toxicity. *Terminalia chebula* is an active ingredient of the well known herbal preparation, Triphala, which is used for the treatment of enlarged liver, stomach disorders and pain in eyes. This review gives a bird’s eye view on the biological and pharmacological properties of various extracts and isolated phytoconstituents of *Terminalia chebula* to enrich our knowledge about this plant.

Vinotha *et al.*, (2013), reported that *Enicostemma littorale*, Blume (Gentianaceae) was a glabrous perennial herb and it was found in open, sandy places among sparse grass close the beach throughout the dry zone in Sri Lanka. It was
traditionally used to treat inflammatory and painful conditions like arthritis, back pain; Diabetes mellitus and to regulate bowel functions. Phyto, physicochemical standardization of dried, matured whole plants of *E. littorale* had been carried out. The study included organoleptic characters along with estimation of its physicochemical parameters such as loss on drying, pH, ash values, extractability in water and ethanol and preliminary phytochemical screening. The generated information of that study would provide data which was helpful in the correct identification and authentication of this medicinal plant and might help in preventing its adulteration.

Antony *et al.*, (2014), observed, that Herbs and herbal derivatives were of great research interest owing to their wide applications in therapeutics. Several folk evidences had been recorded in the formulations of ancient world’s medicinal system, which had attracted researchers for their scientific validation. Various herbal compounds had been identified and showed their therapeutic efficiency against pathophysiological conditions. Employing these herbal compounds for synthesizing nanoparticles for biomedical applications had been ventured in recent times. Green synthesis is the procedure of synthesizing nanoparticles from herbal/ biogenic resources and several metallic nanoparticles had been synthesized by this process. The metal nanoparticle-herb combination might show better efficacy against different pathophysiological conditions. This review tries to put forward the different metal nanoparticles formed from different herbal resources and their role in health and diseases. Although green synthesis of nanoparticle was an emerging area of research but very few data were available regarding their physiological effects, compatibility and toxicity. This review was an effort to elaborate in detail the role of medicinally important herbs in synthesizing metal nanoparticles, their physiological compatibility and therapeutic efficacy. Further, considerations and discussions were also made on limitations (toxicity) of the green synthesis of nanoparticles along with their future prospects in health and diseases. This review opened door to a completely new dimension in medicinal plant research combining the nanotechnology with herbs i.e. Herbonanoceuticals.
2.2. Pharmacognostical standardization

Pazhanichamy et al., (2010), explained that the study was based on anatomical and morphological investigations of *Costus igneus*. It is an important plant because of its value for medicinal uses. Plant samples were cultivated in Periyar Maniammai University nursery under net house and the morphological, anatomical features of various plant parts of *Costus igneus* such as young root, leaf and rhizome were investigated in detail and demonstrated by illustrations. The total ash value, acid insoluble ash and water-soluble ash of rhizome were significantly (p<0.001) higher than stem and leaf. Moisture content, protein and carbohydrate were significantly higher in leaf. In Fluorescent analysis, leaf, stem and rhizome powder were treated with various chemicals and were studied under UV light and daylight.

Safdar et al., (2010), identified bioactive chemical compounds from flower of *Calendula officinalis*, and their antimicrobial activity. *Calendula officinalis* L. (Family Compositae) commonly known as Marigold or Calendula is an annual plant which grows upto 1.5 feet tall. The percentage of moisture content was 18.79% per gram and total ash content was 98.74 mg per gram of air-dried flowers indicating the presence of organic compounds. For isolation and identification of compounds, Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR) were carried out. In 9:1 of chloroform: methanol solvent system Rf value under UV light and Iodine vapours for methanolic extract were 0.93 and 0.93; ethanolic extract 0.94 and 0.85, 0.94; chloroform extract 0.90 and 0.91, 0.94; acetone extract 0 and 0.96 and for water extract 0.95and 0 respectively. In 1:1 solvent system Rf value under UV light and Iodine vapours for methanolic extract 0.95 and 0; ethanolic extract 0.94 and 0.86; chloroform extract 0.89 and 0.88, 0.93; acetone extract 0 and 0.93 and for water extract 0 respectively. The FTIR spectrum obtained from methanolic extract showed that flower extract of *C. officinalis* composed of organic compounds mostly containing functional groups OH, CH, C=O, C=C and COOH. With regard to antimicrobial activity, Ethanolic extract showed activity against *E. coli*, *Vibrio cholera* and *Candida albicans*. Methanolic extract showed antimicrobial activity against *Candida albicans* only. Chloroform extract showed antimicrobial activity against all microbes while acetone extract showed antimicrobial activity against *E. coli*. 
Seal, (2011), analyzed the nutritional value of some wild edible fruits like of *Morus indica* Linn. (Moraceae), *Myrica nagi* Thunb (Myricaceae), *Myrica esculenta* Buch-Ham ex D.Don. (Myricaceae), *Parkia roxburghii* G.Don. (Mimosaceae), *Prunus nepalensis* Ser (Steud) (Rosaceae) and *Terminalia bellerica* Roxb. (Combretaceae), by determining the proximate and phytochemical composition using standard method of food analysis like AOAC. The present study revealed that for different plant species the crude fat content ranged between 0.36±0.04-5.07±0.05%. The crude protein content was determined highest in the fruits of *P.roxburghii* (19.75±0.03%) while the available carbohydrate content was highest in *M.indica* (84.04±0.12%). The nutritive value ranged from 366.57±0.62-395.04±0.54 Kcal/100g in the various edible fruits. Micronutrients, such as, zinc, copper, manganese and chromium were analyzed in the different plant specimens. The result indicated that nutritional value and mineral contents of these wild fruits under investigation were richer than that of commercial fruits and very much comparable with the various wild fruits reported earlier.

Bag *et al.*, (2012), found that the leaves, bark and fruit of *Terminalia chebula* possessed high antioxidant activity and phenolics to be responsible for this activity. Aqueous extract of *Terminalia chebula* inhibited xanthine / xanthine oxidase activity and was also an excellent scavenger of DPPH radicals. *Terminalia chebula* in a polyherbal formulation (Aller-7/ NR-A2) inhibited free radical induced hemolysis and also significantly inhibited nitric oxide release from lipopolysaccharide stimulated murine macrophages. Six extracts and four compounds of *Terminalia chebula* fruit exhibited antioxidant activity at different magnitudes of potency. Strong antioxidant activity of aqueous extract of *Terminalia chebula* was observed by studying the inhibition of radiation induced lipid peroxidation in rat liver microsomes at different doses, and methanolic extract was also found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals in vitro. Acetone extract showed stronger antioxidant activity than alpha-tocopherol and HPLC analysis with diode array detection indicated the presence of hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives, flavonol aglycones and their glycosides, as main phenolic compounds.
Table: 2.1. Various pharmacological activities of *Terminalia chebula* Retz.

<table>
<thead>
<tr>
<th>Pharmacological activities</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>Antioxidant</td>
<td>24-30</td>
</tr>
<tr>
<td>Antibacterial</td>
<td>47-58</td>
</tr>
<tr>
<td>Antifungal</td>
<td>59-62</td>
</tr>
<tr>
<td>Antiviral</td>
<td>63-70</td>
</tr>
<tr>
<td>Antiprotozoal</td>
<td>71-73</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>31,32</td>
</tr>
<tr>
<td>Radioprotective</td>
<td>25,34</td>
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<tr>
<td>Antimutagenic</td>
<td>33</td>
</tr>
<tr>
<td>Chemopreventive</td>
<td>35</td>
</tr>
<tr>
<td>Hepatoprotective</td>
<td>36-38</td>
</tr>
<tr>
<td>Cardioprotective</td>
<td>39,40</td>
</tr>
<tr>
<td>Cytoprotective</td>
<td>41-44</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>45,46</td>
</tr>
<tr>
<td>Renoprotective</td>
<td>45</td>
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<tr>
<td>Antiinflammatory</td>
<td>74,76</td>
</tr>
<tr>
<td>Antiarthritic</td>
<td>75</td>
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<tr>
<td>Adaptogenic</td>
<td>77</td>
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<tr>
<td>Antianaphylactic</td>
<td>78</td>
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<td>Hypolipidemic</td>
<td>79</td>
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<tr>
<td>Hypcholesterolemic</td>
<td>80</td>
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<tr>
<td>Gastrointestinal motility</td>
<td>81</td>
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<tr>
<td>Antiulcer</td>
<td>82</td>
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<tr>
<td>Antispasmodic</td>
<td>83</td>
</tr>
<tr>
<td>Anticaries</td>
<td>53,84</td>
</tr>
<tr>
<td>Wound healing</td>
<td>85</td>
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<tr>
<td>Purgative</td>
<td>86</td>
</tr>
<tr>
<td>Antiallergic</td>
<td>76</td>
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<tr>
<td>Immunomodulatory</td>
<td>87</td>
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</table>
Shalini and Prema, (2012), indicated that there was a demand for natural source of pesticides in food, cosmetic & therapeutic industry. Due to their low cost, high stability, compatibility and environment friendly, it was found that the test plants were good source of natural antimicrobial agents due to the presence of phytochemicals. Plant extracts appeared to be one of the better alternatives as they were known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. The phytochemical screening of both methanol and aqueous crude plant extracts of *Annona squamosa* *Catharanthus roseus*, *Sapindus emarginatus* and *Wrightia tinctoria* revealed the presence of various secondary metabolites such as alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, coumarin glycosides, terpenoids and saponins. Further the presence of phytochemicals was detected by TLC, which was a standard technique for separating organic compounds. The FTIR analysis of the methanol crude extract of the plants provided the information about the distribution of functional groups which formed the basis for comparison of compositional differences between isolates and among samples. The antimicrobial activity was done for both methanol and aqueous plant extracts. The study revealed that the leaf extracts of *Wrightia tinctoria* showed highest antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antifungal activity of methanol plant extracts showed 30% inhibition against *Curvularia* sp.

According to Devi and Divakar (2012), the leaves of *Wrightia tinctoria* (Roxb) were collected dried and studied to determine various parameters of Pharmacognostical standards such as ash values, extractive values, phytochemical tests and microscopical characters of leaf powder. The shade dried powder and various solvent extracts (viz., methanol, 70% ethanol, aqueous, dichloromethane, chloroform, ethyl acetate and petroleum ether) had been analyzed for their phytoconstituents and fluorescence characters. The methanolic extract was found to contain presence of triterpenes. The data generated for the Pharmacognostical evaluation on *Wrightia tinctoria* leaves might be useful for establishing the standardization protocols. The HPTLC analysis data indicated that the collected *Wrightia tinctoria* leaves contain 47.6mg of lupeol/ g of the total methanolic extract.
Prakash et al., (2012b), said that herbal drug product had a special place in the world of pharmaceuticals. *Terminalia chebula* was a deciduous tree, used in traditional medicines. It was reported to contain various biochemical compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, flavonoids etc. It had been reported as antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc.

Singh et al., (2012), carried out a chemical analysis of leaf, pulp and seed powder of Bael (*Aegle marmelos* L.) for proximate composition, available carbohydrates, mineral content, dietary fiber and anti-nutritional factors. The values were calculated for 100 g of Bael (*Aegle marmelos* L.) leaf, pulp and seed powder. It was found that bael leaf, pulp and seed were good source of protein, fat, minerals, crude fibre, energy and the available rich source of carbohydrates and dietary fibre. They also contained anti-nutrient content which helped in controlling blood sugar. Thus, it was concluded that this hypoglycemic medicinal plants had no side effects and provide various nutrients which were not provided by allopathic medicines. So, the diabetic patients could be encouraged to include this medicinal plant in their daily diet as per the physician’s advice to control blood sugar level.

Harpreet and Saroj (2013), observed that Herbal medicines were in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin and lower costs. *Terminalia chebula* Retz. was an important medicinal plant which had been extensively used in ayurveda, unani and homoeopathic medicine and has become cynosure of modern medicine. It is called “King of Medicines” in Tibet and is always listed first in ayurvedic meteria medica because of its extraordinary power of healing. It had been reported to exhibit a variety of biological activities including, antidiabetic, antimutagenic, antibacterial, antifungal and antiviral activities. All these activities were due to the presence of various phytochemicals in plants. Thus, phytochemical analysis of plant is necessary and provides useful information. The review gave a bird’s eye view, mainly on the biological and pharmacological activities of some compounds of *Terminalia chebula*, clinical studies and plausible medicinal applications, along with their safety evaluation.
Amita and Shalini (2014), defined Standardization as the best technical application consensual wisdom inclusive of processes for selection in making appropriate choices for ratification coupled with consistent decisions for maintaining obtained standards. This view includes the case of "spontaneous standardization processes", to produce de facto standards. Plant-derived substances had recently become of great interest owing to their versatile applications. Medicinal plants were the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. World Health Organization (WHO) encouraged, recommended and promotes traditional/herbal remedies in national health care programmes because those drugs were easily available at low cost, safe and people have faith in them. Extraction methods used pharmaceutically involved the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffused into the solid plant material and solubilize compounds with similar polarity. Phytopharmaceutical and secondary plant product of medicinal importance such as alkaloids, glycosides, terpenoids, Flavonoids and lignans.

Nitu and Ramanjaneyulu (2015), explained that one of the impediments in the acceptance of the herbal products worldwide was the lack of standard quality control profiles. Hence various methods were developed by WHO for the standardization of polyherbal formulations. The standardization of *Trachyspermum ammi* and its marketed polyherbal formulations “Ajmodadi churna” of four different companies were done. An attempt had been made to develop standardization method based on the Pharmacognostic and physicochemical parameters of *Trachyspermum ammi* and its marketed polyherbal formulations “Ajmodadi churna” of four different companies. The Pharmacognostic and physicochemical profile of *Trachyspermum ammi* was taken as a reference standard in comparing with four marketed formulations of Ajmodadi churna. Histological parameters like endodermis, epidermis, fibers, cortex, sclereids, oil globules etc were seen. Extractive value, ash value, moisture content was found to be 7.4, 1.2, 4.3 %w/w respectively. The following study would improve the quality of drugs and also motivated the practitioners to get more involved in the standardizations of formulations.
2.3. Phytochemical Evaluation

Vanaja et al., (2005), reported that *Wrightia tinctoria* R. Br. could be very effective against jaundice in Indian indigenous system of medicine. The juice of the tender leaves was used efficaciously in jaundice; also crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it was used for Psoriasis and other skin diseases. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Morphological and anatomical aspects as well as differential microchemical response had been worked out to identify the diagnostic features of the leaf. Physical constant values involving moisture content, ash and extractives as well as qualitative and quantitative estimation of various phytochemicals had been studied. The presence of lipid, saponin, tannin, alkaloid, phenol, steroid, flavonoid, and some other chemical constituents were recorded.

Based on the HPLC and FTIR results Thenmozhi et al., (2011), reported that the phytochemicals like carbohydrates, cardio glycosides, saponins, oil & fats, terpenoids, alkaloids, tannin, flavonoids, quinones were present in methanolic extract of *Eclipta alba* and *Emilia sonchifolia*. The results revealed the presence of a good number of phytochemicals in their extract which were responsible for antimicrobial, antioxidant and curing activities against several diseases. Hence sensitive and comprehensive analytical techniques were needed to acquire a better understanding of the pharmacological basis of the herb and to enhance the product quality control.

Manjulika et al., (2014), identified phytochemical analysis was a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently might lead to drug discovery and development. Chief phytoconstituents of the six selected medicinal plants of different families were identified in order to relate their presence with bioactivities of the plants. Screening of six selected medicinal plants was performed for the presence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins, anthraquinones, anthocyanins and leucoanthocyanins using standard methods. All the selected medicinal plants were found to contain tannins and flavonoids. Moreover, terpenoids were also present in all the selected plants except *Phoenix dactylifera*. On the other hand, saponins and
steroids were absent in all plants except *Swertia chirata* and phlobatannins were absent in all plants except *Raphanus sativus*. In addition, carbohydrates, glycosides and coumarins were present in all the selected plants except *Phoenix dactylifera* and *Raphanus sativus*. Alkaloids were present in all the selected plants except *Ficus religiosa, Phoenix dactylifera* and *Raphanus sativus*. Proteins were present only in *Ficus religiosa* and *S. chirata*. Whereas emodins, anthraquinones, anthocyanins and leucoanthocyanins were absent in all the selected six plants. It is evident from the study that *Swertia chirata* was of highest therapeutic efficacy possessing majority of phytochemical classes of compounds and *Phoenix dactylifera* was of lowest therapeutic potential due to the absence of majority of phytoconstituents.

Tashsin *et al.*, (2015), stated that India had been known for its rich biological diversity. Five medicinal plants such as *Bryophyllum pinnatum, Ipomea aquatica, Ricinus communis, Terminalia bellerica* and *Tinospora cordifolia*, were selected. To investigate the presence of phytochemicals. Soxhlet apparatus was used for the organic solvent extraction. Solvents used were water, methanol, ethanol, and acetone. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, were detected in all of the plants tested. Our findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justified their use in the traditional medicines for the treatment of different diseases.

### 2.4. Phytochemistry

Muhammad *et al.*, (2012), discussed trust on traditional medicines in the treatment of ailment was at a halt and a lot of population, especially rural population, still believed on herbs due to their easy accessibility and cost effectiveness. Due to greater attentiveness concerning significance of conventional medicine in health care, research on medicinal plants would be valuable. The plants of genus *Terminalia*, comprising of 250 species, were widely distributed in tropical areas of the world. Fruits of *Terminalia chebula* (Family: Combretaceae) commonly known as black Myrobalan in English and Harad in Hindi, were widely grown in Pakistan and India among many Asian and African countries and was a popular folk medicine. *Terminalia chebula* has been studied for its homeostatic, antitussive, laxative, diuretic and cardiotonic activities. This article showed a vivid account of *Terminalia chebula*
as a natural product and aims to (i) to refresh the importance of *Terminalia chebula* to the medicinal plant researchers and (ii) to presents new information such of *Terminalia chebula*.

### 2.5. Fractionization

Ullah *et al.*, (2009), revealed that the *n*-hexane, carbon tetrachloride, chloroform soluble fractions of methanol extract from the plant *Centella asiatica* (Apiaceae) was subjected to antioxidant, antimicrobial and brine shrimp lethality bioassay. All the fractions showed moderate to potent antioxidant activity, of which the chloroform and aqueous soluble fraction demonstrated the strongest antioxidant activity with the IC50 value of 4.0 \(\mu\text{g/ml}\) and 7.0 \(\mu\text{g/ml}\), respectively. In case of antimicrobial screening, crude extracts showed notable antibacterial and antifungal activity against sixteen microorganisms. The *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates of the methanolic soluble fractions showed average zone of inhibition ranged from 7-15 mm, 8-12 mm, 8-16 mm and 8-13 mm, respectively, at a concentration of 400 \(\mu\text{g/disc}\). However, in the brine shrimp lethality bioassay, all the crude extracts possessed considerable cytotoxic activity. It was evident that, the *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions had significant cytotoxic potentials having LC50 1.254, 0.826, 3.866 and 5.366\(\mu\text{g/ml}\) respectively.

Mudi and Bukar (2011), reported that the leaves of *Calotropis procera* were air dried, grounded and soaked with ethanol. The extracts obtained (29.79g, CP1) was fractionated sequentially using aqueous methanol with petroleum ether, chloroform and ethyl acetate respectively. The residue of ethanol extract (marc) was extracted with 5M HCl, basified and extracted with chloroform. These were labeled as CP1-01 to CP1-05 for the plant. Each of these fractions was phytochemically screened to detect the class of secondary metabolite present. The fractions obtained from the plant were found to be selectively active against brine shrimp larvae. These fractions were also subjected to antimalaria parasites bioassay. Fractions CP1, CP1-04 and CP1-05 were found to be active against tested organisms, with CP1-04 being the most active. CP1-04 was further subjected to activity guided column chromatography that led to the isolation of two pure compounds CP1-04-1 and CP1-04-61. Compound
CP1-04-61 was found to be active against the malarial parasite. This was further purified and subjected to qualitative and quantitative analysis.

Tensingh and Astalakshmi (2014), studied with the fruit extracts of *Terminalia chebula* Retz. contained different types of phytochemicals such as glycosides, alkaloids, flavonoids, phenolic compounds, saponin, steroids, quinine and tannin. The antibacterial activity of crude extract of *Terminalia chebula* Retz. was studied against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*. The antibacterial activity was studied by disc diffusion method. Extracts with the different solvent of *Terminalia chebula* Retz. exhibited the antibacterial activity against bacterial strains. In general, all extracts inhibited the growth of all test microorganisms and in disc method, with the range of concentration of 100µl, 150µl and 200µl of the extract, the growth of all microorganisms was inhibited and also showed dose dependent activity. Of the eleven solvent used methanol, ethanol and acetone seemed to be the best solvent when compare to other solvents.

**2.6. Optimization of extraction condition**

Hismath *et al.*, (2011), optimized the extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves using response surface methodology (RSM). A central composite rotatable design (CCRD) was applied to determine the effects of acetone concentration (%), extraction time (mins), and extraction temperature (°C) on total phenolic content (TPC) from neem (*Azadirachta indica*) leaves. The independent variables were coded at five levels and their actual values were selected based on the results of single factor experiments. Results showed that acetone concentration and extraction time were the most significant (p<0.001) factor affecting the TPC. The optimum extraction conditions were found to be acetone concentration of 48.49%, extraction time of 59.25 mins, and extraction temperature of 40.88°C. Under the optimized conditions, the experimental value for TPC was 4661.17 mg GAE/100 g DW, which reasonably close to the predicted value (4649.16 mg GAE/100 g DW).
According to Prakash et al., (2012a), *Terminalia chebula* was a moderate tree used in traditional medicines. It contained Chebulinic acid, Quercetin, Tannic acid and many other compounds. The phenolic compounds like Total Phenolic, Chebulinic acid and Quercetin were extracted. The studies on optimization of physico-chemical parameters like effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol and methanol as solvents and pH for the extraction of Total Phenolic Content, Chebulinic acid and Quercetin were done. The highest Total Phenolic Content concentration for optimized conditions was 2.25mg/DW. For the extraction of Chebulinic acid, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 3.4mg/ml. For the extraction of Quercetin, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH were methanol, 2 days, 1hrs, 125 microns, 80% (v/v), 1:1 ratio and 6.0 respectively. The highest Quercetin concentration for optimized conditions was 0.54mg/cl.

Andressa et al., (2013), *Limonium brasiliense* was a common plant on the southern coast of Brazil. The roots were traditionally used for treatment of premenstrual syndrome, menstrual disturbances and genito-urinary infections. Pharmaceutical preparations obtained from its roots and used for these purposes were marketed in Brazil in the 1980s and 1990s. Currently, the Brazilian Drug Agency (National Health Surveillance Agency, ANVISA) had canceled the registration of these products, and their use was discontinued because of a lack of studies to characterize the plant raw material and ensure the effectiveness and safety of its use. They developed and validate an analytical method to determine the content of total polyphenols (TP) in an extract from *Limonium brasiliense* roots, by the UV/Vis Spectrophotometric method. *Limonium brasiliense* roots were extracted in acetone:water mixture (7:3, v/v-10% w/v). The crude extract was used to develop a method for TP assay. The method was validated according to national and international guidelines. The optimum conditions for analysis time, wavelength, and
standard substance were 30 min, 760 nm, and pyrogallol, respectively. Under these conditions, validation by UV/Vis spectrophotometry proved the method to be linear, specific, precise, accurate, reproducible, robust, and easy to perform. This methodology complies with the requirements for analytical application and to ensure the reliability of the results.

Sulaiman and Fazilah (2015), reported *Moringa oleifera* was the most commonly cultivated plant all over the world. It had high economic impact due to the medicinal and nutritional values. The seeds of *Moringa oleifera* also contained various constituents that were useful for therapeutic purposes. The aim of this study was to quantify some important antioxidant compounds of *Moringa oleifera* seed extract. Total phenolics (TP), total tannins (TT) and total flavonoids (TF) content were determined by colorimetric method. The results showed that the seed extract contain total phenolics of 10.179 ± 2.894 (mg Gallic acid equivalents / g dry matter) which is higher compared to flavonoid 2.900 ± 0.0002 (mg Quercetin equivalents / g dry matter) and tannic acid of 0.890 ± 0.020 (mg Gallic acid equivalents / g dry matter). Total phenolic content of the seeds was likely to be a key for determining the free radical scavenging and ROS reducing ability of the seeds.

2.7. Estimation of Bioactive compound

Bhat *et al.*, (2012), analyzed the *Melissa parviflora*is an aromatic perennial herbaceous plant of Lamiaceae family. The different extracts were subjected to preliminary phytochemical screening for the presence of carbohydrate, flavonoids, phenolics etc. This included organoleptic properties, pH of aqueous solution, ash values, extractive values, successive extractive values, and loss on drying, HPTLC finger printing profile and preliminary phytochemical screening. Total phenolic content was measured by Folin Ciocalteu method in term of Gallic acid equivalent in mg/g of the extract. The amount of phenolic content was found to be 68.41 µg/ml (0.68% w/w). The total flavonoid content was found to be 28.41 µg/ml (0.28 % w/w). The findings of that study might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.
Mazandarani et al., (2012), researched 8 kinds of solvents extracts from roots of *Onosma dichroanthum* Boiss. were used to examine the effects of extraction solvent on total phenolics (TP), total flavonoids (TF), total anthocyanin (TA) content and antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH), total antioxidant capacity (TAC), reducing power (RP) and antioxidant activity (AA) were determined spectrophotometrically. Results showed that extraction solvent had significant effects on TP, TF, TA content and antioxidant activity of acetone extract. The highest content of TP, TF and TA were found in acetone extracts. The TP varied from 4.5 ± 0.7 to 125.6 ± 3.01 mgGAE g⁻¹ dry weight, TF contents were between 9.8 ± 3 to 41 ± 2.3 mgQUE g⁻¹and TA were 11.5± 3.4 to 47.8± 6.8 mgECGgr⁻¹. Effective concentration (EC50) (antioxidant activity) in TAC, RP and 2,2- diphenyl-1-picrylhydrazyl (DPPH) methods were measured at 0.495, 0.844 mg/ml and 4.21 mg dw, respectively and amount of antioxidant activity (AA%) was reported at 38.02%. The greater amount of phenolic compounds which led to more potent radical scavenging effect was shown by acetone extract. Additionally, amount of phenolic compounds and antioxidant activities increased in acetone extract. Thus, a positive correlation existed between antioxidant activity and their total phenolic content. Acetone solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced.

Vijay and Rajendra (2014), evaluated the total phenol, tannin, alkaloid and flavonoid contents in petroleum ether, ethyl acetate and methanol extracts of *Hibiscus tiliaceus* wood. Extraction of powdered wood material was carried out by continuous hot percolation method in soxhlet apparatus using petroleum ether, ethyl acetate and methanol as solvents. Gallic acid was used as standard for the determination of total phenol and tannin by Folin-ciocalteu method. Total alkaloid content was determined by chloride colorimetric method using quercetin as a standard. The results showed that ethyl acetate extract had high concentration of total phenol, tannin, alkaloid and flavonoid contents as compared by bromocresol green solution using atropine as a standard. Total flavonoid content was determined by aluminium to petroleum ether, ethyl acetate and methanol extracts. Ethyl acetate extract contained the total phenol of 30.18 and tannins of 83.03 as mg of gallic acid equivalents (GAE), alkaloids of 66.01 as mg of atropine equivalents (AE) and flavonoids of 91.01 as mg of quercetin equivalents (QE).
2.8. Characterization of Bioactive compound

Jeganathan and Kannan (2008), made an attempt to develop a HPTLC (High Performance Thin Layer Chromatography) method of quantitative estimation of marker compounds, ellagic acid and gallic acid in laboratory prepared authentic formulation and commercial formulation of *Triphala churanam*. The two formulations were subjected to methanol and ethyl acetate extraction using soxhlet apparatus and quantified using HPTLC at wavelength of 280nm. Ellagic acid were found to contain 0.201% w/w and gallic acid 0.656% w/w in methanol extract while it shows 0.573%w/w ellagic acid and 2.664% w/w in gallic acid. Linearity studies indicated that ellagic acid, gallic acid were in the linear range of 125-500ng and 1.25-5.00µg, respectively, while the percentage recovery study revealed 99.2% w/w of ellagic acid and 98.13% w/w of gallic acid, thus providing the accuracy and precision of the analysis.

Anil and Nandhini (2010), performed reversed-phase preparative HPLC method with UV spectrophotometric detection had been developed for the simultaneous isolation of eight hydrolysable tannins from dried fruits of *Terminalia chebula*, a traditional herbal medicine. Isolation of phytoconstituents was achieved by preparative HPLC using C18 column and acetonitrile – 0.2% formic acid in water as mobile phase. The purities of the phytoconstituents were determined by HPLC and their structures were elucidated by spectroscopic (UV, 1H-NMR, ESI-MS) techniques. These phytoconstituents could be used as marker compound to develop suitable identification test for raw materials, to determine the assay of active constituents of known therapeutic activity as well as stability of the extracts.

Bade *et al.*, (2010), reported that hydrolysable tannins gallic acid (3, 4, 5-trihydroxy benzoic acid) and ellagic Acid (dimer of 3, 4, 5-trihydroxy benzoic acid) were isolated from the water soluble extract (WE) of *Terminalia belerica* Roxb. (Combretaceae) leaf. Isolation was carried out by different chromatographic techniques. The characterization and identity of compounds was verified through various physical and spectroscopic methods like UV-Visible spectroscopy, Infra Red Spectroscopy, Proton and Carbon NMR spectroscopy and Mass spectrometry. Total polyphenolic content was found to be 17.23%w/w using UV-Visible spectroscopy atmax 725 nm of leaf water soluble extract.
Walia et al., (2010), compared the antioxidant efficacy and the phenolic content of two hexane extracts viz. ‘Hex 1’ and ‘Hex 2’ of fruits of Terminalia chebula prepared by maceration and sequential method respectively. The extracts were tested for their relative levels of antioxidant activity and the total phenolic content using DPPH, deoxyribose, reducing power, chelating power, lipid peroxidation and Folin-Ciocalteu method. Furthermore, the UV–VIS spectrum of extracts and the correlation between total phenolic content were examined in order to give an orientation to the search of phytochemicals responsible for their activity. From the results, it was concluded that phenolic compounds were predominant in the ‘Hex 2’ prepared by sequential extraction method. The antioxidative potential of ‘Hex 2’ was also far superior to the ‘Hex 1’ prepared by maceration method. Such study would contribute to further knowledge relating to the extraction of plant materials by different methods.

Belur and Pallabhanvi (2011), observed Gallic acid was produced from Terminalia chebula extracts using a novel cell-associated tannase (CAT) of Bacillus massiliensis. The stability of CAT was ascertained during storage at 4°C, found that 100% activity was retained in first six days. The incubation of CAT-Terminalia chebula extract under anaerobic condition gave 327 mg/L gallic acid as against 207mg/L in aerobic conditions. Extraction and subsequent HPLC analysis revealed that substantial amount of gallic acid was present with other gallic acid esters. This was the first report of production of gallic acid from plant extracts using naturally immobilized tannase.

Bag et al., 2012, reported fruits of Terminalia chebula were rich in tannins (about 32%-34%) and its content varied with geographical distribution. The tannins of Terminalia chebula were of pyrogallol (hydrolysable) type. A group of researchers found 14 components of hydrolysable tannins (gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, 1,6-di-o-galloyl-D-glucose, casuarin, 3,4,6 tri-o-glloyl-D-glucose, terchebulin) from Terminalia chebula fruits. Other constituents include phenolics such as chebulinic acid, ellagic acid and anthraquinones. Some of the other minor constituents were polyphenols such as corilagin, galloyl glucose, punicalagin, terflavin A, maslinic acid. Besides, fructose,
amino acids, succinic acid, betasitosterol, resin and purgative principle of anthraquinone were also present. Flavonol, glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds were also isolated. Twelve fatty acids were isolated from *Terminalia chebula* of which palmitic acid, linoleic acid and oleic acid were main constituents. Triterpenoid glycosides such as chebulosides I and II, arjunin, arjun glucoside, 2α-hydroxyursolic acid and 2α-hydroxymicromeric acid also had been reported. The leaves were found to contain polyphenols such as punicalin, punicalagin, terflavins B, C, and D. The plant was found to contain phloroglucimol and pyrogallol, along with phenolic acids such as ferulic, p-coumaric, caffeic and vanillic acids.

**Table 2.2. Structure and activities of some active compounds and their derivatives from *Terminalia chebula* Retz.**

<table>
<thead>
<tr>
<th>Class of compounds</th>
<th>Compounds</th>
<th>Plant parts</th>
<th>Activities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acid</td>
<td>Elagic acid</td>
<td>Fruit</td>
<td>Antibacterial activity</td>
<td>31, 50</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Chebulic acid</td>
<td>Fruit</td>
<td>Antioxidant</td>
<td>36, 41</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Neochebulic acid</td>
<td>Fruit</td>
<td>Antioxidant</td>
<td>36</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Gallic acid</td>
<td>Fruit</td>
<td>Antibacterial activity</td>
<td>41, 51, 63</td>
</tr>
<tr>
<td>Phenolics</td>
<td>2,4-chebulyl-beta-D-glucopyranose</td>
<td>Fruit</td>
<td>Antiproliferative activity</td>
<td>31</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Chebulinic acid</td>
<td>Fruit</td>
<td>Antiproliferative activity</td>
<td>31</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Hydrobenzoic acid derivatives</td>
<td>Fruit</td>
<td>Antioxidant</td>
<td>30</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>Hydrobenzoic acid derivatives</td>
<td>Fruit</td>
<td>Antioxidant</td>
<td>30</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Falvonol aglycones</td>
<td>Fruit</td>
<td>Antioxidant</td>
<td>30, 50</td>
</tr>
</tbody>
</table>
Pitchai et al., (2012), reported that Diabetes mellitus was a progressive disease characterized by insulin deficiency and insulin resistance. The targets insulin receptor and PPAR\(\gamma\) associated protein [PDB: 1IRK, 3KDU] were obtained from protein data bank. Chemskech 12.0 software was used to draw the three dimensional structure of the phytocompounds. The drug likenesses of the compounds were evaluated by checking the Lipinski and ADMET properties by using Accord for Excel. Among the 11 compounds, eight compounds were satisfied and 4 were not satisfied the rules of Lipinski properties. In the prediction of ADMET [Absorption, Distribution, Excretion, Metabolism, and Toxicology] properties for the chosen compounds, catechin, costunolide, eremanthin, and saponin were found to be toxic. After screening 4 ligands namely novel gymnemic diacetate, novel gymnemic triacetate, novel dihydroxy gymnemic triacetate, gallic acid were tested, through molecular docking interactions using Discovery Studio 2.1 version. All the 4 compounds interacted with insulin receptor and were predicted to promote the insulin signaling pathway. At the same time only 2 compounds interacted with PPAR\(\gamma\) and were predicted to promote PPAR signaling pathway. Hence, these 2 novel compounds namely gymnemic diacetate and gymnemic triacetate were identified as potent medicinal compounds as dual agonistic ligands for insulin receptor and PPAR\(\gamma\).

Hosana et al., (2012), observed pharmacology over the past hundred years used traditional pharmacology tools such as in vivo and in vitro models for finding a drug molecule. These in silico methods include data bases, similarity searching, pharmacophores, homology models, Quantitative Qstructure Activity Relationship and data analysis tools. Such methods have been frequently used in the drug target discovery, drug design, drug docking or screening, drug metabolism prediction, toxicity properties and physicochemical characterization. From the study it could be concluded that all compounds had more or less inhibition effect over all the bacterial target proteins. By enlarging the dock score values Carvacrol, Rosmarinic Acid, Chlorogenic Acid and Gallic Acid showed higher level of dock score when compared with other compounds i.e. these compounds had reasonably high interaction on the active sites of the protein. Hence Coleus aromaticus might be a promising vital drug with minimum side effects in future. Future studies could be focused on Aromaticus plants to derive and identify new compounds for treating.
Janakiraman et al., (2012), revealed the presence of phytochemical screening for steroids, alkaloids, sugars, phenolics, flavonoids, saponin, tannins and amino acids with varied degree. TLC profile showed distinct bands with varied Rf values. GC-MS revealed the presence of 25 different phytocompounds viz., n-Hexadecanoic acid (35.13%), Phytol (9.62%), 2-Ethylacridine (7.41%), Cyclotrisiloxane, hexamethyl-(6.68%), Bicyclo [3,1,1] heptanes, 2,6,6-trimethyl-(6.11%), etc. The FTIR spectrum confirmed the presence of alcohols, acids, alkenes, aromatics, nitro compounds, alkyl halides, aliphatic amines, alkynes, primary and secondary amines with different peak values. The present study confirmed that *Andrographis precatorius* might be used as a natural source for pharmaceutical purposes due to presence of biologically active compounds.

Surya Prakash and Gopal (2014), said that Bhuvnesvara vati (BV) was an Ayurvedic formulation containing *Emblica officinalis, Terminalia belerica, Terminalia chebula, Trichyspermum ammi, Aegle marmelus* and two minerals namely rock salt and Soot as main ingredients. Finger printing methods were developed for well-known Ayurvedic formulation. Three sample batch of BV were prepared in the laboratory and two different marketed formulations were procured from Ayurvedic medicine shop. A HPLC method was developed for the estimation of gallic acid and tannic acid in laboratory and marketed formulations. The concentration of gallic acid present in raw material was found to be 3.174±0.049% w/w in *Emblica officinalis*, 8.920±0.173% w/w in *Terminalia belerica*, 4.092±0.117% w/w in *Terminalia chebula*, 1.831±0.973% w/w in *Aegle marmelus* and 0.264±0.365% w/w in *Trichyspermum ammi*. Gallic acid content in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356% and 2.632±0.239% w/w respectively. Two marketed formulation of Bhuvnesvara vati M-I and M-II showed gallic acid concentration to be 2.019±0.872 % and 2.019±0.872 % w/w respectively. The concentration of tannic acid present in raw material was found to be 6.172±0.365% w/w in *Emblica officinalis*, 8.667%±0.0319% w/w in *Terminalia belerica*, 13.956%±0.745% w/w in *Terminalia chebula*, 4.789±0.983% w/w in *Aegle marmelus* and 0.668±1.002% w/w in *Trichyspermum ammi* respectively and in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356%, 2.632±0.239% w/w respectively. In order to obtain precision and
accuracy, the recovery study was performed and result obtained with mean value 99.69% for gallic acid and 99.38% for tannic acid, which proved reproducibility of the result. This showed significant precision of methods at 95% confidence level. The mean of % RSD value was found to be 0.357 for gallic acid & 0.353 for tannic acid. Results of statistical analysis shows that present HPLC method for determination of gallic acid and tannic acid is simple, precise, accurate and suitable for routine analysis of gallic acid and tannic acid in BV. The developed fingerprints could be used as a standard and gallic acid and tannic acid could be used as a possible marker compound for fingerprinting of BV.

Nitu and Shiva (2015), analyzed the Chromatographic fingerprint of herbal drugs represented a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products. In the present research article TLC and HPLC Chromatogram of Aegle marmelos Corr was taken as standard for comparing its fingerprinting profile with three marketed formulations containing Aegle marmelos Corr as one ingredient. Bilva patra showed four spots at 0.89, 0.75, 0.65, and 0.52 in thin layer chromatography. Bilwadi churna also showed three spots at 0.89, 0.80 and 0.63. Pushyanug churna showed two spots at 0.89 and 0.  Whereas Gangadhar churna showed two spots of which only one spot was matching with Bilva Patra (0.63). On the inspection of various chromatogram of Bilva Patra and its formulations in Polyherbal drugs, gave various peaks from 4.8-10. It seemed that the peaks at 4.8,5.8, and 9.2 might be beneficial for standardizing the Bilva Patra in the polyherbal formulations, but when the chromatogram of polyherbal formulations had been recorded, it was found that the peaks obtained at 9.2 had been retarded to a negligible amount, but only the peak of 5.8 min could be taken as standard.

2.9. Biological activities of Bioactive compounds
2.9.1. Antibacterial activity of Bioactive compounds

Kumar et al., (2009), reported that aqueous fruit extract of Terminalia chebula Retz showed anti-bacterial activity against gram-positive bacteria (Bacillus subtilis, Syaphylococcus aureus and Staphylococcus epidermis) and gram-negative bacteria (E. coli, Shigella flexineria and Pseudomonas auriginosa).
Moorthy et al., (2012), carried out an *in-vitro* antibacterial study using methanolic and petroleum ether extracts from the leaves of *Wrightia tinctoria* (Roxb.) R.Br. using disc diffusion and broth dilution methods were employed for the assessment of antibacterial activity against 14 microorganisms. Methanolic extract of *Wrightia tinctoria* leaves showed significant antimicrobial activity against *Staphylococcus aureus* (27.2mm), *Staphylococcus epidermidis* (23.2mm) and *Bacillus subtilis* (20.2mm), whereas petroleum ether leaves extract showed significant antibacterial activity against *Staphylococcus aureus* (25.0mm), *S.epidermidis* (18.5mm) were observed. According to broth dilution method, the methanolic extract of plant material showed the MIC values against *Staphylococcus aureus*, whereas the petroleum ether extract of *Wrightia tinctoria* showed the MIC values against *Staphylococcus aureus* and *Staphylococcus epidermidis* with a significant inhibitory activity.

Mostafa et al., (2011), observed medicinal plants according to Ayurveda for its broad spectrum medicinal value including in the treatment of enteric disorders. Leaf extract in water as well as in various organic solvents (methanol, ethanol, ethyl acetate and chloroform) were analyzed to testify its antibacterial activities against four different bacteria causing enteric disorders, viz., *Escherichia coli*, *Salmonella sp*, *Shigella sp* and *Vibrio cholerae* invitro along with *Saccharomyces cerevisiae*. The analysis was carried out by taking the extracts at a concentration of 10mg/ml and their activities were recorded by estimating zone of inhibition as produced by disc-diffusion method on Mueller-Hinton agar media. While all the organisms were resistant to chloroform extract and some of them of ethyl acetate, the methanol as well as the aqueous extract of the plant showed the potential bactericidal activity, however nothing was evident against the yeast candidate. When compared with the traditional antibiotics, this activity was especially competent against *Escherichia coli* and *Shigella sp* followed by *Vibrio sp* and *Salmonella spp*. The broth dilution assay revealed that the bactericidal values occurred within range of 5000 to 8000 µg/ml.

Sintubin et al., (2011), said that biogenic silver nanoparticles were produced by *Lactobacillus fermentum* which served as a matrix preventing aggregation. The antibacterial activity of the biogenic silver was compared to ionic silver and chemically produced nanosilver. The minimal inhibitory concentration (MIC) was
tested on Gram-positive and Gram-negative bacteria and was comparable for biogenic silver and ionic silver ranging from 12.5 to 50 mg/L. In contrast, chemically produced nanosilver had a much higher MIC of at least 500 mg/L, due to aggregation upon application. The minimal bactericidal concentration (MBC) in drinking water varied from 0.1 to 0.5 mg/L for biogenic silver and ionic silver, but for chemically produced nanosilver concentrations, up to 12.5 mg/L was needed. The presence of salts and organic matter decreased the antimicrobial activity of all types of silver resulting in a higher MBC and a slower inactivation of the bacteria. The mode of action of biogenic silver was mainly attributed to the release of silver ions due to the high concentration of free silver ions measured and the resemblance in performance between biogenic silver and ionic silver. Radical formation by biogenic silver and direct contact was found to contribute little to the antibacterial activity. In conclusion, biogenic nanosilver exhibited equal antimicrobial activity compared to ionic silver and could be a valuable alternative for chemically produced nanosilver.

Chandhini et al., (2014), showed that the phytochemical analysis revealed the presence of active ingredients such as steroids, saponins, phenols, flavonoids, terpenoids, alkaloids and tannins in the rhizome extract of Drosera hamiltonii followed by others. Total flavonoid content was quantitatively estimated which recorded maximum in Kanchipuram accession (5.25 mg Quercetin Equivalents (QE) /g). The rhizome extracts were evaluated for antioxidant activities by DPPH (1, 1–Diphenyl -2- picryl - hydrazyl) radical scavenging assay. Among the three accessions with different solvents extractions, maximum antioxidant activity was found in the ethanolic rhizome extract (90.9%) of Drosera hamiltonii followed by others. HPLC analysis of these extracts showed that the main components of the active principles namely 2H4MB were present in the rhizome extract of Drosera hamiltonii. Different concentrations of ethanolic rhizome extract were tested using the agar disc diffusion technique for the activity against Bacillus cereus, Pseudomonas aeruginosa, and Bacillus subtilis. It was found to be inactive against Staphylococcus aureus and Escherichia coli.
2.9.2. Antifungal activity of Bioactive compounds

According to Saheb et al., (2005), aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species (*T. alata*, *T. arjuna*, *T. bellerica*, *T. catappa*, *T. chebula*) were tested by paper disc method against five plant pathogenic fungi like *Aspergillus flavus*, *Aspergillus niger*, *Alternaria brassicicola*, *Alternaria alternata* and *Helminthosporium tetramera*. Generally the activity of aqueous leaf extracts were relatively less than the activity of ethyl acetate extracts, while the alcoholic extracts possessed the highest activity against all tested pathogens. Extract of *Terminalia arjuna* showed maximum zone of inhibition (23) against *Aspergillus niger*. It is followed by the extract of *Terminalia bellerica* and *Terminalia chebula*. In case of *Terminalia catappa*, the extract was more effective against *Helminthosporium tetramera*. The positive results so obtained were compared with that of the reference standard fungicide (Carbendazim). It was found that most of the extracts were more effective against fungi than the control fungicide.

Elumalai et al., (2010b), noticed that development of biologically inspired experimental processes for the synthesis of nanoparticles was evolving into an important branch of nanotechnology. Metallic nanoparticles were traditionally synthesized by wet chemical synthesis techniques where the chemicals used were quite often toxic and flammable. A cost effective and environment friendly synthesis of silver nanoparticles from 1mM AgNO₃ solution through the leaf extract of *Euphorbia hirta* L, as reducing as well as capping agent was carried out. Nanoparticles were characterized using UV-Visible Absorption spectroscopy. Further the nanoparticle synthesis by green route was found highly toxic against 7 clinically isolated fungal species. At a concentration of 50 μl silver nanoparticles revealed higher antifungal activity against *Candida albicans*, *Candida kefyr*, *Aspergillus niger* whereas intermediated activity showed against *Candida tropicalis*, *Candida krusei*, *Aspergillus flavus*, *Aspergillus fumigatus*. The inhibitory activities of all the silver nanoparticles reported were comparable with standard antimicrobics Ketoconazole (30mg) and Itraconazole (30mg).

Savithramma et al., (2011), reported that the synthesis of metal nanoparticles using biological systems was an expanding research area due to the potential applications in nanomedicines. Nanoparticles synthesized by chemical method were
not eco-friendly. The biological synthesis of silver nanoparticles was convenient and extracellular method, which were environmentally safe. The silver nanoparticles were synthesized rapidly by using the stem barks of endemic medicinal plants *Boswellia ovalifoliolata* and *Shorea tumbuggaia*. After assessing the formation of silver nanoparticles with the help of UV-Visible spectroscopy and were characterized by using EDAX and SEM. Diversity has been observed in size and shape of the silver nanoparticles synthesized in two plants. Phytosynthesized silver nanoparticles were tested for the antifungal activity. Against the test cultures of *Aspergillus*, *Fusarium*, *Curvularia*, and *Rhizopus* species. The antifungal property of silver nanoparticles was analyzed by measuring the inhibitory zone. The silver nanoparticles synthesized from bark extract of *Boswellia ovalifoliolata* and *Shorea tumbuggaia*. *Boswellia ovalifoliolata* showed moderately toxic to the *Aspergillus*, *Curvularia and Rhizopus* species and highly toxic to *Fusarrium* species. Biologically synthesized silver nanoparticles of *Shorea tumbuggaia* bark extract were moderately toxic to *Aspergillus, Fusarium and Rhizopus* species and highly toxic to *Curvularia* species. The study served as an eye-opener to comprehend the development of value added products from medicinal plants of India for biomedical and nanotechnology based industries.

Olaleye *et al.*, (2015), analyzed the antimicrobial activities and preliminary phytochemical screening of methanolic extract of *Vitellaria paradoxa* was also performed against clinical isolates obtained from UITH which included *Staphylococcus aureus, Escherichia coli, Aspergillus niger* and *Candida albicans*. Leaf extracts of *Vitellaria paradoxa* were prepared using methanol as solvent. The extracts were tested using agar diffusion and broth dilution method. *Escherichia coli* was resistant to all the extracts of *Vitellaria paradoxa*. Antimicrobial activity was recorded by the methanolic extract of *Vitellaria paradoxa* with zone of inhibition of 19mm on *Candida albicans*. The minimum inhibitory concentration of the extract ranged from 40mg/ml to 160mg/ml for *Vitellaria paradoxa* leaf extract. Methanolic leaf extract of *Vitellaria paradoxa* were found to be bactericidal and fungicidal on *Staphylococcus aureus* and *Aspergillus niger*, *Candida albicans* respectively. *Vitellaria paradoxa* leaf extract contained saponin, tannins, flavonoid, phenolics, steroids, alkaloids, phlobatannins and glycoside. Therefore suggest the possibility of
using *Vitellaria paradoxa* extracts as antimicrobial agents, was suggested which could be a great asset to drug development for purpose of health care delivery in Nigeria.

### 2.9.3. Antioxidant activity of Bioactive compounds

Pfundstein *et al.*, (2010), determined antioxidant capacities of the raw fruit extracts of *Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrid*. The major isolated substances using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) in vitro assays indicated that chebulic ellagittannins showed high activity which might be correlated with high potential as cancer chemopreventive agents. Therefore, further studies (metabolism, bioavailability and toxicity) of the polyphenolics in *Terminalia* species using preclinical models and in vivo human intervention trials were warranted.

Hazra *et al.*, (2010), observed the ability of the extracts of the fruits in exhibiting their antioxidative properties followed the order *Terminalia chebula* > *Emblica officinalis* > *Terminalia bellerica*. The same order was followed in their flavonoid content, whereas in case of phenolic content it becomes *Emblica officinalis* > *Terminalia bellerica* > *Terminalia chebula*. In the studies of free radicals' scavenging, where the activities of the plant extracts were inversely proportional to their IC50 values, *T. chebula* and *E. officinalis* were found to be taking leading role with the orders of *Terminalia chebula* > *Emblica officinalis* > *Terminalia bellerica* for superoxide and nitric oxide, and *Emblica officinalis* > *Terminalia bellerica* > *Terminalia chebula* for DPPH and peroxynitrite radicals. Miscellaneous results were observed in the scavenging of other radicals by the plant extracts, viz., *Terminalia chebula* > *Terminalia bellerica* > *Emblica officinalis* for hydroxyl, *Terminalia bellerica* > *Terminalia chebula* > *E. officinalis* for singlet oxygen and *Terminalia bellerica* > *Emblica officinalis* > *Terminalia chebula* for hypochlorous acid. As a whole, the studied fruit extracts showed quite good efficacy in their antioxidant and radical scavenging abilities, compared to the standards.

Kathirvel and Sujatha (2012), obtained phenolic, tannin, flavonoid and flavonol content in various solvent extracts like acetone > ethyl acetate > methanol > water > chloroform > petroleum ether. Parameters tested in different concentrations of
crude extracts showed an excellent potential of which acetone and ethyl acetate revealed good IC50 values. About 0.13 mg against the standard α-tocopherol (0.197 mg) and ascorbic acid (0.18 mg) was obtained as IC50 value for the scavenging activity in acetone extract. EC50 value for reducing power was 0.0375 mg in acetone extract against the standards like α-tocopherol (0.197 mg) and ascorbic acid (0.18 mg). With such stronger phytochemical properties, *Terminalia chebula* leaves could be utilized as an effective and safe source of functional food material such as natural antioxidants.

Rajinder *et al.*, (2015), reported Gallic acid (3,4,5-trihydroxybenzoic acid) was an organic acid and naturally occurring polyhydroxyphenolic compound abundantly found in various fruits and vegetables. This compound was widely used to cure various disorders and had anti-inflammatory, antibacterial, antifungal, antiviral, antidiabetic, antimalarial and antiallergic activities. Therefore, this study was planned to investigate its other medicinal values such as antimutagenic and antioxidant activities. The antimutagenic activity of gallic acid was checked by using Ames assay. Antioxidant activities was determined through various *in vitro* assays like DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, lipid peroxidation, deoxyribose degradation (site-specific and non-site specific modes), and reducing power assay. Gallic acid showed strong free radical scavenging and antimutagenic effects in both *in vitro* antioxidant and antimutagenic assays.

### 2.9.4. Cytotoxicity of Bioactive compounds

Saleem *et al.*, (2002), reported that 70% methanol extract of *Terminalia chebula* fruit, showed its effect on the growth of several malignant cell lines including a human (MCF-7) and mouse (S115) breast cancer cell line, a human Osteosarcoma cell line (HOS-1), a human prostate cancer cell line (PC-3) and a non-tumorigenic, immortalized human prostate cell line (PNT1A) using assays for proliferation ([3H]-thymidine incorporation and coulter counting), cell viability (ATP determination) and cell death (flow cytometry and Hoechst DNA staining). In all cell lines studied, the extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. Flow cytometry and other analyses showed that some apoptosis was induced by the extract at lower concentrations, but at higher concentrations, necrosis was the major mechanism of cell death. ATP assay
guided chromatographic fractionation of the extract yielded ellagic acid, 2,4-chebulyl-b-D-glucopyranose (a new natural product), and chebulinic acid which were tested by ATP assay on HOS-1 cell line in comparison to three known antigrowth phenolics of *Terminalia*, gallic acid, ethyl gallate, luteolin, and tannic acid. Chebulinic acid (IC$_{50}$ 53.2 mM 0.16), tannic acid (IC$_{50}$ 59.0 mg/ml 0.19) and ellagic acid (IC$_{50}$ 78.5 mM 0.24), were the most growth inhibitory phenolics of *Terminalia chebula* fruit in our study.

Sathyanarayanan *et al.*, (2009), investigated for the preliminary phytochemical analysis and characterization for *Wrightia tinctoria* by various instrumental techniques. Indole derivatives such as isatin, induridine, tryphanthrine and fatty acids were identified. Methanolic extract of leaf parts of *Wrightia tinctoria* (WT) had been studied against replication of HCV in Huh 5.2 cells. The 50% effective concentration for inhibition of HCV in RNA subgenomic replicon replication in huh 5-2 cells (luciferase assay) by CWT was found to be 15 <g/mL. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells by 50% was greater than 50 <g/mL.

Kibria and Hussain (2012), reported that the methanolic crude extracts (n-hexane, ethyl acetate and chloroform soluble fractions) of *Terminalia chebula* were screened for cytotoxic activity using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate was used as a positive control. From the results of brine shrimp lethality bioassay it could be well predicted that methanolic crude extracts (n-hexane, ethyl acetate and chloroform soluble fractions) of *Terminalia chebula* possessed cytotoxic principles, (LC$_{50}$ 1.413 µg/ml, 1.492 µg/ml and 1.496 µg/ml respectively) with the positive control with vincristine sulphate, (LC$_{50}$ 0.563 µg/ml).

### 2.10. Phytosynthesis of silver nanoparticles

Ahmad *et al.*, (2010), observed that Luteolin was a common flavone found in the aerial parts of basil plant. It is a known antioxidant, a free radical scavenger, an agent in the prevention of inflammation and a promoter carbohydrate metabolism. The formation of enol form of the luteolin freely liberated reactive hydrogen which was responsible for the conversion of silver to silver ion (Ag$^+$ to Ag$^0$) (Figure: 2.1. a and b). There was liberation of reactive hydrogen in the keto form of the rosemarinic
acid to form an intermediate, unstable enol form of the same (rosemarinic acid). This structure was however unstable due to the presence of two hydroxyl group on one carbon atom and reverts back to its keto form (Figure: 2.1. c and d).

Figure: 2.1. Mechanism of biosynthesis of Silver Nanoparticles.

Thus in both the cases the reactive hydrogen got liberated which participated in the synthesis of silver nanoparticles. The antioxidant effectiveness of many polyphenols was essentially a result of the ease with which hydroxyl group was donated to a free radical and the ability of the aromatic structure to support an unpaired electron.

Korbekandi and Iravani (2010), observed Silver nanoparticles which were also used in medical devices. (e.g., polymethylmethacrylate bone cement, surgical masks and implantable devices) to increase their antimicrobial activities.

Table: 2.3. Silver nanoparticles used in pharmaceutics, medicine and dentistry

<p>| Treatment of dermatitis; Inhibition of HIV-1 replication |
| Treatment of ulcerative colitis &amp; acne |
| Antimicrobial effects against infectious organisms |
| Remote laser light-induced opening of microcapsules |
| Silver/dendrimer nanocomposite for cell labelling |
| Molecular imaging of cancer cells |
| Enhanced Raman scattering (SERS) spectroscopy |
| Detection of viral structures (SERS &amp; Silver nanorods) |</p>
<table>
<thead>
<tr>
<th>Pharmaceuticals &amp; Medicine</th>
<th>Dentistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating of hospital textile (Surgical gowns, face mask)</td>
<td>Additive in polymerizable dental materials Patent</td>
</tr>
<tr>
<td>Coating of catheter for cerebrospinal fluid drainage</td>
<td>Silver-loaded SiO2 nanocomposite resin filler (Dental resin composite)</td>
</tr>
<tr>
<td>Coating of surgical mesh for pelvic reconstruction</td>
<td></td>
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</tbody>
</table>
The biosynthesis of silver nanoparticles using *Wrightia tinctoria* leaf extract is very simple and economic.

Prashanth *et al.*, (2011), reported that the biosynthesis of silver nanoparticles of varying sizes ranging from 33-45.9 nm using aqueous extract of eighteen medicinal plants at room temperature. The synthesized nanoparticles are evaluated for its antimicrobial activity against human pathogens as well as fungal phytopathogens. Synthesized nano particles were characterized using UV-visible spectrophotometer and two nano particles which showed highest inhibitory activity against microbes were also characterized with Scanning Electron Microscope (SEM) and X-ray diffractometer (XRD). Novelty of this present study is that the plant extract is very cost effective and eco friendly and thus can be economic and effective alternative for the large scale synthesis of silver nanoparticles.

Kumar *et al.*, (2012), demonstrated green rapid biogenic synthesis of silver nanoparticles (Ag NPs) using *Terminalia chebula* aqueous extract in this present study. The formation of silver nanoparticles was confirmed by Surface Plasmon Resonance (SPR) at 452 nm using UV–visible spectrophotometer. The reduction of silver ions to silver nanoparticles by *Terminalia chebula* extract was completed within 20 min which was evidenced potentiometrically. The hydrolysable tannins such as di/tri-galloyl-glucose present in the extract were hydrolyzed to gallic acid and glucose that served as reductant while oxidised polyphenols acted as stabilizers.

Lalitha *et al.*, (2013), noticed that the field of nanotechnology was one of the most active researches nowadays in modern material science and technology. Eco friendly methods of green mediated synthesis of nanoparticles were the present research in the limb of nanotechnology. The synthesis of nanoparticles from 1mM AgNO$_3$ solution through aqueous leaf extract of *Azadirachta indica* was used as reducing as well as capping agent. Synthesized nanoparticles were characterized under UV-Vis Spectroscopy at the range of 350-420nm. The peak showed at 351nm. Further that range and size was confirmed by Particle Size Analyzer. The chemical groups studied using FT-IR analysis. Green synthesized silver nanoparticle showed zone of inhibition against isolated Gram positive (*Salmonella typhi*) and Gram negative (*Klebsiella pneumoniae*) bacteria. The leaf extract showed higher antioxidant
activity found by DPPH assay and Hydrogen Peroxide assay. Based on the result obtained it could be said that the plant resources could be efficiently used in the production of silver nanoparticle and it could be utilized in various fields such as biomedical, nanotechnology and so on.

Kirthika et al., (2014), Synthesized and characterized silver nanoparticles from five different herbal plants (Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus). The qualitative analysis of plant extracts was performed to determine the presence of secondary metabolites. The plant mediated silver nanoparticles were synthesized. The colour changed into brown to black color indicating the formation of AgNPs. The characterization of synthesized AgNPs was carried out by different methods such as UV-Vis Spectra, FE-TEM, Particle size analysis, Zeta potential analysis, XRD and FTIR. The antimicrobial activity of synthesized silver nanoparticles also examined against three fungi and bacteria. The UV wave length of AgNPs is from 300 to 450 nm. The average size of AgNPs 581 d.nm, zeta potential is -13.3 mV. The FTIR results showed that AgNPs contained the functional groups. In antimicrobial activity of all AgNPs synthesized by five plants inhibited the growth of bacteria and Terminalia chebula showed maximum effect. The XRD pattern clearly confirmed that the synthesized silver nanoparticles were crystalline in nature. TEM results showed that synthesized silver nanoparticles were round in shape. The green synthesis of nanoparticles showed that cost-effective, environmentally friendly, and safe for human therapeutic use. Colour change, UV-Vis spectra, TEM and XRD analysis confirmed the stability of synthesized AgNPs.

2.11. Optimization and Characterization of Silver Nanoparticles

Li et al., (2007), analyzed the effect of Capsicum annuum L. proteins on the formation of silver NPs was investigated using X-ray photoemission spectroscopy (XPS), electrochemical measurements, Fourier-transform infrared spectroscopy (FTIR) and differential spectrum techniques. The morphology and crystalline phase of the nanoparticles were determined from transmission electron microscopy (TEM), selected area electron diffraction (SAED) and X-ray diffraction (XRD) spectra. The results indicated that the proteins, which have amine groups, played a reducing and controlling role during the formation of silver nanoparticles in the solutions, and that the secondary structure of the proteins changed after reaction with silver ions. The
crystalline phase of the NPs changed from polycrystalline to single crystalline and increased in size with increasing reaction time. A recognition–reduction–limited nucleation and growth model was suggested to explain the possible formation mechanism of silver nanoparticles in *Capsicum annuum* L. extract.

Kulkarni *et al.*, (2011), showed that the plant extract was prepared in water and ethanol and treated with different concentrations of silver nitrate to obtain nanoparticles. The synthesis of nanoparticles was confirmed by change in colour from pale green to reddish brown. Further, a peak between 400nm to 440nm was obtained on UV-Visible spectrometer which confirmed the biosynthesis of silver nanoparticles. Presence of silver nanoparticles was confirmed by carrying out Scanning Electron microscopy with EDS that gave a strong silver signal. Silver nanoparticles also showed antibacterial activity against four disease causing microorganisms (*Escherichia coli*, *Pseudomonas aeroginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*).

Haq and Ullah (2011), observed that the bulb of *Allium sativum*, root of *Rheum australe* and fruit of *Terminalia chebula* were selected for trace elements analysis using Atomic Absorption Spectrophotometer. The plant samples were digested with concentrated Nitric acid (HNO₃) and concentrated perchloric acid (HClO₄). The analysis for trace elements in the sampled plants indicated the presence of Sodium, Potassium, Calcium, Magnesium, Copper, Zinc, Iron, Cobalt, Manganese, and Lead in all samples of all selected plant species at different levels which played a vital role in cure of diseases. The concentration of Cu was 0.626 mg/L, Zn was 0.569mg/L, Fe was 0.711mg/L in *Terminalia chebula*. The results that provided justification for the usage of *Terminalia chebula* in daily diet for nutrition as well as for medicinal usage in the treatment of growth retardation and hair loss, delayed wound healing and emotional disturbance. Toxic element Lead was also found but at low concentration. These results could reveal the importance of these plants, and they were used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials in herbal remedies and in pharmaceutical companies.

Sathyavani *et al.*, (2011), reported that the synthesized silver nanoparticles were generally found to be spherical in shape with size 31 nm by Atomic Force Microscopy (AFM). The molar concentration of the silver nanoparticles solution used
was 1100 nM/10 mL. The results exhibited that silver nanoparticles mediated a dose-dependent toxicity for the cell tested, and the silver nanoparticles at 500 mM decreased the viability of HEp 2 cells to 50% of the initial level. LDH activities were found to be significantly elevated after 48 hours of exposure in the medium containing silver nanoparticles when compared to the control. Caspase 3 activation suggested that silver nanoparticles caused cell death through apoptosis, which was further supported by cellular DNA fragmentation and the silver nanoparticles treated with HEp2 cells exhibited extensive double strand breaks, thereby yielding a ladder appearance, while the DNA of control HEp2 cells supplemented with 10% serum exhibited minimum breakage.

Yilmaz et al., (2011), reported, the synthesis of silver nanoparticles by employing a shadow-dried Stevia rebaudiana leaf extract in AgNO₃ solution. Transmission electron microscopy and X-ray diffraction inspections indicated that nanoparticles were spherical and polydisperse with diameters ranging between 2 and 50 nm with a maximum at 15 nm. Ultraviolet–visible spectra recorded against the reaction time confirmed the reduction of silver nanoparticles indicating that the formation and the aggregation of nanoparticles took place shortly after the mixing, as they persisted concurrently with characteristic times of 48.5 min and 454.5 min, respectively. Aggregation was found to be the dominant mechanism after the first 73 minutes. Proton nuclear magnetic resonance spectrum of the silver nanoparticles revealed the existence of aliphatic, alcoholic and olefinic CH₂ and CH₃ groups, as well as some aromatic compounds but no sign of aldehydes or carboxylic acids. Infrared absorption of the silver nanoparticles suggested that the capping reagents of silver and gold nanoparticles reduced in plant extracts/broths were of the same chemical composition of different ratios. Ketones were shown to play a reasonably active role for the formation of nanoparticles in plant extracts/broths.

Dipankar and Murugan (2012), reported that the reaction mixture which turned to brownish grey colour after 7 days of incubation and exhibited an absorbance peak around 460 nm revealed the characteristic of Ag nanoparticle. Scanning electron microscopy (SEM) and EDX analysis showed silver nanoparticles were pure and polydisperse and the size were ranging from 44 to 64 nm. X-ray diffraction (XRD) studies revealed that most of the nanoparticles were cubic and face centered cubic in
shape. Fourier Transform InfraRed spectroscopy (FTIR) showed nanoparticles were capped with plant compounds. Biosynthesized silver nanoparticles showed potent antibacterial activity against human pathogenic bacteria. Phytosynthesized nanoparticles exhibited strong antioxidant activity as well as cytotoxicity against HeLa cervical cell lines.

Vanaja et al., (2013), indicated phytosynthesis process of silver nanoparticles using plant extract was simple, cost-effective, and ecofriendly. It was also reported that green nanoparticles were prepared by using stem extract of *Cissus quadrangularis* and the physical and chemical factors such as time duration, metal ion concentration, pH, and temperature that play vital role in the nanoparticles synthesis were assessed. The maximum synthesis of silver nanoparticles was attained within 1 hour, at pH 8 and 1 mM AgNO$_3$ concentration, at 70°C. The nanoparticles obtained were characterized by UV–visible spectroscopy. Silver nanoparticles that were synthesized under these conditions showed crystalline nature confirmed by X-ray diffraction and showed mostly spherical and some rod and triangle shapes with sizes ranging from 37 to 44 nm, which were characterized by Scanning Electron Microscopy. Fourier Transform InfraRed spectroscopy showed that the functional groups were carboxyl, amine, and phenolic compounds of stem extract which were involved in the reduction of silver ions. Thus, synthesized silver nanoparticles showed more antibacterial activity against *Klebsiella planticola* and *Bacillus subtilis*, which was analyzed by disc diffusion method.

Mohamed and Abdul (2014), reported that the environmentally friendly synthesis of nanoparticles process was a revolutionary step in the field of nanotechnology. In recent years plant mediated biological synthesis of nanoparticles had been gaining importance due to its simplicity and eco-friendliness. In this study, a simple and an efficient eco-friendly approach for the biosynthesis of stable, monodisperse silver nanoparticles using aqueous extracts of four *Terminalia* species, namely, *Terminalia catappa*, *Terminalia mellueri*, *Terminalia bentazoe* and *Terminalia bellerica* were described. The silver nanoparticles were characterized in terms of synthesis, capping functionalities (polysaccharides, phenolics and flavonoidal compounds) and microscopic evaluation by UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy and Transmission Electron Microscopy. The results
showed a simple and feasible approach for obtaining stable aqueous monodispersive silver nanoparticles.

2.12. Antimicrobial activity of Silver Nanoparticles:

Kulkarni et al., (2011), said that the antibacterial activity of silver nanoparticles was reported to a large extent. The silver nanoparticles obtained from Anthoceros also showed antibacterial activity against four strains of laboratory pathogens viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Bacillus subtilis. The zone of inhibition was measured and it was evident that the nanoparticles synthesized were good candidates for their usage as and/or in antibacterial drugs. Mechanism of action of silver nanoparticles as antibacterial agents was not very well known but it seemed that the nanoparticles interfered in the respiratory metabolism of the organisms and therefore, show antibacterial activity. Since the nanoparticles thus synthesized showed antibacterial activity, they could be used in various fields such as paint industry, pharmaceutical industry and so on.

Savithramma et al., (2011), tested phytosynthesized silver nanoparticles for the antifungal activity. Using the test cultures of Aspergillus, Fusarium, Curvularia, and Rhizopus species. The antifungal property of silver nanoparticles was analyzed by measuring the inhibitory zone. The silver nanoparticles synthesized from bark extract of Boswellia ovalifoliolata and Shorea tumbuggaia. Boswellia ovalifoliolata showed moderately toxic to the Aspergillus, Curvularia and Rhizopus species and highly toxic to Fusarrium species. Biologically synthesized silver nanoparticles of Shorea tumbuggaia bark extract were moderately toxic to Aspergillus, Fusarium and Rhizopus species and highly toxic to Curvularia species. The important outcome of the study was the perception of the development of value added products from medicinal plants of India for biomedical and nanotechnology based industries.

Prashanth et al., (2011), revealed the synthesis of silver nanoparticles of varying sizes using aqueous extract of fifteen medicinal plants at room temperature. The synthesized nanoparticles were evaluated for its antimicrobial activity against human pathogens as well as fungal phytopathogens. Since kuduveli, vasambu, vilangam, Pavu and vettiver showed high antimicrobial activity against bacterial human pathogen, these particles were studied with antibiotics for its comparative inhibitory activity against Staphylococcus aureus and Micrococcus sp., and with azole
compounds against *Candida albicans*. All the five samples were showed good inhibitory activity against *Staphylococcus aureus* whereas vasambu and vilangam did not show activity against *Micrococcus* sp. In case of azole compounds vasambu and vilangam showed good inhibitory activity against *Candida albicans* even triclosan did not show inhibitory activity.

Dipankar and Murugan (2012), reported that the antibacterial effect of 1 hour silver nanoparticles (IhAgNPs) at different concentrations (50–250 μg/ml) was quantitatively assessed on the basis of the zone of inhibition. IhAgNPs exhibited strong antibacterial activity against all human pathogens even at the lowest concentrations used, except against *Klebsiella pneumoniae*, for which the growth was inhibited only at 100 μg/ml. The IhAgNPs exhibited a maximum effect against *Escherichia coli* with a zone of inhibition of 15.7 ± 0.6 mm. When these results were compared with those for standard antibiotics, it was found that the IhAgNPs were more effective against *Staphylococcus aureus* and *Enterobacter faecalis* than all the test antibiotics at higher concentrations. The MIC of the IhAgNPs and standard antibiotics evaluated against the human pathogens. It was found that the MIC value of the IhAgNPs was lower than that of kanamycin against *Escherichia coli*. The antimicrobial effect was dose-dependent and increased linearly with the increased concentration of the test sample. The nanoparticle concentration was limited to a moderate dose (250 μg/ml) because higher doses may be toxic toward the host of the pathogens.

Kumar *et al.*, (2012), showed that the green synthesized silver nanoparticles has effective results towards Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922). The MIC/MBC was determined in batch cultures with varying concentration of silver nanoparticles. Gram-positive *Staphylococcus aureus* was more resistant to silver nanoparticles compared to Gram-negative *E. coli*, based on their studies with single strain of each culture. Diameter of Inhibition Zones (DIZ) is measured. Different activity was observed with *Escherichia coli* where initially, inhibitory zones were formed which was able to inhibit the growth of bacteria but few resistant colonies were seen growing on the zones formed initially. Thus silver nanoparticles have shown higher sensitivity towards *Staphylococcus aureus* strain than *Escherichia coli*. Here we have
measured the zone of diameter only of the cleared zone area excluding the growth of resistant colonies in the zones formed. Antibacterial activity using Staphylococcus aureus and E. coli was performed by evaluation of MIC/MBC. The lowest concentration of 15 μg/mL (Ag NPs) showed cell growth on plates for 1 × 109 CFU/mL but had shown no growth for concentrations ranging from 1 × 108 to 1 × 104 CFU/mL indicating bactericidal activity. The lowest concentration of 15 μg/mL of silver nanoparticles was found effective in the study of MIC/MBC and can kill the bacteria up to 1 × 108 CFU/mL concentrations, as no cell growth can be seen upon plating, followed by incubation at 37 °C for 24 h, which shows that it is responsible in complete killing of bacteria up to this concentration. However the test tube with 109 CFU/mL concentrations of bacteria showed very little turbidity (in the case of Escherichia coli)/and no turbidity (in the case of Staphylococcus aureus) which showed selective bacteriostatic nature of nanoparticles as colonies might form on plating. Thus it is concluded that bio-inspired silver nanoparticles are a potential bacteriostatic as well as bactericidal agent.

Dwivedi, (2013), emphasized for the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. Among the various agents used for nanoparticle synthesis, plants have found important application. The biomolecules found in plants induced the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). The aqueous leaves extract of Terminalia chebula was used as reducing and stabilizing agent for the synthesis of silver nanoparticle. Synthesized nanoparticle was confirmed by the change of color from transparent yellow to dark brown indicated the formation of silver nanoparticles. UV-Vis Absorption Spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The UV visible spectrum of colloidal solutions of SNPs was 199.00nm. The antimicrobial assays were done on human pathogens like Escherichia coli, Staphylococcus aureus, Salmonella typhi and Klebsiella pneumonia. The zone of inhibition of synthesized silver nanoparticles were found to be 18 mm for Salmonella typhi, 16 mm for S. aureus, 15 mm for E. coli and 20 mm for Klebsiella pneumoniae. The plant based route could be considered to be an environmental friendly, safe and economic biological method for the silver nanoparticles production.
Kumarasamyraja and Jeganathan (2013), investigated the Bio synthesis of silver Nanoparticles using aqueous extract of *Acalypha indica* and its antimicrobial activity against different micro organisms. About 10 ml of aqueous extract of *Azadirachta indica* added with 90 ml of AgNO₃ (1mM) solution, the resulting mixture was incubated at 37°C under static condition. The development of yellowish brown colour indicated the formation of Ag-Np’s. The Ag-Np’s monitored with the help of UV-visible spectrophotometer at the wavelength of 200– 800 nm. The observed absorbance peak at 400 nm indicated the formation of Ag-Np’s. The particle size of Ag-Np’s was determined by using particle analyzer and the results showed that average size range was found to be 0.516 nm. TEM technique was employed to visualize the size and shape of Ag-Np’s. The antibacterial activity of *Azadirachta indica* Ag-Np’s was evaluated against both Gram positive and Gram negative pathogenic microorganism by disc diffusion method. The diameter of inhibition zone of *Azadirachta indica* Ag-Np’s was analyzed at different concentrations ranging from 100 to 300μg/ml. The maximum zone of inhibition was observed with *Pseudomonos aeruginosa* (16 mm), followed by *Escherichia coli* (14 mm), *Bacillus subtilis* (13 mm) and *Staphylococcus aureus* (13 mm) when compared with standard drug Amikacin. The antifungal activity of *Azadirachta indica* Ag-Np’s at 300 μg/ml concentration was found to be 23mm and12 mm for *Candida albicans* & *Aspergillus niger* respectively when compared with the standard antifungal drug ketokonazole. It was observed from the results that biologically synthesized Ag-Np’s from *A. indica* aqueous extract showed effective antimicrobial and antifungal activity against selected microorganisms which were comparable with standard.

Rathinamoorthy and Thilagavathi (2014), reported the qualitative and quantitative analysis of the major bioactive constitutions of methanol extract of *Terminalia chebula* from the various parts of the Coimbatore district, Tamil nadu, India for their authentication. Phytochemical analysis, High-performance liquid chromatography (HPLC), Agar diffusion test, Minimum inhibitory concentration (MIC) were also reported. The proximate analysis and phytochemical analysis revealed that *Terminalia chebula* had most of the important phyto-constituents like Alkaloids, proteins, saponins, tannins, flavonoids, phenols, terpenoids, carbohydrates, triterpenoids, thiols, steroids and glycosoides were analyzed qualitatively. High-performance liquid chromatography analysis was confirmed the presence of these
components objectively. The Quantitative analysis revealed the presence of saponin, alkaloids, Tannin and flavanoids, which in turn confirmed their potential for medicinal use. The *in-vitro* antibacterial test by agar diffusion test was conducted for finished textile samples and it was observed that, the treated textiles material had the potential antibacterial property against wide spectrum of human pathogenic strains like *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 1687 *pneumoniae* (MTCC 6644), *Proteus vulgaris* (MTCC 742), *Salmonella typhi* (MTCC 733), *B. licheniformis* (MTCC 429), *Micrococcus luteus* (ATCC 49732) and *Pseudomonas Sp.* (MTCC 6628 ), *Corynebacterium Sp* (MTCC 8730 and ATCC 3021). Minimum inhibitory concentration (MIC) values were determined and compared with the positive control (Tetracycline) used. Study justified the potential and application of traditional medicine in healthcare sector as an alternative eco friendly way against human pathogenic bacterial strains in wound beds.