Chapter – IV
Antimicrobial Activity
1. Introduction

1.1 Introduction

Plants are used as medicines since time immemorial. India has rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha, and Unani besides folklore practices. Rigveda is one of the oldest repositories of human knowledge\(^1\) in which the uses of medicinal plants were mentioned. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is traditional medicines and systematic investigation of this may result in the discovery of novel drugs\(^2\). Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century\(^3\). Natural antimicrobials have been often derived from plants, animal tissues or microorganisms\(^4\). The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants\(^5\). The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agent. Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections.
2. Review of Literature

Primordial people used plants to cure a variety of human ailments. About 85% of Indians use higher plants as effective anti-microbials for the treatment of various diseases\(^6\). Ethanol extracts of 78 traditional medicinal plants from India were used for treating infectious diseases and show bacterial and fungal activity at 1.6 mg/ml\(^7\). The 50% ethanol extracts of 285 plant materials were screened for 61 biological activities and revealed effective anti-bacterial, and a wide range of pharmacological activities\(^8\). Anti-microbial and phytochemical studies exposed 45 Indian medicinal plants effective against multi-drug-resistant bacteria\(^9\).

The in vitro antimicrobial activity of *Cassia alata* leaf extracts was investigated against *S. aureus*, *S. aureus coagulase positive*, *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *E. coli*, *V. cholerae*, *S. typhi*, *S. dysenteriae* and *K. pneumoniae*. The acetone and ethanol (95%) extract of *Cassia alata* showed the inhibitory effects very close and the same in magnitude with that of standard antibiotics ampicillin and tetracycline used\(^10\).

*Achyranthes bidentata* Blume belonging to the family Amaranthaceae was investigated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* organisms, using agar diffusion method. The petroleum ether, chloroform, methanol and aqueous extracts showed significant antibacterial activity. The activity of methanol extract was less than that of standard antibiotic ampicillin\(^11\).

Ethanol, petroleum ether and chloroform extracts of two medicinal plants *Lawsonia inermis* L. and *Mimosa pudica* L. were proven for antibacterial properties against 15 Gram-positive and Gram-negative human pathogenic bacteria. Among the three types of extracts tested, ethanol extract was found to possess maximum
antibacterial activity. In ethanol extract of *Lawsonia inermis*, the zone of inhibition was ranging from 7.20 mm (*E. coli*) to 17.25 mm (*S. dysenteriae*). Lowest (156.25 μg/ml) and highest (2500 μg/ml) MIC was observed for *S. dysenteriae* and *E. coli*, respectively. The methanol leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritiana* showed significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis pv. malvacearum* and antifungal activity against *Aspergillus flavus, Dreschlera turcica* and *Fusarium verticillioides* when compare to root/bark extracts. Among the five plants viz., *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritiana*, leaf and bark extract showed significant antibacterial activity against the test pathogens. Leaf extract showed significant activity when compared with the bark/root extract of all the test plant extract. Bark extract of all the five plant extracts was almost similar or higher activity when compared with the streptomycin sulphate.

Antimicrobial activity of 18 ethnomedicinal plant extracts were evaluated against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Erwinia sp, Proteus vulgaris*) and one fungal strain (*Candida albicans*). The collected ethnomedicinal plants were used by Paliyar tribe from Tamil Nadu, India, in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders. Out of 18 plants, 10 plants exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5 and 5 mg/disc. *Acalypha fruticosa, Peltophorum pterocarpum, Toddalia asiatica, Cassia auriculata, Punica granatum* and *Syzygium lineare* were most active. Aqueous extracts of ten medicinal plants were examined for their antibacterial potential.
against some reference strains of human pathogenic bacteria. *Anethum graveolens*, *Elettaria cardamomum, Foeniculum vulgare, Trachyspermum ammi* and *Viola odorata* were found to be effective compared to standard antibiotics, viz. ampicillin, cefixime, chloramphenicol, co-trimoxazole, gentamicin, imipenem, pipericillin/tazobactam and tobramycin was assessed by the disc diffusion method. *V. odorata* was the most effective antibacterial with minimum inhibitory concentration values ranging from 1 to 2%\textsuperscript{15}.

A screening of the antibacterial potential of essential oils of *Syzygium neesianum* Arn (leaves) *Elaeocarpus lanceifolius* Roxb. (Leaves), *Glycosmis tirunelveliensis* (Leaves), and *Boesenbergia tiliafolia* (rhizome), traditionally used by the folk were collected from Agasthiyamalai Biosphere reserve, Tamil Nadu, South India. were investigated by agar disc diffusion method against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli*. The essential oils of *S. neesianum* Arn (Leaves) was active against four of the bacteria tested, showing the highest zone of inhibition values 31mm against *S. aureus* and the lowest zone of inhibition value 21mm against *E.coli* respectively\textsuperscript{16}.

The antibacterial activity of the methanolic extract the leaves ,and tannins of *Solanum trilobatum* Linn., collected from Western Ghats, Tamil Nadu assayed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris Salmonella typhi, and Streptococcus pyogenes*. The minimum inhibitory concentration (MIC) of tannins isolated in the study against the tested organisms ranged between 1.0, and 4.0mg/ml\textsuperscript{17}. The crude extracts of aerial parts of *Artemisia annua* Linn (Asteraceae) were investigated against five Gram-positive bacteria and three Gram-negative bacteria. The methanol extract showed the strongest activity against most bacteria used. The MIC value for the *P.aeruginosa* was 2.0mg/mL. Furthermore
B. pumilus, B. subtilis, B. cereus and M. luteus showed a MIC value of 0.5mg/mL whereas S. aureus required nearly 0.25mg/mL of the methanol extract for inhibition\textsuperscript{18}.

The crude methanolic extracts of Oldenlandia umbellate, Rubiaceae, were tested against both gram positive and gram negative bacteria. It was found that the methanolic extract of roots and aerial portion (except leaves) possessed more antibacterial activity than the extract obtained from the leaves. The active compound 1,2-dihydroxy anthraquinone known as Alizarin was also separated. The plant extract gave a zone of inhibition of around 18 – 21mm for all strains namely E. coli, S. aureus, Proteus sp, Klebsiella sp. and Pseudomonas sp\textsuperscript{19}. The preliminary antibacterial activity of 12 medicinal plants used in Indian folk medicine was made. The antibacterial activity was done by both gar disc diffusion method and agar well diffusion method against five bacterial strains. Among the plants Bauhinia variegate L. exhibited notable antibacterial activity\textsuperscript{20}.

Antimicrobial activity of Alpinia calcarato Rosc. a medicinal plant used in India, Sri Lanka, showed significant results against, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli, K. pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi, Vibrio cholera and Bacillus subtilis. The alcohol extracts of Alpinia calcarato Rosc showed antibacterial effect against K. pneumoniae, and Bacillus subtili\textsuperscript{21}. The antimicrobial activity of the extracts of Andrographis paniculata Nees., Phyllanthus niruri Linn., Terminalia bellerica Roxb., Terminalia chebula Retz., and Vitex negundo Linn., medicinal herbs practiced in traditional folk medicine in India were screened for the antimicrobial activity. The MIC value of Phyllanthus niruri Linn., leaf extract was 50µg/ml against Staphylococcus aureus, and Salmonella typhi. The MIC value of Terminalia bellerica Roxb. fruit extract against Escherichia coli, and Staphylococcus aureus were 50µg/ml and 200µg/ml respectively.\textsuperscript{22}
The antibacterial potential of three medicinal plants *Siegesbeckia orientalis* Linn., *Berberis tinctoria* Lesch., and *Justicia betonica* Linn., used by the tribal of Nilgiris for the treatment of various skin ailments were tested against various pathogenic bacteria. Benzene extract of *Siegesbeckia orientalis* Linn., exhibited higher activity against *Staphylococcus aureus* followed by chloroform extract against *V.cholerae*. The leaf extract of *Justicia betonica* Linn. showed moderate activity against the tested organisms. The anti-enterobacterial potential of nine ethnobotanically selected plants traditionally used in different parts of India for the treatment of gastrointestinal disorders was reported. These plants were screened for antibacterial activity against 11 strains of bacteria, including *Vibrio cholerae*. The extracts of *T.Chebula* and *S.cumini* had strong bacterial activity of MIC ranging from 0.25 to 4 mg/ml, against *V.cholerae*. *S.nigrum* was active against *V.cholerae* and did not show activity against *E.coli* and *P.aeruginosa*.  

Methanol extracts of 23 plants were screened for their antibacterial activity against multi-drug resistant bacteria, viz. *Staphylococcus aureus*, *Salmonella typhi*, and *Salmonella paratyphi*. The highest level of activity against *Staphylococcus aureus* was noted with *Cryptolepis buchanani* extract showing the MIC value of 1mg/ml. The MIC value of *Manilkara hexandra* extract against *Staphylococcus epidermis* was 2mg/ml. Leaf extract of *Mangifera indica* displayed good inhibitory efficacy against all pathogens with MIC value of 2 4 mg/ml. *Cyperus rotundus* (rhizome ), *Caesalpinia bonducella* (seed), *Tinospora cordifolia* (stem), *Gardenia gummifera* (buds and shoots), *Ailanthus excels* (leaves), *Acacia arabica* (leaves), *Embelia ribes* (seeds) and *Ventilago maderspatana* (steam and bark) from Melghat forest were screened for their antibacterial potential against *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella flexneri*, *Salmonella paratyphi*, *Salmonella
typhimurium, Pseudomonas aeruginosa, Enterobacter aerogenes by disc diffusion method by Tambekar et al. Caesalpinia bonducella, Gardenia gummifera and Acacia arabica showed remarkable antibacterial potential. The phytochemical analysis of Ventilago madraspatana showed the presence of reducing sugars, anthroquinone, alkaloid, tannins and phenols. Acetone extract of Ventilago maderaspatana proved antibacterial to Klebsiella pneumonia. Escherichia coli, Salmonella typhi, Proteus vulgaris, Salmonella paratyphi, Salmonella typhimurium and Pseudomonas aeruginosa were resistant to Cyperus rotundus, Caesalpinia bonducella, Tinospora cordifolia, Ailanthus excelsa, Embelia ribes and Ventilago madraspatana with 10mg/disc.

Ventilago maderspatana was antibacterial against S.aureus, K.pneumoniae, P.vulgaris, S. flexneri and E.aerogenes. The antibacterial activity of the extracts of Ventilago madraspatana stem-bark, Rubia cordifolia root and Lantana camara root-bark was evaluated by the agar-well diffusion method. Chloroform and ethanol extracts of V. maderaspatana showed broad-spectrum activity against most of the bacteria except S. aureus, E. coli and V. cholerae. The activity of the chloroform and methanol extracts of R. cordifolia and L. camara was found to be more specific towards the gram-positive strains. The water extracts of V. madraspatana and L. camara were found to be inactive. Emodin and physcion were isolated from the stem-bark of V. madraspatana and the MICs of emodin in the range 0.5–2.0 µg/mL against three Bacillus sp.

A broad spectrum of antibacterial activity was exhibited by the methanol extracts of leaves, root and stem barks of Terminalia complanata and Flacourtia zippelii and their subsequent fractions (petrol, dichloromethane, ethyl acetate). Fractionation enhanced the activity particularly in the ethyl acetate fractions of the stem and root barks of T. complanata. Ethanolic extracts of Phoebe lanceolata stem bark and Stephania glabra tubers were evaluated for their antibacterial and antifungal activities against five
bacterial species, *Staphylococcus aureus* (along with ten hospital strains), *Staphylococcus mutans, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae* and six fungal species *Aspergillus niger, Aspergillus fumigatus, Penicillium citranum, Microsporum gypseum, Microsporum canis, Trichophyton rubrum*, obtained from different culture media. The plants/parts extracts were found active against most of the tested microorganism with MIC range of 50-100μg/ml. The MIC was taken at the lower concentration where inhibition ceased. Novobiocin (15μg/ml) and erythromycin (15μg/ml) were used as positive controls for bacterial and fungus species respectively.

Two alkaloids, stephanine and crebanine, were isolated from tubers of the traditional Chinese medicinal plant *Stephania dielsiana*, using an activity-directed isolation method, and inhibitory activity of methanol extract, stephanine and crebanine against ten animal pathogenic bacteria and eight plant pathogenic fungi was evaluated in vitro. *S. dielsiana* exhibited high inhibitory activity against five gram-positive and four gram-negative animal pathogenic bacteria, with MIC values of 0.625–7.5 g/l; stephanine and crebanine had high inhibitory activity against gram-positive animal pathogenic bacteria, with MIC values of 0.078–0.312 g/l. A novel hasubanalactam alkaloid, named glabradine, had been isolated from the tubers of *Stephania glabra*. It was evaluated for antimicrobial activity against *Staphylococcus aureus, S. mutans, Microsporum gypseum, M. canis* and *Trichophyton rubrum* and displayed effective antimicrobial activity superior to those of novobiocin and erythromycin used as positive controls.

*Flacourtia indica* bark is used as astringent. Dried leaves are used as carminative, expectorant, tonic and astringent. Its fruit is used as food for the local people. *Flueggea leucopyrus* leaves are used as vermifuge. It is also used to cure uterine polyps. The juice of the leaves is used to destroy maggots in sores. *Ventilago*
maderaspatana Gaertn is used as carminative, stomachic, stimulant, debility and in mild fever. Powdered bark is used against skin diseases and itch. Stephania wightii Dunn has been used by the kanikars as analgesic, anti-inflammatory, anthelmintic and emetic. As there are no reports on the pharmacology especially against hepatotoxicity and antimicrobial activities an attempt was made.
3. Materials and Methods

The fresh medicinal plants had been chosen for this research namely Flacourtia indica (Flacouriaceae), Flueggea leucopyrus (Phyllanthaceae), Stephania wightii Dunn. (Menispermaceae) and Ventilago maderaspatana Gaertn. (Rhamnaceae), were collected from Agasthia hills, Tirunelveli district, Tamilnadu, India. The plants were identified by Dr.V.Chelladurai, Research Officer (Botany), Retired, Survey of Medicinal and Aromatic plants Unit- Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India. The collected plant materials were air dried under shade at room temperature and ground with a grinder into powder.

3.1 Preparation of Extracts

Extracts were prepared by cold extraction method in which 100g of dry powdered material was soaked in different solvents chloroform, ethanol and water for 12 hours at room temperature and shaken occasionally. Each extract was filtered with Whatman filter paper No.1. The filtrates were dried, and dried extracts were collected and stored in a refrigerator until further use.

3.2 Microbial activity

The bacterial stains Staphylococcus aureus, Escherichia coli, Pseudomonas auruginosa, Streptococcus mutans, Salmonella typhi, Proteus vulgaris, Klepsiella pneumoniae, Bacillus cereus and Lactobacillus species were obtained from Post Graduate Department of Microbiology, Sri Paramakalyani college, Alwarkurichi.

3.3 Preparation of discs

The sterile discs (Hi-Media) were used for the preparation of different concentrations such as 50, 100, 200 and 300 mcg/disc of compound and 0.5, 1.0, 1.5, 2.0,
and 2.5 mg of crude extracts. The discs were prepared aseptically and allowed to dry and then stored in a sterile container till usage.

3.4 Preparation of media

Composition of Muller – Hinton agar (MHA) media

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>300.00g</td>
</tr>
<tr>
<td>Caesin acid hydrolysate</td>
<td>17.50g</td>
</tr>
<tr>
<td>Starch</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Agar</td>
<td>17.00 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.3± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

Sterile Muller – Hinton agar (MHA) was prepared and 15 ml of media were poured into each plate. After solidification the plates were dried for 30 minutes in an incubator to remove the excess moisture from the surface. The broth cultures were incubated at 37°C for 3-5 hours.

3.5 Kirby – Bauer disc diffusion method

Disc diffusion method was employed for screening the antibacterial properties of various cold extracts of *Flacourtia indica* (Flacouriaceae), *Flueggea leucopyrus* (Phyllanthaceae)\(^35\), *Stephania wightii* Dunn. (Menispermaceae) and *Ventilago maderaspatana* Gaertn. (Rhamnaceae)\(^36\) such as chloroform, ethanol, and water extracts and tetrahydropalmatine, compound isolated from the tuber of *Stephania wightii* were tested to check their potentiality of antimicrobial activities.

A sterile cotton swab was dipped into the 3 – 5 hours old culture of organisms and the swab inside the wall of tubes was rotated to remove the excess inoculums. The test organisms were swabbed on the surface of the dried overnight agar plates. The prepared discs were placed on surface of the inoculated plates with the help of sterilized
forceps. Prepared discs were placed in equal distance to avoid overlapping zone of inhibitions. Antibiotics amikacin, ampicillin, kanamycin, gentamycin, norfloxacin, penicillin-G, tetracyclin and ciprofloxacin were used as positive reference. The inoculated plates were incubated at 37°C for 24 hours for bacteria. The zone of inhibition were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for the three replicates.
4. Result and Discussion

4.1 Screening of antibacterial activity

The preliminary in vitro antimicrobial activity of Flacourtia indica leaves and stem, Flueggea leucopyrus leaves and stem, Ventilago maderaspatana leaves and stem, Stephnia wightii tuber and tetrahydropalmatine are screened and results are recorded in Table 29 – 32 and plates 1-10.

The aqueous extract of Flacourtia indica is inactive against the bacteria studied. A mild activity is observed in chloroform extract such as 10 mm against Bacillus cereus and 9mm against Klebsiella pneumonia in the concentration of 2.5mg/disc. The ethanol extract show mild activity and maximum zone of inhibition in 2.5mg/disc against Lactobacillus species and the concentrations of 2.0, 1.5, and 1.0 mg/disc have shown minimum activity of 8mm.

The chloroform extract of Flueggea leucopyrus does not display any activity against the bacteria studied. A moderate activity is observed in ethanol extract such as 11mm zone of inhibition against Staphylococcus aureus and Escherichia coli whereas 9mm each against Staphylococcus mutans and Bacillus cereus, 8mm against Lactobacillus species and low activity of 7mm zone of inhibition against Proteus vulgaris and Salmonella typhi in the concentration of 2.5mg/disc. In the concentration of 2mg/disc 10 mm zone of inhibition against Staphylococcus aureus and 8mm against, Staphylococcus mutans, Bacillus cereus, Escherichia coli, and Lactobacillus species and minimum zone of inhibition of 7mm against Proteus vulgaris and Salmonella typhi. In the concentration of 1.5mg/disc the inhibition activity of 8mm against Staphylococcus aureus, Staphylococcus mutan, Bacillus cereus, Escherichia coli, Lactobacillus species 7mm against Proteus vulgaris and Salmonella typhi. The
maximum activity of 8mm zone of inhibition have shown against *Staphylococcus mutans*, *Lactobacillus* species and minimum activity of 7mm against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* in the concentration of 1.0mg/disc. In the concentration of 0.5mg/disc 8mm zone of inhibition was shown against *Lactobacillus* species, and 7mm against *Staphylococcus aureus*, *Staphylococcus mutans*, *Bacillus cereus*. In aqueous extract of *Flueggea leucopyrus* a mild activity of 7mm is shown against only *Pseudomonas aeruginosa* in all the concentrations.

The chloroform extract of *Ventilago maderaspatana* is inactive against all the tested bacteria. In the ethanol extract, a moderate activity is observed of 10mm zone inhibition against *Bacillus cereus* and 8mm zone inhibition against *Klebsiella pneumonia*, *Proteus vulgaris*, *Lactobacillus* species and 7mm against *Staphylococcus aureus*, *Staphylococcus mutans*, *Escherichia coli*, and *Salmonella typhi* in the concentration of 2.5mg/disc. A mild activity of 7mm zone of inhibition against, *Klebsiella pneumonia*, *Proteus vulgaris*, *Staphylococcus mutans*, *Salmonella typhi* and *Lactobacillus* species are shown in the concentration of 2.0, 1.5, 1.0 and 0.5mg/disc. 8mm zone of inhibition is observed against *Bacillus cereus* in the concentration of 2.0, 1.5 and 1.0mg/disc whereas 7mm zone inhibition is seen in the concentration of 0.5mg/disc.

A significant activity has shown in the aqueous extract of concentration 2.5mg/disc of 15mm zone of inhibition against *Salmonella typhi*, *Bacillus cereus*, 11mm against *Klebsiella pneumonia*, 8mm each against *Proteus vulgaris*, *Staphylococcus mutans* and 7mm zone of inhibition against *Staphylococcus aureus*. In the concentration of 2.0mg/disc a maximum zone of inhibition 15mm is seen against *Bacillus cereus* followed by 13mm against *Salmonella typhi* 11mm of inhibition against *Klebsiella pneumonia*, and 8mm against *Proteus vulgaris* 8mm zone of inhibition is
exhibited by *Proteus vulgaris*. *Salmonella typhi* in the concentration of 1.5mg/disc. 7mm of zone of inhibition is shown against *Bacillus cereus, Klebsiella pneumonia, Escherichia coli*, in the concentration of 1.5mg/disc. 7mm zone of inhibition is shown against *Salmonella typhi* in the concentration of 0.5mg/disc and against *Lactobacillus* species a 7mm of inhibition is seen in 2.5, 2.0, 1.5and 1.0mg/disc concentration.

The chloroform extract of *Stephania wightii* show a maximum activity of 15mm zone of inhibition against *Bacillus cereus*, 13mm zone of inhibition against *Proteus vulgaris*, 12mm zone against *Klebsiella pneumonia*, 11mm zone of inhibition against *Staphylococcus aureus, Escherichia coli*, and *Lactobacillus* species followed by 10mm zone of inhibition against *Salmonella typhi* in the concentration of 2.5mg/disc. A moderate activity of 11mm zone of inhibition against *Bacillus cereus, Proteus vulgaris* followed by 10mm zone of inhibition against *Staphylococcus aureus, Lactobacillus* species 9mm zone of inhibition against *Klebsiella pneumonia* and minimum of 8mm zone of inhibition against *Salmonella typhi* in the concentration of 2.0mg/disc. In the concentration of 1.5mg/disc a maximum activity is shown of 11mm zone of inhibition against *Proteus vulgaris* and 10mm zone of inhibition of against *Bacillus cereus, Staphylococcus aureus* and *Lactobacillus* species. A moderate activity of 9mm zone of inhibition is seen against *Bacillus species* and 7mm zone of inhibition against *Klebsiella pneumonia, Proteus vulgaris*, and *Lactobacillus* species in the concentration of 1.0mg/disc. In the concentration of 0.5mg/disc an activity of 7mm zone of inhibition exhibited by both *Bacillus cereus, and Proteus vulgaris*.

The ethanol extract of *Stephania wightii* pronounced the highest antibacterial activity of 19mm zone of inhibition against *Proteus vulgaris*, 15mm of zone of inhibition against *Klebsiella pneumonia, 14 mm zone of inhibition against*
Staphylococcus aureus and Staphylococcus mutans, 11 mm zone of inhibition against Pseudomonas aeruginosa and 9 mm zone of inhibition against Bacillus cereus in the concentration of 2.5mg/disc. In the concentration of 2.0mg/disc a maximum zone of inhibition is shown by Proteus vulgaris. 12 mm zone of inhibition against Klebsiella pneumonia, 11 mm against Staphylococcus aureus and Staphylococcus mutans, and 10 mm against Pseudomonas aeruginosa. A maximum zone of inhibition is shown by Klebsiella pneumonia, and Proteus vulgaris, of 12 mm followed by 11 mm zone of inhibition against Staphylococcus aureus and Staphylococcus mutans, and 9 mm zone of inhibition against Pseudomonas aeruginosa in the concentration of 1.5mg/disc. In the concentration of 1.0mg/disc higher activity is shown against Klebsiella pneumonia, and Proteus vulgaris, of 10mm zone of inhibition and 9mm zone of inhibition against Staphylococcus aureus and Staphylococcus mutans and 8mm zone of inhibition is seen against Pseudomonas aeruginosa in the concentration of 1.0mg/disc. In the concentration of 0.5mg/disc a maximum activity is shown against Klebsiella pneumonia and 8mm zone of inhibition was seen against Staphylococcus aureus and Staphylococcus mutans, Pseudomonas aeruginosa and Proteus vulgaris, of 8 mm zone of inhibition.

The aqueous extract of Stephania wightii is inactive against all the pathogens analyzed.

The isolated compound from the tubers of S. wightii, tetrahydropalmatine show maximum activity of 16mm zone of inhibition against Klebsiella pneumonia, 12mm zone of inhibition against Bacillus cereus and Proteus vulgaris and 10mm zone of inhibition against Staphylococcus aureus, Staphylococcus mutans, and Escherichia coli and 9mm zone of inhibition against Pseudomonas aeruginosa.

The chloroform extracts of Flacourtia indica, Flueggea leucopyrus, and Ventilgo maderasptana is inactive against all the tested pathogens except the chloroform extract of F. indica against Bacillus cereus (10mm) and Klebsiella pneumonia. The chloroform
extract of *S. Wightii* has greater activity against *Bacillus cereus*, and *E.coli* compared with that of standard Gentamycin, and Ampicillin respectively and same as that with Tetracyclin against *Klebsiella pneumonia*.

The aqueous extracts of *Flacourtia indica*, *Flueggea luecopyrus*, and *S.wightii* was inactive against all the pathogens tested, except *Flueggea luecopyrus* which has the least activity against *Pseudomonas aeruginosa*. The aqueous extracts (2.0mg/ml) of *V.maderaspatana* show a greater activity against *Bacillus cereus* than standard antibiotic Gentamycin. The aqueous extracts of *V.maderspatana* show no activity only against *Pseudomonas aeruginosa*.

The ethanolic extract of *F.indica* (2.5ml/mg) show more activity only against *Lactobacillus species*. The ethanolic extracts (2.5mg/ml) of *F.leucopyrus* show higher activity against *Staphylococcus aureus* and *E.coli* than standard antibiotic Norfloxacin and Ampicillin respectively. The ethanolic extracts of *V.maderaspatana* show activity against all the test pathogens except against *Pseudomonas aeruginosa* and it show the activity as that of the antibiotic Gentamycin. The ethanolic extracts of *S.wightii* show a remarkable activity against *Staphylococcus aureus, Staphylococcus mutans, Klebsiella pneumonia* and *Proteus vulgaris*. The ethanolic extracts (2.5mg/ml) show greater activity against *Staphylococcus aureus* than the antibiotics kanamycin and Norfloxacin and equal that of gentamycin and ciprofloxacin. Against *Klebsiella pneumonia*, and *Proteus vulgaris* the ethanolic extracts (2.5mg/ml) show a greater activity than tetracyclin.

The isolated compound from the tubers of *S. wightii*, tetrahydropalmatine pronounce activity against all the bacteria strains tested except *S.typhi* and *Lacobacillus* species. The compound (300µcg) pronounce higher activity against *Klebsiella*
pneumonia than standard antibiotic tetracycline, against Bacillus cereus its activity is greater than standard antibiotic gentamycin and against Staphylococcus aureus, E.coli the activity is similar to that of the standard antibiotic norfloxacin, and ampicillin respectively.

The evaluation of antibacterial activity of different extracts of ethnomedicinal plants of kanikaras has revealed that the plants possess potential antibacterial activity against the pathogenic bacteria. The present study has also revealed the importance of natural products to control antibiotic resistant bacteria which are being threat to human health.
Table. 29 Antibacterial Activity of various extracts of Flacourtia Indica. (Zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Name of the micro organisms</th>
<th>Control</th>
<th>Chloroform Extract(mg/disc)</th>
<th>Ethanol Extract(mg/disc)</th>
<th>Water Extract (mg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AK(^{30})</td>
<td>A(^{10})</td>
<td>K(^{30})</td>
<td>G(^{10})</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
<td>-</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td><em>Staphylococcus mutans</em></td>
<td>22</td>
<td>-</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><em>Bacillus Species</em></td>
<td>28</td>
<td>-</td>
<td>22</td>
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<td><em>Escherichia coli</em></td>
<td>24</td>
<td>10</td>
<td>16</td>
<td>24</td>
</tr>
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<td><em>Klebsiella pneumoniae</em></td>
<td>24</td>
<td>-</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>26</td>
<td>-</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>16</td>
<td>-</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>24</td>
<td>-</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td><em>Lactobacillus species</em></td>
<td>10</td>
<td>10</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: AK\(^{30}\) – Amikacin; A\(^{10}\) – Ampicillin; K\(^{30}\) – Kanamycin; G\(^{10}\) – Gentamycin; NX\(^{10}\) – Norfloxacin; P\(^{10}\) – Penicillin-G; T\(^{30}\) – Tetracyclin; Cfp\(^{30}\) – Ciprofloxacin.
### Table. 30 Antibacterial Activity of various Extracts of *Flueggea leucopyrus* (Zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Name of the micro organisms</th>
<th>Control</th>
<th>Chloroform Extract(mg/disc)</th>
<th>Ethanol Extract(mg/disc)</th>
<th>Water Extract (mg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AK&lt;sup&gt;30&lt;/sup&gt;</td>
<td>A&lt;sup&gt;10&lt;/sup&gt;</td>
<td>K&lt;sup&gt;30&lt;/sup&gt;</td>
<td>G&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
<td>-</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td><em>Staphylococcus mutans</em></td>
<td>22</td>
<td>-</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><em>Bacillus Species</em></td>
<td>28</td>
<td>-</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>24</td>
<td>10</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>24</td>
<td>-</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>26</td>
<td>-</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>16</td>
<td>-</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>24</td>
<td>-</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td><em>Lactobacillus species</em></td>
<td>10</td>
<td>10</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

**Note:**

AK<sup>30</sup> – Amikacin;  A<sup>10</sup> – Ampicillin;  K<sup>30</sup> – Kanamycin;  G<sup>10</sup> – Gentamycin;

NX<sup>10</sup> – Norfloxacin;  P<sup>10</sup> – Penicillin-G;  T<sup>30</sup> – Tetracyclin;  Cl<sup>30</sup> – Ciprofloxacin.
### Table 31 Antibacterial Activity of various Extracts of Ventilago Madarasapatana (Zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Name of the micro organisms</th>
<th>Control</th>
<th>Chloroform Extract(mg/disc)</th>
<th>Ethanol Extract(mg/disc)</th>
<th>Water Extract (mg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AK&lt;sup&gt;30&lt;/sup&gt; A&lt;sup&gt;10&lt;/sup&gt; K&lt;sup&gt;30&lt;/sup&gt; G&lt;sup&gt;10&lt;/sup&gt; NX&lt;sup&gt;10&lt;/sup&gt; P&lt;sup&gt;10&lt;/sup&gt; T&lt;sup&gt;30&lt;/sup&gt; Cl&lt;sup&gt;30&lt;/sup&gt;</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20 - 12 14 10 - - 14</td>
<td>- - - - -</td>
<td>7 7 7 7 7</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Staphylococcus mutans</td>
<td>22 - 18 20 14 - 16 20</td>
<td>- - - - -</td>
<td>7 8 8 8 10</td>
<td>- 7 7 15 15</td>
</tr>
<tr>
<td>Bacillus species</td>
<td>28 - 22 10 28 - 20 32</td>
<td>- - - - -</td>
<td>7 7 7 7 8</td>
<td>- 7 7 7 7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>24 10 16 24 20 - 14 22</td>
<td>- - - - -</td>
<td>- - - 7 7</td>
<td>- 7 7 11 11</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>24 - 18 24 26 - 12 32</td>
<td>- - - - -</td>
<td>7 7 7 7 8</td>
<td>- 7 7 7 7</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>26 - 20 24 26 - 14 30</td>
<td>- - - - -</td>
<td>7 7 7 7 8</td>
<td>- 7 7 8 8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>16 - 12 16 26 - - 38</td>
<td>- - - - -</td>
<td>- - - - -</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>24 - 20 22 26 - 20 28</td>
<td>- - - - -</td>
<td>7 7 7 7 7</td>
<td>7 7 8 13 15</td>
</tr>
<tr>
<td>Lactobacillus species</td>
<td>10 10 18 20 20 - 18 24</td>
<td>- - - - -</td>
<td>7 7 7 7 8</td>
<td>7 7 7 7 7</td>
</tr>
</tbody>
</table>

Note: AK<sup>30</sup> – Amikacin; A<sup>10</sup> – Ampicillin; K<sup>30</sup> – Kanamycin; G<sup>10</sup> – Gentamycin; NX<sup>10</sup> – Norfloxacin; P<sup>10</sup> – Penicillin-G; T<sup>30</sup> – Tetracyclin; Cl<sup>30</sup> – Ciprofloxacin.
### Table. 32 Antibacterial Activity of various Extracts of *Stephania wightii* & Tetrahydropalmitine (Zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Name of the microorganisms</th>
<th>Control (mg/disc)</th>
<th>Chloroform Extract (mg/disc)</th>
<th>Ethanol Extract (mg/disc)</th>
<th>Water Extract (mg/disc)</th>
<th>Tetrahydropalminate (mhecg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AK(^{30}) A(^{10}) K(^{30}) G(^{10}) NX(^{10}) P(^{10}) T(^{30}) CF(^{30})</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
<td>0.5 1.0 1.5 2.0 2.5</td>
<td>50 100 200 300</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
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<td>14</td>
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<tr>
<td><em>Staphylococcus mutans</em></td>
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<td>14</td>
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<tr>
<td><em>Bacillus Species</em></td>
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<td>10</td>
<td>9</td>
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<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td><em>Klebsiella pneumonia</em></td>
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<td>32</td>
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<td><em>Proteus vulgaris</em></td>
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<td><em>Pseudomonas aeruginosa</em></td>
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<td>8</td>
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<tr>
<td><em>Salmonella typhi</em></td>
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<tr>
<td><em>Lactobacillus species</em></td>
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<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

*Note:* AK\(^{30}\) – Amikacin; A\(^{10}\) – Ampicillin; K\(^{30}\) – Kanamycin; G\(^{10}\) – Gentamycin; Nx\(^{10}\) – Norfloxacin; P\(^{10}\) – Penicillin-G; T\(^{30}\) – Tetracyclin; CF\(^{30}\) – Ciprofloxacin.
Plate 1: Antibacterial activity of *flacourtia indica*

(A - Chloroform Extract    B - Ethanol extract    C - Aqueous extract)

*Bacillus sp.*

*Pseudomonas aurogenosa*

*Salmonella typhi*

*Escheria coli*
Plate 2: Antibacterial activity of *flacourtia indica*

(A - Chloroform Extract    B - Ethanol extract    C - Aqueous extract)

*Proteus vulgaris*  

*Klebsiella pneumoniae*

*Streptococcus mutans*  

*Staphylococcus aureus*

*Lactobacillus sp.*
Plate 3: Antibacterial activity of *Stephania wightii*
(A - Chloroform Extract  B - Ethanol extract  C - Aqueous extract)

*Bacillus sp.*

*Pseudomonas aurogenosa*

*Salmonella typhi*

*Escheria coli*
Plate 4: Antibacterial activity of *Stephania wightii*

(A - Chloroform Extract   B - Ethanol extract   C - Aqueous extract)

Proteus vulgaris

Klebsiella pneumoniae

Streptococcus mutans

Staphylococcus aureus

Lactobacillus sp.
Plate 5: Antibacterial activity of *fluggea leucopyrus*

(B - Ethanol extract   C - Aqueous extract)

*Bacillus sp.*

*Pseudomonas aurogenosa*

*Salmonella typhi*

*Escheria coli*
Plate 6: Antibacterial activity of *flueggea leucopyrus*

(B - Ethanol extract    C - Aqueous extract)

*Bacteriologically streaked plates*

Proteus vulgaris

Klebsiella pneumoniae

Staphylococcus mutans

Staphylococcus aureus

Lactobacillus sp.
Plate 7: Antibacterial activity of *v.madraspatana* (C - Aqueous Extract)

*Bacillus* sp.

*Pseudomonas aurogenosa*

*Salmonella typhi*

*Escheria coli*

*Proteus vulgaris*

*Klebsiella pneumoniae*

*Streptococcus mutans*

*Streptococcus aureus*

*Lactobacillus sp.*
Plate 8: Antibacterial activity of tetrahydropalmatine

A. Bacillus sp.
B. Pseudomonas aurigenosa
C. Salmonella typhi
D. Escheria coli
E. Proteus vulgaris
F. Klebsiella pneumoniae
G. Streptococcus mutans
H. Control
Plate 9: Antibacterial activity of antibiotics

Bacillus sp.

1. AK\textsuperscript{30} 2. A\textsuperscript{10} 3. K\textsuperscript{30} 4. G\textsuperscript{10} 5. Nx\textsuperscript{10} 6. P\textsuperscript{10} 7. T\textsuperscript{30} 8. Cf\textsuperscript{30}

Pseudomonas aurogenosa

Salmonella typhi

Escheria coli
Plate 10: Antibacterial activity of antibiotics

Proteus vulgaris

Klebsiella pneumoniae

Staphylococcus mutans

Staphylococcus aureus

Lactobacillus sp.

1. AK^{30}
2. A^{10}
3. K^{30}
4. G^{10}
5. Nx^{10}
6. P^{10}
7. T^{30}
8. CF^{30}
References


