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# Antibacterial activity of medicinal plants used in Ayurvedic medicine towards food and water borne pathogens

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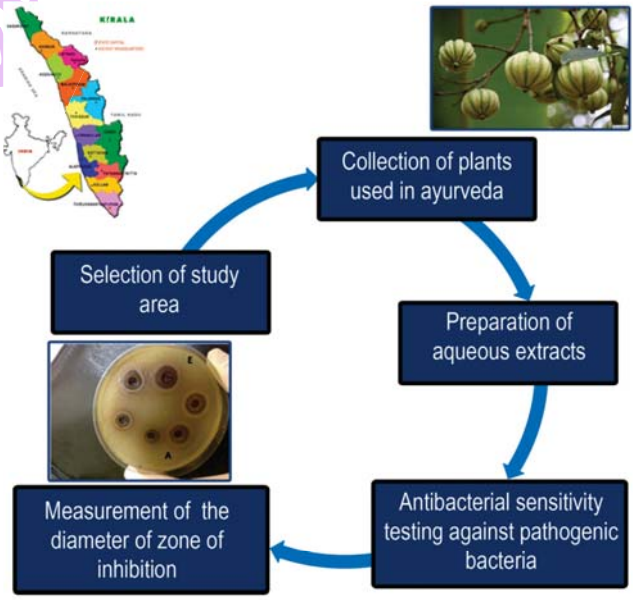
## Abstract

**Aim :** In the emerging scenario of increasing multiple antibiotic resistance among pathogenic bacteria, plant based extracts having antibacterial activity are being explored as a means to check the emergence of drug resistant mutants. In the present study, aqueous extracts from various parts of twenty four different plants used in Ayurvedic medicine were evaluated for their antibacterial activity against water and food borne pathogens such as *Salmonella, Escherichia coli, Vibrio, Aeromonas, Bacillus* and *Staphylococcus*.

**Methodology :** Antibacterial activity of plant extracts from various parts of twenty four different plants was evaluated by agar well method. Antibiotic sensitivity testing was carried out by Kirby – Bauer method. The diameter of inhibition zones was measured in millimetres. Inhibition zone with diameter less than 12 mm were considered as having no antibacterial activity. Diameters between 12 and 16 mm were considered as moderately active, and greater than 16 mm were considered as highly active.

**Results :** Out of 33 extracts prepared from various officinal parts, 23 extracts showed antibacterial activity ranging from narrow spectrum to broad spectrum. *Tamarindus indica, Garcinia gummi-gutta* and *Allium sativum* possessed excellent broad spectrum antibacterial activity. While inhibitory activity against *Salmonella* was widespread among many plants, some of the plant extracts showed specific activity towards *Staphylococcus aureus*.

**Interpretation :** The study revealed that the Gram positive bacteria were more susceptible to crude plant extracts than Gram negative ones. Some of the plant extracts showed superior antibacterial activity when compared to antibiotics. Antibacterial properties of plants analysed might be helpful in discovery of new plant based bactericidal compounds to control drug resistant bacteria.



## Introduction

Ayurveda known as the 'science of life' is a system of traditional medicine native to the Indian subcontinent and a form of alternative medicine which is more than 5000 years old. Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. The core concept lies on the mutual relationship of man and nature. Plants have provided a source of insight for developing novel drug compounds, as they were used traditionally for the welfare of human beings (Maurice *et al.*, 1999). Indigenous plants have served as a major source of drugs for centuries and have been used by our ancestors (Hong-Fang *et al.*, 2009).Though the accepted modern medicine has gradually evolved by the scientific and observational hard work of many scientists, the basis of its development remains rooted in traditional medicine and therapies (Bhushan *et al.,* 2004). Most of the drugs available in the market today have been derived from the natural products.

Repeated and improper use of antibiotics in the treatment of human as well as animal production system has resulted in the assortment of drug resistant mutants. Drug resistance among bacteria is of concern among health practioners as the infection caused by them are difficult to treat. This resistance in bacteria is a serious threat in healthcare now a days and can arise in a significant minority of infected patients and mostly for the one who have other basic health conditions, regular medications and repeated exposure to antibiotics (Hooper *et al.,* 2012). While the innovation of new antibiotics is not keeping rapidity with the emergence of drug resistant mutants, different options are being sought to reduce the selection pressure for drug resistant mutants in the environment. While cautious use of antibiotics is a vital step, other step is screening of plant based alternatives i.e., secondary metabolites that have bactericidal activity. In this view, the antibacterial activity of some medicinal plants used in Ayurvedic medicinal practices were evaluated against some food and water borne pathogenic bacteria.

## Materials and Methods

**Selection of plant materials and preparation of aqueous extracts :** Twenty four plant species belonging to 20 different families were selected for evaluating the antibacterial properties. Based on the therapeutic uses of different parts of the plant, in some cases more than one part of the same plant viz. roots, stem, leaves, flowers and fruits were used. Details of the plant analysed is presented in Table 1. Fresh plants were collected randomly from local areas and were identified with the help of herbarium maintained in the School of Environmental Sciences, Mahatma Gandhi University, Kerala, India.

The plant parts were cleaned and washed in sterile distilled water. In order to obtain plant extracts, about 100g of each washed plant parts were crushed with mortar and pestle by

**Table 1 :** List of plants and the officinal parts evaluated

| Botanical name | Vernacular name (Malayalam) | Family | Officinal parts used |
|---|---|---|---|
| *Nerium oleander* L. | Arali | Apocynaceae | Leaf |
| *Clerodendrum infortunatum* L. | Thettaparamaram | | Leaf |
| *Chromolaena odorata* (L.) King and Robinson | Communist Pacha | Asteraceae | Leaf and stem |
| *Abelmoschus esculentus* L.Moench. | Venda | Malvaceae | Fruit |
| *Morus alba* L. | Mulberry | Moraceae | Leaf |
| *Ficus religiosa* L. | Arrayal | Moraceae | Leaf |
| *Averrhoa bilimbi* L. | Irumpanpuli | Oxalidaceae | Leaf and fruit |
| *Cassia fistula* L. | Kanikkonna | Fabaceae | Leaf |
| *Tamarindus indica* L. | Valanpuli | Fabaceae | Leaf and fruits |
| *Ocimum americanum* L. | Kattuthulasi | Lamiaceae | Leaf |
| *Leucas aspera* (Willd.) link | Thumba | Lamiaceae | Leaf, stem,flowers |
| *Curcuma longa* L. | Manjal | Zingiberaceae | Rhizome |
| *Zingiber officinale* Roscoe | Inchi | Zingiberaceae | Rhizome |
| *Calotropis gigantea* L. | Erukku | Asclepiadaceae | Leaf and flower |
| *Garcinia gummi-gutta* (L.) Roxb. | Kudampuli | Clusiaceae | Leaf and fruits |
| *Piper betle* L. | Vettila | Piperaceae | Leaf |
| *Moringa oleifera* Lam. | Muringa | Moringaceae | Leaf and fruit |
| *Psidium guajava* L. | Pera | Myrtaceae | Leaf and fruit |
| *Aloe vera* L. Burm.F | Kattarvazha | Xanthorrhoeaceae | Leaf |
| *Murraya koenigii* L. | Kariveppu | Rutaceae | Leaf |
| *Azadirachta indica* A. Juss. | Aryaveppu | Meliaceae | Leaf |
| *Scoparia dulcis* L. | Kallurukki | Scrophulariaceae | Leaf |
| *Coriandrum sativum* L. | Malli | Apiaceae | Leaf |
| *Allium sativum* L. | Velluthulli | Lilliaceae | Bulb |

adding sterile distilled water. The extracts were sieved through a fine mesh cloth. After centrifugation at 1500 rpm for 20 minutes, the supernatants were sterilized using a membrane filter (0.45-micron sterile filter). These crude extracts were transferred into sterile test tubes.

**Bacterial strains :** Pathogenic bacteria belonging to both Gram positive and Gram negative bacterial strains were used in the present study to evaluate the broad spectrum activity of the medicinal plants. The Gram positive strains used in the present study were *Staphylococcus aureus* and *Bacillus subtilis*, while Gram negative strains includes eight strains of *Salmonella*, two strains of *Vibrio*, three strains of *Escherichia coli* and *Aeromonas hydrophila*. *A. hydrophila*, *Stapylococcus aureus* and *B. subtilis* cultures were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India. Among *Salmonella*, the strains *S. paratyphi*, *S. mgulani*, *S. bareily*, *S. enteritidis*, *S. senftenberg*, *S. typhi*, *S. bovis, S. weltevreden* and *S. worthington* were included in the present study. While *S. paratyphi, S. mgulani, S. typhi, S. weltevreden* and *S. senftenberg* were isolated from seafood, *S. worthington, S. bareily* and *S. enteritidis* were isolated from chicken. The serotypes of *E.coli* include O25, O86 and O63. The strains of *Vibrio* selected in the present study were *V. cholerae* and V. vulnificus. *E. coli* and *Vibrio* strains were isolated from the Cochin estuary in a previous investigation funded by Department of Science and Technology, Govt. of India.

**Antibacterial sensitivity testing using agar well method (Cup Plate Method) :** Using a sterile cotton swab, the bacterial cultures enriched in sterile nutrient broth for 6-8 hrs at 37ºC were swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Agar wells were prepared with the help of sterilized cork borer with 10mm diameter. Using a micropipette, 100 µl of plant extracts were added to different wells in the plate. The plates were incubated in an upright position at 37ºC for 24 hrs. The diameter of inhibition zones was measured in millimetres. Inhibition zone with diameter less than 12 mm were considered as having no antibacterial activity. Diameters between 12 and 16 mm were considered as moderately active, and greater than 16 mm were considered as highly active (Bauer *et al*.,1966).

**Antibiotic sensitivity testing (Kirby-Bauer Method) :** The test bacterial pathogens were also tested for their sensitivity against antibiotics such as Pencillin, Ampicillin, Chloramphenicol, Ciprofloxacin, Kanamycin, Erythromycin, Lincomycin, Gentamycin, Vancomycin, Amikacin, Nitrofurantoin, Novobiocin, Nalidixic acid, Streptomycin and Tetracycline by the disk diffusion method. The antibiotic discs were aseptically placed over the seeded MHA plates and were incubated at 37ºC for 24 hrs. The diameter of the inhibition zones were measured in millimetres and were compared with the standard Kirby-Bauer chart to group them into resistant and sensitive. Based on this interpretation chart (Bauer *et al.,* 1966), the inhibition zone size were categorised as susceptible (S), intermediate (I) or resistant (R).

## Results and Discussion

In the present study, crude extracts of 24 plants, which were used in Indian medicinal practices, were tested against both Gram negative and Gram positive strains. On the basis of the medicinal aspects of different parts of the plants, 33 extracts were prepared. Fourteen extracts showed excellent antibacterial activity (zone of diameter greater than 16 mm) and nine extracts showed moderate activity. Ten extracts possessed no antibacterial activity which includes stem of *C. odorata*, leaf of *N. oleander, A. vera, F. religiosa, C. gigantea, C. fistula, S. dulcis,* flower of *C. gigantea,* stem of *L. aspera* and rhizome of *Z. officinale*. The results are given in Table 2.

Leaf extracts of *Clerodendrum infortunatum*, *Averrhoa bilimbi*, *Moringa oleifera*, *Murayya koenigii*, *Azadirachta indica* and *Chromolaena ordata* showed good to moderate antibacterial activity against *Salmonella* strains, with diameter of inhibition zone ranging up to 24 mm, depending on the type of plant. Maximum activity against *Salmonella* was shown by curry leave (*M. koenigii*) extract. Curry leaves are an indispensable ingredient in many of the Indian dishes. While we found the activity of the above leaf extracts were specific to Gram negative forms, Modi *et al.* (2010) reported antibacterial activity of ethanolic extracts of the leaves of *C. infortunatum* against *E. coli*, *B. subtilis* and *S. aureus*. Stanley *et al.* (2014) also reported moderate bactericidal activity of solvent based leaf extracts of *C. odorata.* Contradictory to our observations some of the researchers (Ifeanyichukwu *et al.,* 2015) reported broad spectrum activity of the above leaf extracts. Notable among is the widespread antibacterial activity of neem extract against *A. hydrophila* and *V. cholera* (Raut *et al.,* 2014), *S. aureus* (Mistry *et al.,* 2014) and *B. subtilis* (Raut *et al.,* 2014).

Fruit extracts of *A. bilimbi*, *Tamarindus indica* and *Garcinia gummigutta* showed excellent broad spectrum antibacterial activity. These fruit extracts were able control the growth of both gram negative and gram positive bacteria. The fruit extract of *A. bilimbi* controlled the growth of all test pathogens analysed in this study, except *A. hydrophila*, *V. vunificus* and *E. coli*, while that of T. indica showed excellent activity against all the strains except *S. aureus* and *E. coli* O86. The zone of inhibition caused by fruit extract of T. indica against S. senftenberg was 36 mm. Whole fruits of A. bilimbi and T. indica and the pods of *G. gummigutta* are widely used in the preparation of many south Indian dishes. *A. bilimbi* fruits are also used to make pickles. Antibacterial activity of these fruits besides their culinary value makes them an attractive ingredient to various dishes. Another plant which showed remarkable broad spectrum antibacterial activity was *G. gummigutta.* In case of *T. indica* and *G. gummigutta* leaf extracts also showed good broad spectrum

**Table 2 :** Diameters of inhibition zones of plant extracts against different pathogenic bacterial strains by agar well method

| Plants | | Diameter of inhibition zone (millimeters) Bacterial strains used | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Parts used | *SP* | *SM* | *SBa* | *SE* | *SS* | *SB* | *ST* | *SWe* | *SW* | *AH* | *VC* | *VV* | *EC O25* | *EC O86* | *EC O63* | *BS* $G^{+ve}$ | *SA* $G^{+ve}$ |
| *Nerium oleander* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clerodendrum infortunatum* | L | 12 | 18 | 12 | 20 | 20 | 0 | 17 | 12 | 16 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chromolaena odorata* | L | 15 | 15 | 15 | 0 | 0 | 14 | 0 | 12 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chromolaena odorata* | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Abelmoschus esculentus* | Fr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| *Morus alba* | L | 0 | 0 | 15 | 12 | 15 | 0 | 14 | 12 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ficus religiosa* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Averrhoa bilimbi* | L | 0 | 0 | 0 | 21 | 13 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Averrhoa bilimbi* | Fr | 12 | 12 | 18 | 16 | 15 | 16 | 14 | 15 | 16 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 17 |
| *Cassia fistula* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tamarindus indica* | L | 15 | 14 | 16 | 16 | 0 | 18 | 15 | 15 | 14 | 14 | 15 | 16 | 0 | 16 | 17 | 0 | 16 |
| *Tamarindus indica* | Fr | 24 | 22 | 20 | 20 | 22 | 36 | 22 | 22 | 22 | 17 | 18 | 16 | 14 | 15 | 0 | 17 | 0 |
| *Ocimum americanum* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 15 | 0 |
| *Leucas aspera* | L | 0 | 14 | 0 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Leucas aspera* | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Leucas aspera* | Fl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 13 | 0 |
| *Curcuma longa* | Rh | 0 | 15 | 0 | 14 | 15 | 0 | 14 | 0 | 14 | 12 | 0 | 0 | 0 | 0 | 0 | 14 | 14 |
| *Zingiber officinale* | Rh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Calotropis gigantea* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Calotropis gigantea* | Fl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Garcinia gummi-gutta* | L | 12 | 12 | 13 | 12 | 12 | 16 | 17 | 12 | 12 | 0 | 15 | 0 | 0 | 13 | 0 | 12 | 12 |
| *Garcinia gummi-gutta* | Fr | 19 | 21 | 19 | 0 | 19 | 24 | 21 | 21 | 17 | 20 | 26 | 19 | 16 | 20 | 17 | 22 | 20 |
| *Piper betle* | L | 0 | 0 | 12 | 12 | 12 | 16 | 0 | 12 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Moringa oleifera* | L | 16 | 0 | 15 | 16 | 13 | 14 | 12 | 15 | 14 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 25 |
| *Moringa oleifera* | Fr | 0 | 0 | 15 | 20 | 19 | 17 | 14 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| *Psidium guajava* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| *Psidium guajava* | Fr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 23 |
| *Aloe vera* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Murraya koenigii* | L | 0 | 12 | 12 | 24 | 12 | 0 | 12 | 16 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Azadirachta indica* | L | 12 | 12 | 14 | 12 | 12 | 12 | 12 | 13 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Scoparia dulcis* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Coriandrum sativum* | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Allium sativum* | Bulb | 27 | 28 | 31 | 30 | 27 | 26 | 27 | 29 | 29 | 28 | 20 | 17 | 29 | 22 | 26 | 38 | 28 |

SP: *Salmonella paratyphi,* SM: *Salmonella mgulani,* SBa: *Salmonella bareily,* SE: *Salmonella enteritidis,* SS: *Salmonella senftenberg,* SB: *Salmonella bovis,* ST: *Salmonella typhi,* SWe: *Salmonella weltevreden,* SW: *Salmonella worthington,* AH: *Aeromonas hydrophila*, VC: *Vibrio cholerae*, VV: *Vibrio vulnificus*, EC: *Escherichia coli*, SA: *Staphylococcus aureus*, BS: *Bacillus subtilis,* $G^{+ve}$ Gram Positive; L: Leaf, S: Stem, Fl: Flower, Fr: Fruit, Rh: Rhizhome

antibacterial activity. Fruit extract of *G. gummigutta* was able to inhibit the growth of all pathogenic strains analysed in this study except *S. enteritidis*. Antibacterial activity of *A. bilimbi* (Nwaivu *et al.,* 2012), *T. indica* (Trila *et al.,* 2014; Routhu *et al.,* 2015) and *G. gummigutta* (Semwal, 2015) has been reported previously, though with minor variations in activity against different pathogens. This variation could be due to the strain variations among the pathogens or due to variations in the plant species according to geographical locations. Antimicrobial activity of *T. indica* is attributed to the presence of phenolic acids and flavanoids (Trila *et al.,* 2014).

Moderate antibacterial activity has been observed in the leaf extracts of *Leucas aspera*, *Piper betle* and *Ocimum americanum.* While the leaf extract of *O. americanum* showed good activity against *A. hydrophila* and *B. subtilis, L. aspera* leaf extracts were effective in inhibiting *S. mgulani*, *S. bovis* and *B. subtilis*. Flower extracts of *L. aspera* could control growth of *V. cholera* and *B. subtilis*. This variation in the activity of different parts of the plant is interesting and needs further studies to identify the phytochemical responsible for the antibacterial activity. Our observation about the antibacterial activity of flower extracts of *L. aspera* is in tune with the findings of Chew *et al* (2012).

**Table 3 :** Classification based on the antibacterial activity, based on inhibition zone diameter in millimetres

| Plants with officinal parts | | |
|---|---|---|
| **No activity< 12mm** | **Moderate between 12-16mm** | **High> 16mm** |
| *Chromolaena odorata* (S) | *Chromolaena odorata* (L) | *Clerodendrum infortunatum* (L) |
| *Nerium oleander* (L) | *Piper betle*(L) | *Moringa oleifera* (L) |
| *Aloe vera* (L) | *Psidium guajava* (L) | *Moringa oleifera* (Fr) |
| *Ficus religiosa* (L) | *Morus alba* (L) | *Psidium guajava* (Fr) |
| *Calotropis gigantea* (L) | *Azadirachta indica* (L) | *Murraya koenigii* (L) |
| *Calotropis gigantea* (Fl) | *Ocimum americanum* (L) | *Averrhoa bilimbi* (L) |
| *Cassia fistula* (L) | *Leucas aspera*(L) | *Averrhoa bilimbi* (Fr) |
| *Scoparia dulcis* (L) | *Leucas aspera* (Fl) | *Garcinia gummi-gutta* (L) |
| *Leucas aspera* (S) | *Cucurma longa* (Rh) | *Garcinia gummi-gutta* (Fr) |
| *Zingiber officinale* (Rh) | | *Tamarindus indica* (L) |
| | | *Tamarindus indica* (Fr) |
| | | *Abelmoschus esculentus* (Fr) |
| | | *Allium sativum* (B) |
| | | *Coriandrum sativum* (L) |

L- Leaf, S - Stem, Fl- Flower, Fr- Fruit, R- Root, Rh- Rhizome, B - Bulb

**Table 4 :** Diameters of inhibition zones of antibiotics against different pathogenic bacterial strains

| **Antibiotics** | **Zone of inhibition (mm)** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***SP*** | ***SM*** | ***SBa*** | ***SE*** | ***SS*** | ***SB*** | ***ST*** | ***SWe*** | ***SW*** | ***AH*** | ***VC*** | ***VV*** | ***EC O25*** | ***EC O86*** | ***EC O63*** | ***BSS*** $G^{+ve}$ | ***A*** $G^{+ve}$ |
| Pencillin (P) | 12 | 12 | 12 | 14 | 14 | 17 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 |
| Ampicillin (A) | 0 | 12 | 0 | 12 | 13 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 |
| Nalidixic acid (Na) | 0 | 20 | 24 | 26 | 24 | 0 | 24 | 24 | 0 | 20 | 18 | 0 | 22 | 21 | 0 | 0 | 0 |
| Chloramphenicol (C) | 21 | 25 | 28 | 28 | 26 | 20 | 31 | 28 | 20 | 26 | 18 | 0 | 25 | 28 | 28 | 0 | 29 |
| Ciprofloxacin (Cf) | 23 | 26 | 27 | 29 | 28 | 20 | 28 | 27 | 21 | 25 | 30 | 25 | 25 | 27 | 0 | 23 | 31 |
| Kanamycin (K) | 17 | 14 | 15 | 15 | 15 | 0 | 0 | 15 | 14 | 12 | 18 | 17 | 12 | 12 | 0 | 15 | 16 |
| Erythromycin (E) | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 15 | 0 | 20 |
| Lincomycin (L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 22 |
| Gentamycin (G) | 15 | 15 | 16 | 15 | 16 | 0 | 10 | 16 | 16 | 11 | 20 | 17 | 13 | 13 | 20 | 12 | 16 |
| Vancomycin (Va) | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amikacin (Ak) | 16 | 15 | 19 | 17 | 18 | 0 | 16 | 19 | 16 | 12 | 17 | 21 | 14 | 12 | 13 | 18 | 22 |
| Tetracycline (T) | 14 | 14 | 16 | 18 | 18 | 24 | 23 | 16 | 13 | 19 | 19 | 12 | 16 | 16 | 0 | 0 | 25 |
| Streptomycin (S) | 14 | 14 | 16 | 15 | 15 | 0 | 16 | 16 | 13 | 15 | 0 | 0 | 13 | 15 | 13 | 12 | 12 |
| Nitrofurantoin (Nf) | 0 | 15 | 12 | 15 | 15 | 17 | 12 | 12 | 16 | 12 | 0 | 0 | 13 | 0 | 13 | 0 | 16 |
| Novobiocin (Nv) | 0 | 14 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 13 | 18 | 0 | 0 | 0 | 0 | 16 |

SP: *Salmonella paratyphi,* SM: *Salmonella mgulani,* SBa: *Salmonella bareily,* SE: *Salmonella enteritidis,* SS: *Salmonella senftenberg,* SB: *Salmonella bovis,* ST: *Salmonella typhimurium,* SWe: *Salmonella weltevreden,* SW: *Salmonella worthington,* AH: *Aeromonas hydrophila*, VC: *Vibrio cholerae*, VV: *Vibrio vulnificus*, EC: *Escherichia coli*, SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, $G^{+ve}$ : Gram Positive

Antibacterial activity of *P. betle* leaf extract was limited to various serotypes of *Salmonella*. All the Gram positive bacterial strains as well as those belonging to Gram negative strains such as *A. hydrophila*, *Vibrio* and *E. coli* were not inhibited by the leaf extract of this plant. However, solvent based extracts of *P. betle* are reported to inhibit *E. coli, S. aureus* and *V. cholera*. *P. betle* leaves are widely used by Indian public for chewing along with tobacco.

Another plant which showed specific antibacterial activity against Gram positive pathogen *S. aureus* was *M. oleifera*, which corroborate the findings of Peixoto *et al.* (2011). The leaf and fruit extracts of this plant inhibited the growth of S. aureus with zone of inhibition ranging from 25 and 22 mm respectively. However, these extracts failed to inhibit the growth of other Gram positive pathogen *B. subtilis* analysed in this study. The fruit extract of this plant exhibited moderate activity against *Salmonella* serotypes, except *S. paratyphi* and *S. mgulani*. Growth of *E. coli* was not inhibited by the leaf or fruit extracts of *M. oleifera*, which corroborates the findings of previous researchers (Piexoto *et al.,* 2011; Trondel *et al.,* 2014). Growth of S. aureus was also inhibited

by the leaf and fruit extracts of *Psidium guajava*, the guava plant. While leaf extract was specific in its action against *S. aureus* (diameter of inhibition zone 15 mm), fruit extract showed broad range antibacterial activity and inhibited the growth of *S. bovis, E. coli* and *S. aureus*, with diameter of inhibition zone ranging from 22 to 23 mm.

Gram negative pathogen *A. hydrophila* was inhibited by the leaf extract of *Coriandrum sativum*, which is widely used spice in Indian cookery. Similarly the extracts from the bulb of garlic *Allium sativum* showed very good broad spectrum antibacterial activity against the various test pathogens evaluated in the present study. Maximum zone of inhibition of this extract was recorded against *B. subtilis* (38 mm) and minimum was against *V. vulnificus* (17 mm). Indu *et al.* (2006) reported the potential of bulb extract of *A. sativum* in controlling the growth of both gram positive and negative bacterial strains. Antibacterial activity of *C. sativum* is attributed to the presence of essential oils in this leaves.

Based on the diameter of inhibition zones obtained from the agar well method, the activity of plant extracts were classified as no or least (<12 mm), moderate (12-16 mm) and high (> 16 mm). The results are represented in the Table 3. Out of the 33 aqueous extracts prepared from the officinal parts of 24 plants, 10 extracts showed least activity against the test organisms. Fourteen plants were found to have excellent antibacterial property and nine with moderate activity.

The plant extracts which control the growth of both Gram positive and negative strains were categorised to have broad spectrum activity and those which control any one were categorised as narrow spectrum. Of the 23 plant extracts with bactericidal activity, 12 possess and 11 with narrow spectrum activity. Results revealed that Gram positive pathogens included in this study are more susceptible to the crude plant extracts tested when compared to Gram negative ones. Similar observations were reported previously (Janakiraman *et al.,* 2012). Both these groups of researchers also reported that Gram-negative bacteria were more resistant to the action of antibacterial compounds than Gram-positive strains. This is attributed to the presence of hydrophilic outer membrane rich in lipopolysacchride molecules and enzymes associated with periplasmic space which is capable of breaking down the foreign molecules (Shan *et al.,* 2007) In case of Gram-positive bacteria such outer membranes and cell wall structures were lacking (Chowdhury *et al.,* 2004).

Generally it was observed from this study that strains of *Salmonella* were highly susceptible to the action of plant extracts. Strains of *E. coli* were resistant to the bactericidal properties of the extracts of plant officinal parts. The resistance is in the order *E. coli* > *Vibrio* > *B. subtilis* > *A. hydrophila* > *S. aureus*> *Salmonella*. When the activity of officinal parts of the plants was compared, the bulb extract were found to have excellent activity in inhibiting the growth of both Gram positive and negative strains. The potential of the plant's officinal parts is in the sequence of Bulb > Fruit > Flower > Rhizome > Leaf.

The pathogenic strains were also tested against 15 standard antibiotics that were commonly used for treating microbial infections (Table 4) for an authentication with the antibacterial activity of plant extracts. Antibiotics namely nalidixic acid, chloramphenicol and ciprofloxacin showed excellent activity. The zone of inhibition observed from the fruit extract of *Tamarindus indica* and *Garcinia gummi-gutta*, bulb extract of *Allium sativa* clearly implicit their potency to control the growth of pathogenic strains, compared to above tested antibiotics. The diameter of the inhibition zone obtained against garlic extract was comparable to those obtained against chloramphenicol and ciprofloxacin. The other antibiotics namely tetracycline, amickacin, novobiocin, nitrofurantoin, streptomycin, gentamycin, vancomycin, erythromycin, kanamycin and lincomycin showed only moderate antibacterial activity against the test pathogens. The extracts of plants give promising results when compared to the activity of antibiotics.

The use of different plant based natural compounds as antibacterial agents is an interesting strategy to combat emerging multidrug resistant (MDR) pathogens. The increasing consumer demand for effective, safe, natural products, calls for the research interest in the study of phytochemicals. Ingenious screening programs are required to discover the plant based antimicrobials with diverse chemical structures and mechanisms of action. Screening strategies adopted in this study provides good route to select particular plants, that in the near future could provide useful therapeutic tools in controlling the antibiotic resistant bacteria.

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