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Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria-a Comparative Analysis

[1]H.V. Girish and [2]S. Satish

[1]Department of Studies in Botany, Plant Biotechnology Laboratory,
University of Mysore, Manasagangotri, Mysore- 570 006, Karnataka, India
[2]Department of Studies in Microbiology, Herbal Drug Technology Laboratory,
University of Mysore, Manasagangotri, Mysore- 570 006, Karnataka, India

Abstract: The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine. Based on ethno pharmacological and taxonomic information, antibacterial activities of aqueous and methanol extracts of some medicinal plants were determined by *in vitro* by agar diffusion-method against some human pathogenic bacteria. The leaves of five different plants, belonging to the different family and which have some ethnomedicinal applications were studied for antibacterial activity. Powdered leaf materials of all selected plants were extracted with aqueous and methanol. The solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residue was dissolved in methanol (1:10 w/v) and tested for antibacterial activity. The antibacterial screening of aqueous and methanol extract carried out *in vitro* on the following bacteria viz., *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis* and *Yersinia enterocolitica*. It has been showed that the methanol extracts had wider range of activity on these organisms than the aqueous extracts, which indicates that the methanol extracts of all selected plants may contain the active components. This study supports, the traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases.

Key words: Antibacterial · Medicinal plants · Human pathogens

INTRODUCTION

Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential [1]. Human infections particularly those involving microorganisms i.e. bacteria, fungi, viruses, they cause serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases [2, 3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [4].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [5, 6]. Different extracts from traditional medicinal plants have been tested. Many reports have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles [7]. The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, neutralceuticals, cosmetics and food supplements. In this regard, plants have given western pharmacopoeia about

Corresponding Author: Dr. S. Satish, Department of Studies in Microbiology, Herbal Drug Technology Laboratory, University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India

7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, e.g. quinine, artemisinin, taxol, camptothecin, etc. [8]. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens [9, 10].

Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens [11]. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potentiality of plant extracts on standard microorganism strains as well as on the multi-drug resistant bacteria.

MATERIALS AND METHODS

Selection of Medicinal Plants for the Study: Five medicinal plants viz. *Boerhaavia diffusa, Cassia auriculata, Cassia Lantana, Eclipta alba* and *Tinospora cardiofolia* were selected based on ethanomedical importance. Healthy, disease free leaves of selected plants were collected in around Mysore, Mysore District, Karnataka (India) were used for the preparation of aqueous and solvent extracts.

Test Microorganisms: Authentic pure cultures of human pathogenic bacteria like *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella* sp., *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella* sp., *Staphylococcus aureus* and *Yersinia enterocolitica,* were obtained from Department Microbiology, Mysore Medical College and Research Institute, Rajiv Gandhi University, Mysore, India.

PREPARATION OF EXTRACTS

Grinding of the Selected Plant Materials: After drying at 37°C for 24 h the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components.

Preparation of Leaf Aqueous Extract: Fifty grams of selected fresh leaf materials was macerated with 50 ml of sterile distilled water in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) for about 10-15 min. The macerate was first filtered through double layer muslin cloth then centrifuged at 3500 rpm for 30 min. The supernatant was filtered through. Whatman No. 1 filter paper and sterilized at 120°C for 30 minutes. The extracts were preserved aseptically at 5°C for further use [12].

Preparation of Leaf Solvent Extract: Thoroughly washed selected plant leaf materials were dried in shade and then powdered with the help of grinding machine. 50 grams of shade dried powder was filled in the thimble and extracted with 150 ml of methanol successively up to 48 h. The solvent extracts were concentrated separately under reduced pressure [13]. Extract from this method was then weighed and stored at 22°C in desiccators until further use. After complete solvent evaporation, one gram of each concentrated solvent extracts were dissolved in 9 ml of methanol and used for antibacterial assays.

ANTIBACTERIAL ACTIVITY ASSAY

Antibacterial activity of aqueous and solvent extracts of all the selected plant extracts was determined by the cup diffusion method on nutrient agar medium [14]. Both the aqueous and solvent extracts of plants were screened for the antibacterial assay.

Aqueous Extract: The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24 h at 37°C, a loop of inoculum was transferred into 5 ml of nutrient broth and incubated for 2 h at 37°C which served as fresh suspension inoculum. Five wells (5 mm diameter) were made in sterile nutrient agar plate using cork borer (one in the center and four wells at the corner) and inoculum containing 10^6 CFU/ml of test bacteria were spread on solid plates with the help of sterile swab moistened with the bacterial suspension. Then 50 µl of aqueous extract of all the leaves were placed in the wells made in inoculated plates. The treatment also includes 50 µl of sterilized distilled water as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment six replicates were maintained.

Solvent Extract: One gram of all the selected plant leaf extract were dissolved in 9ml of methanol. The sterile nutrient agar medium in Petridishes was uniformly smeared with test culture. Well (5 mm) were made in each petridish to which 50 µl of solvent extracts dissolved in methanol were added. For each treatment six replicates were maintained. Methanol served as control.

RESULTS

The antibacterial activity of aqueous and methanol extracts of selected plants against human pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in Table 1.

Activity was analyzed at 50 µl of aqueous extract. Only three plant species viz., *Boerhaavia diffusa, Tinospora cardiofolia* and *Eclipta alba* showed antibacterial activity. *Cassia auriculata and Cassia Lantana* did not show much significant activity. The results revealed that among ten pathogenic bacteria, only *Staphylococcus aureus* was susceptible for *Boerhaavia diffusa* followed by *Bacillus megaterium* and *Streptococus faecalis* by *Tinospora cardiofolia* and *Eclipta alba* respectively. Among the tested pathogens only *Staphylococcus aureus* belongs to Gram-positive bacteria showed maximum inhibition. However, at the test concentration of the plant extracts produced wider zones of inhibition for *S. aureus, Bacillus megaterium, Streptococus faecalis* and *B. substilis* when compared to the zones of inhibition of the gram-negative isolates.

Table 1 indicates that the selected plant extracts inhibited the growth of all the tested microorganisms with the exception. The extracts showed significant antibacterial activity against *Staphylococcus aureus, Bacillus megaterium* and *Streptococus faecalis*. In *Boerhaavia diffusa* maximum inhibition was observed in *Staphylococcus aureus* followed by *Bacillus megaterium* and *Bacillus cereus* respectively at 50 µl concentration. It also observed that still it exerts some degree of sensitivity on Gram-negative bacteria also.

In case of *Tinospora cardiofolia* and *Eclipta alba* similar results were obtained. Both the plant extracts were highly sensitive for Gram-positive bacteria. Even though they showed maximum inhibition for Gram-positive bacteria, the extracts still exerts significant inhibition against Gram-negative bacteria like *Escherichia coli, Pseudomonas aeruginosa* and *Yersinia enterocolitica* respectively.

The results has been showed that, *Salmonella typhi and Pseudomonas aeruginosa* is less sensitive for all tested plant extracts, where as other Gram-negative bacteria showed some degree of susceptible for extract of all selected plants. However, *Cassia auriculata* and *Cassia lantana* showed lesser zone of inhibition in all tested pathogenic bacteria, when compared to the zones of inhibition of other plant extracts. The data revealed that significant inhibitory activity was observed in three important selected plants of methanol extracts.

DISCUSSION

Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [15, 16].

On the basis of the result obtained in this present investigation, we conclude that the methanol extract of *Boerhaavia diffusa, Tinospora cardiofolia* and *Eclipta alba* leaves had significant *in vitro* antimicrobial activity.

This implied that the gram-positive bacteria were more susceptible to the extract than the gram-negative bacteria. Possibly because of the presence of outer

Table 1: Antibacterial activity of aqueous and methanol extracts of selected plant leaves and antibiotic against some human pathogenic bacteria at 50 µl. (Zone of inhibition measured in mm)

	Zone of inhibition (mm)										
Human pathogenic bacteria	*Boerhaavia diffusa*		*Cassia auriculata*		*Cassia lantana*		*Eclipta alba*		*Tinospora cardiofolia*		
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Kanamycin (30 µl)
Bacillus subtilis	8.83±0.30	18.03±0.30	00.00±0.00	7.83±0.22	6.50±0.22	11.16±0.30	7.83±0.22	19.33±0.30	9.16±0.60	19.83±0.30	41.67±0.27
Bacillus cereus	9.73±0.32	19.20±0.60	00.00±0.00	7.00±0.00	00.00±0.00	9.83±0.30	8.16±0.60	17.23±0.60	8.83±0.30	18.86±0.60	33.50±0.27
Bacillus megaterium	00.00±0.00	20.50±0.25	00.00±0.00	9.00±0.00	00.00±0.00	10.00±0.00	9.00±0.00	19.23±0.30	12.50±0.22	22.16±0.30	30.33±0.27
Escherichia coli	8.00±0.00	13.13±0.30	6.00±0.00	8.50±0.22	7.33±0.30	7.00±0.00	10.50±0.22	17.45±0.22	7.16±0.42	21.76±0.30	39.27±0.27
Klebsiella pneumoniae	10.00±0.00	10.16±0.60	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	9.00±0.00	16.16±0.60	8.00±0.00	13.50± 0.22	31.33±0.27
Pseudomonas aeruginosa	9.00±0.00	11.23±0.46	00.00±0.00	11.33±0.30	8.00±0.00	9.53±0.30	10.83± 0.30	14.00±0.00	7.00±0.00	15.67±0.47	30.50±0.25
Salmonella typhi	8.50±0.22	12.63±0.60	00.00±0.00	10.83±0.30	00.00±0.00	7.93±0.22	10.00±0.00	16.00±0.00	7.16±0.42	10.00±0.00	33.00±0.00
Staphylococcus aureus	13.83±0.30	23.13± 0.30	9.46± 0.60	12.13± 0.30	7.93±0.22	18.83± 0.60	11.16± 0.30	20.83± 0.60	10.16± 0.30	20.20± 0.60	25.50± 0.47
Streptococcus faecalis	8.83± 0.30	13.50± 0.25	00.00±0.00	7.16± 0.42	00.00±0.00	9.22± 0.42	11.83± 0.60	18.00±0.00	11.00± 0.00	22.50± 0.40	35.50± 0.00
Yersinia enterocolitica	9.16± 0.30	10.75± 0.30	00.00±0.00	6.50± 0.22	00.00±0.00	8.50± 0.22	9.16± 0.30	15.25± 0.40	10.25± 0.30	16.50± 0.22	25.50± 0.27

Values are the mean of three replicates ± standard error. P<0.05

membrane that serves as an effective barrier in gram-negative species [17, 18]. In addition, since the zone of inhibition is almost equal to the standard, it shows that the test organisms are sensitive to the plant extract. *S. aureus* was the most susceptible bacterium, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of *Boerhaavia diffusa*. In contrast, *Salmonella typhi* and *P. aeruginosa* showed the least susceptibility to the extract. This may be due to the fact that *P. aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs).

The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium [3, 19]. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators [20-23]. The present study justifies the claimed uses of *Boerhaavia diffusa, Tinospora cardiofolia* and *Eclipta alba* in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable plant in large-scale to increase the economic status of cultivars in the country.

The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further chemical and pharmacological investigations to isolate and identify minor chemical constituents in *Boerhaavia diffusa, Tinospora cardiofolia* and *Eclipta alba* and to screen other potential bioactivities may be recommended.

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