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Original Article

IN VITRO ASSESSMENT OF NATURAL HERBAL EXTRACTS FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objective: Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents against infectious pathogens. Objective: The study aimed to investigate *in vitro* antibacterial activity of extracts from some medicinal plants against the most common microbial pathogens including MDR bacteria.

Methods: The processing of plant materials was performed with the washing, drying and grinding of collected plant materials. The plant extracts were prepared by mixing 10 g of powder to 150 ml of ethanol solvent for 5 h at room temperature and sonicated for 15 min; for prepared test samples under laboratory conditions, the air-dried samples were mixed with the respective solvent (1:15 w/v) for 72 h at room temperature with occasional and then filtered through Whattman filter paper No.1. The obtained extract was freed from the solvent by evaporation under reduced pressure and then resuspended in the appropriate solvent to make the solution of known concentration of 10-50 mg/ml. The extract was stored at 4 °C in airtight glass bottle for the antibacterial assay using the Agar-well diffusion method. Ciprofloxacin was used as a control antibiotic.

Results: The growth of *K. pneumoniae, Proteus mirabilis, P. aeruginosa* and *E. coli* were inhibited better with the plant extract *Tinospora cardifolia* leaves than ciprofloxacin antibiotic. Coagulase-negative Staphylococci was inhibited greatly with *Costus igneus* leaf extract. The growth of *Enterococcus faecalic* was inhibited significantly with Tridax procumbens leaf extract than ciprofloxacin**.**

Conclusion: The present study indicates Tridax procumbens, T. cordifolia and Costus igneus methanolic leaf extracts were showed strong antimicrobial activity against all the tested cultures. They were rich in primary and secondary constituents. Most of the biologically active phytochemicals were present in methanolic extract. The tested plant extracts were more efficient than standard antibiotic ciprofloxacin used in the current study.

Keywords: Antimicrobial activity, Plant extracts, Methanol, Microorganisms

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INTRODUCTION

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents. Several works have been done to examine the antimicrobial effects of herbal plant extracts, including roots, stem, leaves, or flowers. Many countries like India and other parts of the world have continued to encourage screening programs of plants used in traditional medicine in order to authenticate their antimicrobial activities and possible inclusion in primary health care [1]. In some rural areas, resorting to natural remedies with "miraculous" plants is preferred to modern medicine. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [2, 3]. Since most of the antibiotics used are associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents [3, 4]. It was found that most plant extracts studied had antimicrobial and antifungal activities. Therefore, there is a need to study on the antibacterial properties of natural herbs inorder to use them as alternative medicine in the treatment of infectious diseases. According to pharmaceutical studies, approximately 10 to 20% of plants are used in a positive way in a health care to treat harmful diseases such as cancer. The classical example is reported on the bark of yew tree, which mainly contains Taxol and is used in ovarian cancer, breast cancer and also used to treat some infectious diseases [5]. The development of new antimicrobial agents' against resistant pathogens is increasing interest. The emergency and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy. MDR bacterial infections often lead to increased mortality, longer length of stays in hospitals, and higher cost of treatment and care [6, 7]. Considering the vast potentially of plants as sources for antimicrobial drugs, this study aimed to investigate *in vitro* antibacterial activity of extracts from some medicinal plants against the most common microbial pathogens, including MDR bacteria [8-10]. An attempt was made in the current study to assess the antimicrobial properties of certain selected herbs such as Tinospora cordifolia (Tippa tegga), Acacia deal roots (Shabdkosh roots), Hemidesmus indicus (Anantamul roots), Cichorium intybus (chicory roots), Tridax procumbes (Gaddi chemanthi leaves), Mimusops elengi (Fruit), Clitoria ternatea (Sankam poolu), Sage (Menthi seeds), Piper betel (Thamalapaku), Moringa oleifera (Drum stick leaves), Broccoli seeds, Costus iqneus (Cengalva kostus), Nepata cataria, Withania somnifera (Ashwagandha) and Ocimum tenuiflorum (Tulasi) against different bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Shigella* species and *Staphylococcus aureus*. Medicinal plants are finding their way into pharmaceuticals, cosmetics along with nutraceuticals.

MATERIALS AND METHODS

Collection and identification of plant samples

The collection of medicinal and other herbal plants is very important prior to their study for various purposes. Plants for the study were selected and collected randomly, based on best cited literature or on the basis of certain specific traditional ethno medicinal uses and are most likely to contain bioactive compounds of medicinal interest. The processing of plant material involves the washing, drying and grinding of collected plant materials. The collected plant materials were washed with tap water and gently brushed to remove soil and other debris. Shade air dry was used for the drying of plant

materials. Plant samples were sliced into small pieces and distributed evenly to facilitate homogenous drying. Shade drying was important aspect to leave plant samples in shaded area or in a room with ambient temperature and adequate ventilation. The process of shade drying is used because it retains the secondary metabolites and prevents the loss of bioactivities of plant material. The dried plant material was stored in sealed containers in a dry and cool area. Storage for expanded periods was avoided in the current study, because some constituents may decompose during storage.

Method of extraction

Successful evaluation of bioactivity of extracts mainly relies on the kind of solvent used in extraction method. For the extraction of dried powdered plant material an aqueous and inorganic solvents like methanol or ethanol were used at 1:2 ratio of dry material to solvent should be used in each case. In our laboratory we use grinded and finely powdered plant material for the extraction. The powdered plant material can be put in flask with a choice of solvent and leave for shaken vigorously for 24h or more on room temperature. The flasks were covered tightly for minimum loss of solvents as some organic solvents are volatile in nature. After extended period of extraction, the residual plant material were separated from the solvent. This process involves a rough clarification by decanting, and followed by filtration. The centrifugation was performed for the powdered plant material those were too fine to be filtered. The separated extract was used to study for antimicrobial activity.

Some of the plant extracts used in the current study were commercially available herbal extract powders. Antibacterial extracts were prepared by mixing 10 g of powder to 150 ml of ethanol solvent for 5 h at room temperature and sonicated for 15 min, for prepared test samples under laboratory conditions the air dried samples were mixed with the respective solvent (1:15 w/v) for 72 h at room temperature with occasional and then filtered through Whattman filter paper No.1. The obtained extract was freed from solvent by evaporation under reduced pressure and then resuspended in the appropriate solvent to make the solution of known concentration of 10-50 mg/ml. The extract was stored at 4 °C in airtight glass bottle for the antibacterial assay.

Microrganisms

The microrgansism *S. aureus, K. pneumoniae, E. coli, Proteus mirabilis, Pseudomonas aeruginosa,* Coagulase negative Staphylococci (CONS), *Enterococcus faecalis, Streptococcus mutans, E. coli ATCC* 25922, *S. aureus* 25927 were collected from the Department of Microbiology and were used for the study.

Evaluation of antimicrobial activity of plant extracts

Most common method used for the evaluation of antimicrobial activity of plant extracts are agar well diffusion, disk diffusion, agar dilution and or broth dilution/micro dilution. These methods for determining antimicrobial activities of plants are based on those described for standardized testing of antibiotics. The suitability of these methods can be affected by several factors such as type of microbial organism, concentration of inoculum, types of media and nature of the extract being tested. These methods can be used to simply determine whether or not antimicrobial activity is present in test plant extracts.

In the present study, sufficient amounts of dried crude plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to obtain stock solutions of 5 mg/ml concentration. For purified compounds, the concentration is normally 1 mg/ml. Ciprofloxain or any other broad-spectrum antibiotic can be used as a positive control and DMSO as negative control. The method of disk diffusion involves the preparation of a petri dish containing 15 ml agar, bacteria of fixed volume were then swabbed across the agar surface. Agar well diffusion method was done with an aliquot of 100 µl of each bacterial isolate (10^6 CFU/ml) on Muller-Hinton agar plates and a volume of 50 µl of extracts was added to each well of 6 mm. The plates were incubated at 37 °C for 24 h. After 24h zones were measured. After proper incubation, the "cleared" zone (zone of inhibition) surrounding the disk is measured and compared with zones for standard antibiotics or literature values of isolated chemicals or similar extracts.

RESULTS

From the results it was found that *S. aureus* was highly sensitive to the plant extract *Tridax procumbens* and when the antimicrobial activity was compared with the Standard antibiotic ciprofloxacin, ciprofloxacin was less effective than Tridax (table 1). The growth of *K. pneumoniae, Proteus mirabilis, P. aeruginosa* and *E. coli* were inhibited better with the plant extract *Tinospora cardifolia* leaves than ciprofloxacin antibiotic (table 2). Coagulase negative Staphylococci was inhibited greatly with *Costus igneus* leaf extract (table 2). The growth of *Enterococcus faecalic* was inhibited significantly with Tridax procumbens leaf extract (table 1) than ciprofloxacin. *Costus igneus* herbal extract was highly effective against *Streptococcus mutants* comparatively than other organisms. *E. coli* ATCC 25922 was inhibited better with *Tinospora cardifolia* and on other hand the other standard culture *S. aureus* 25927 growth was inhibited better with *Costus igneus* (table 1 and table 2). From the above results it was found that, *Tridax procumbens, Tinospora cardifolia* and *Costus igneus* were highly active against tested pathogens comparatively than other plant extracts (fig. 1).

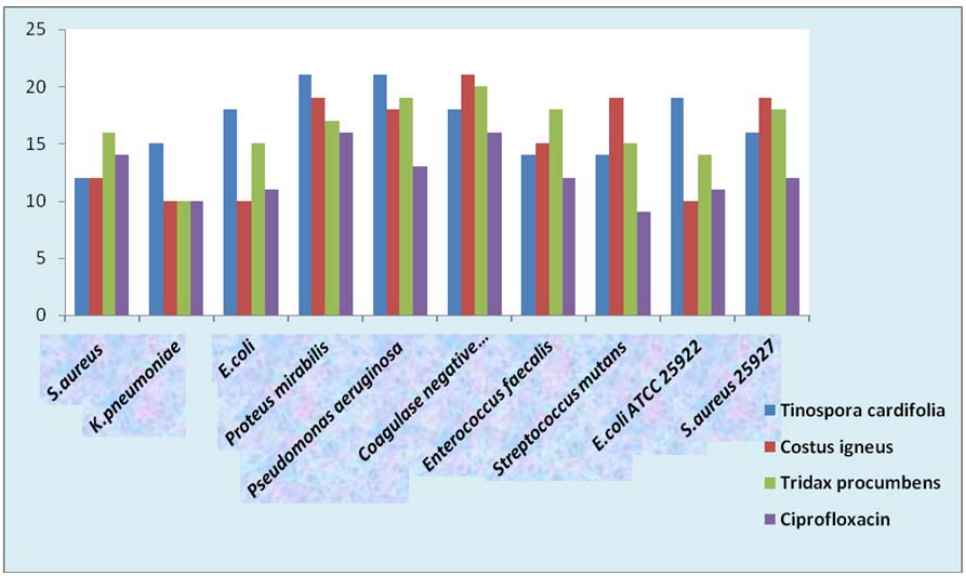

Fig. 1: Antimicrobial activity of potent plant extracts

Table 1: Antimicrobial activity of microbial isolates to various herbal extracts

Test organisms	Zone of inhibition in (mm) with herbal extracts							
	Nepata cataria leaves	Broccoli seeds	Moringa oleifera leaves	Clitoria teenatea flowers	Sage seeds	Mimusops elengi fruits	Tridax procumbens leaves	Standard antibiotic ciprofloxacin
S. aureus	6	5	8	4	6	8	16	14
K. pneumoniae	5	4	7	9	3	7	10	10
E. coli	8	5	9	9	7	10	15	11
Proteus mirabilis	8	8	11	12	10	12	17	16
Pseudomonas aeruginosa	11	5	9	9	6	14	19	13
Coagulase negative staphylococci (CONS)	10	11	6	9	11	19	20	16
Enterococcus faecalis	8	7	10	7	9	11	18	12
Streptococcus mutans	11	9	9	8	7	13	15	09
E. coli ATCC 25922	11	8	10	9	9	11	14	11
S. aureus 25927	9	7	9	5	6	9	18	12

Table 2: Antimicrobial activity

Test organisms	Zone of inhibition in (mm) with herbal extracts							
	Tinospora cardifolia leaves	Ocimum tenniflorum leaves	Piper beetle leaves	Cichorium intybus	Hemidesmus indicus	Acacia delbata leaves	Costus igneus leaves	Withania somnifera leaves
S. aureus	12	13	11	10	6	7	12	14
K. pneumoniae	15	11	9	9	7	8	10	13
E. coli	18	9	6	6	8	7	10	16
Proteus mirabilis	21	13	9	8	12	10	19	19
Pseudomonas aeruginosa	21	12	9	10	11	13	18	14
Coagulase-negative staphylococci (CONS)	18	9	7	9	10	11	21	18
Enterococcus faecalis	14	15	13	12	9	10	15	17
Streptococcus mutans	14	10	9	11	9	12	19	15
E. coli ATCC 25922	19	11	8	9	10	9	10	10
S. aureus 25927	16	14	13	11	10	14	19	15

DISCUSSION

Plants and their derived extracts have been used for many hundreds of years in pharmaceuticals as the alternative medicines and natural therapies. Plant extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens. The emergence of microbial resistance to many presently available antibiotics has resulted in morbidity and mortality from treatment failure and increased health care cost. There is a dire need to find for new, safe, and effective bioactive agents that can fight the problem of multidrug resistance. In the present work, we showed the good efficacy of *T. cordfolia* extract against *E. coli, Proteus, Pseudomonas* and CONS. Similar findings were observed by Getanjali *et al.* (2017) [11]. Although many pharmacologically active secondary metabolites have been discovered in many plants so for, yet the nature must have many more. Nepata cataria has been a representative species of genus Nepeta, which belongs to family Lamiaceae. The plant has been known for its wide range of traditional usages and used to relieve pain, and for the cure of different gastrointestinal and respiratory ailments, female disorders, pneumonia, rheumatism, etc. The chemical diversity of N. cataria has mainly been represented by terpenoids, flavonoids, polyphenols, and steroids; out of these iridoid compounds and terepenoids nepetalactone and its derivatives have been the representative chemical constituents of this plant and genus Nepata. In our study, we have investigated the susceptibility of clinical isolates of the most common pathogens to Nepata cataria in comparison to laboratory reference strains. The good antibacterial activity was achieved with *Pseudomonas aeruginosa,* CONS and Streptococci species and similar findings were reported with [12]. Broccoli has beneficial properties (e. g. gastroprotective, antimicrobial, antioxidant, anticancer, hepatoprotective, anti-inflammatory and immunomodulatory) that contribute to human health. In previous studies, it has been reported that broccoli crude extracts have activity against clinically significant bacteria (Hu *et al.* 2004; Owis 2015) [13, 14]. In the current study, we found that it showed highest activity against CONS. The research on M. oleifera is yet to gain importance in India. It is essential that the nutrients of this wonder tress are exploited for a variety of purpose anti-diabetic, anti-cancer and antimicrobial properties. Saroj *et al.* (1995) studied the ethnolic extract of the leaves of Moringa oleifera for antimicrobial activities against Gram Positive–Bacillus cereus, Bacillus subtilis Staphylococcus aureus, Sarcina lutea: Gram negative–Escherichia coli and Acid fast Mycobacterium phlei and found significant antimicrobial activity of the extract was found in his study. In the present study, similar activity was found with Gram negative bacilli. Syed *et al.* (2020) revealed that Tridax procumbens showed best antimicrobial activity against *E. coli, S. aureus, Enterobacter* and Bacillus species [15] but in our study, Tridax showed best activity against CONS, *P. aeruginosa* and Enterococci species. It was revealed that methanolic extract of Costus igneus was found to contain the highest number of phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids [16]. The leaves are highly active against G*ram-positive Bacillus cerus*, *Bacillus megaterium*, *Micrococcus leuteus*, *Staphylococcus aureus*, *Streptococcus lactis*, and Gram-negative strains *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* [17-19].

CONCLUSION

The present study indicates Tridax procumbens, T. cordifolia and Costus igneus methanolic leaf extracts were showed strong antimicrobial activity against all the tested cultures. They were rich in primary and secondary constituents. Most of the biologically active phytochemicals were present in methanolic extract. The tested plant extracts were more efficient than standard antibiotic ciprofloxacin used in the current study.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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