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Antimicrobial Activity of some Important Edible Leaf Extracts

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ABSTRACT

The plant derived antimicrobial compounds have received considerable attention in recent years due to the emergence of multidrug resistant bacteria, high cost and serious side effects of antibiotics. In the present study, acetone extract of four plants was screened for antibacterial activity against two standard bacterial pathogens, *viz. Escherichia coli* and *Staphylococcus aureus* by agar cup method and disc method. The leaves of the following plants were used in this study *viz.* Moringa (*Moringa oleifera*), Betel (*Piper betle*), Jack (*Artocarpus heterophyllus*) and Basumathi leaf (*Pandanus* sp). Of the four leaves tested, the leaf extracts of betel exhibited activity against the gram negative bacteria, *Escherichia coli.* Betel showed inhibition zone (15.0 mm) in disc method which is on par (17.0 mm) with that of Streptomycin (1000 ppm). However, it had a less inhibitory effect on the Gram positive bacteria, *Staphylococcus aureus.* Against *S. aureus*, moringa is exhibiting inhibition of 16.0 mm followed by jack leaf (9.5 mm). Leaves of moringa had shown inhibition zone on par (16.0 mm) with that of Streptomycin (1000 ppm). No significant antibacterial activity was detected in the extract of basumathi leaf against *S. aureus*, but for *E.coli* it was on par with Moringa leaf extract. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated.

Key words: *Moringa*, betel, jack, basumathi leaf, antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*

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INTRODUCTION

The emergence and spread of antimicrobial resistance is a growing problem in both developing and developed countries and threatens to become a global crisis. Multiple microbial resistances among Gram-negative microorganisms have been a long term and well recognized problem with enteric infections[1]. A strategy for the containment of resistance needs to be developed, applied and evaluated. Such a strategy should focus on improving rational use of antimicrobials and reducing opportunities for spread of resistant organisms[2]. One of the important approaches to solve this problem has lead to the screening of several medicinal plants for their potential antimicrobial activity[3].

The use of different parts of several plants or their extract in the treatment of various diseases is an age old practice. Hence, plant derived antimicrobial compounds have received considerable attention in recent years due to the emergence of multidrug resistant bacteria, high cost and serious side effects of antibiotics. More than a hundred species of therapeutically important higher plants listed and described in the ancient Indian treatise have antimicrobial property. A variety of compounds are accumulated in plants accounting for their constitutive antimicrobial activities[4]. Efforts are thus directed to identify plant or their plant products which have broad spectrum antimicrobial property and no ill effect[5,6]. It is expected that screening and scientific evaluation of plant extracts may provide novel compounds of therapeutic value[7].

The extracts from plants contain not only minerals and primary metabolites but also a diverse variety of secondary metabolites. The search for biologically active compounds has been vigorous in recent years due to the growing cases of microbial resistance to the time honored antibiotics[8]. In recent years numbers of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants[9].

Antibacterial properties of various plants parts like leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades[10]. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial activities[11].

Tambekar[12] had reported antibacterial activity of extract of leaves of various edible plants on *Salmonella typhi.* Tambekar[13] studied the extract of 22 plant leaves for their antimicrobial properties and concluded that leaf extracts of various plants such as tulsi, pudina and betle showed antimicrobial activity of *E. coli, S. aureus, S. typhi, E. feacalis* and *V. choleraei.* In a study conducted by

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poonkothai[14] the leaf extracts of *Galinsoga ciliata* displayed higher activity against the Gram positive rather than the Gram negative bacteria. Deshpande[15] reported appreciable antibacterial activity against both Gram positive and Gram negative bacteria in the acetone and methanol extracts of gooseberry and jamun leaves.

In India medicinal plants have an important therapeutic and antimicrobial aid in various ailments. Today, there is widespread interest in drugs derived from plants, which leads to the screening of several medicinal plants for their potential antimicrobial activity[16].

In Ayurveda, it is said the leaves of the Moringa tree prevent 300 diseases. The leaves have been used for preparing a variety of dishes especially in South India. In India, betel leaves (*Piper betle*) have been widely used for chewing and in the preparation of Beeda. The leaves of betel, possessing a sharp burning taste contains a phenol, chavicol that has antiseptic properties. The leaves of jack (*Artocarpus heterophyllus*) are useful in fever, boils, wounds, skin diseases and is used for cooking delicacies like idlis[17].

Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds[18]. Further, scientific investigation and information of the therapeutic potential of the plant material is limited.

However the antimicrobial activity of these plants have not been thoroughly studied. Therefore, the present study has been undertaken to evaluate the antibacterial efficacy of leaf extracts of edible plants against pathogenic bacteria.

MATERIALS AND METHODS

The study was conducted in the department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore-65, for about six months during 2011. The leaves of the following plants were used in this study viz. *Moringa oleifera*, *Piper betle*, *Artocarpus heterophyllus* and *Pandanus*. The leaves were collected and washed thoroughly. They were surface sterilized with 70% alcohol to remove surface contaminats and washed again with the series of sterile water. The leaves were extracted with acetone by solvent extraction method. Five grams of leaf sample was macerated into a fine paste in 30 mL acetone using sterilized pestle and mortar. The extract was kept on shaker for 24 h. Then it was allowed to stand for a five hours. The supernatant was filtered using Whatman's filter paper and the filtrate was concentrated to 5 mL by evaporating it at 60°C.

Organisms tested: The pathogenic bacterial cultures *Escherichia coli* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Agricultural Sciences, GKVK, Bangalore.

Testing the antibacterial activity: The antibacterial activity of the leaf extracts was tested using two methods viz., disc diffusion method[19] and well diffusion assay method.

Bacterial inocula was prepared by inoculating a loop full of test organisms in 100 mL nutrient broth and incubated at 37°C for 24 h to obtain a cell density of 1.5×10^8. Seeding the agar media was done by adding the culture into nutrient agar media @ 2.5 mL per 100 mL media. The nutrient agar medium was melted and cooled to 45°C and the broth culture was added. It was mixed thoroughly and the seeded agar was poured in to sterilized petriplates and allowed to solidify.

Sterilized 7 mm filter paper discs were soaked in the extract, allowed to dry and then placed on the surface of seeded agar plate using sterile forceps. Similarly for the well diffusion assay method, wells were prepared using cork borer in the solidified agar and 0.1 mL leaf extract was poured into the wells. Then the plates were incubated in upright position at 37°C for 24 h and the results were noted in terms of the diameter of the zone of inhibition. 1000 ppm Streptomycin was used as positive control. Experiments were performed in triplicates and average diameters of inhibition zone were recorded.

The data were subjected to statistical analysis by variance ($p = 0.05$) with mean separation by Least Significant Difference (LSD) as per the methods detailed by the[20].

RESULTS AND DISCUSSION

In the present study, acetone extract of four leaf extracts were screened for antibacterial activity against two standard bacterial pathogens one representing G+ve and one representing.

G-ve by two methods. The experimental results were shown in Table 1. of the four leaves tested, the leaf extracts of betel exhibited activity against the gram negative bacteria, *Escherichia coli*. Betel showed inhibition zone (15.0 mm) in disc method and 13.5 mm in well method which is on par (17.0 mm and 16.5 mm) with respective methods with that of Streptomycin (1000 ppm). However, it had less inhibitory effect on the Gram positive bacteria, *Staphylococcus aureus* (10 and 8 mm). Against *S. aureus*, moringa is exhibiting inhibition of 16.0 mm in disc method 13.5 mm in well method followed by jack leaf (9.5 and 10 mm) in respective methods. Leaves of moringa had shown inhibition zone on par with that of 1000 ppm of Streptomycin (16.0 mm). No significant antibacterial activity was detected in the extract of basumathi leaf against.

S. aureus (5 mm), but for *E. coli* (8 mm) in disc method it was on par with Moringa leaf extract.

Table 1: Antimicrobial activity of important edible leaf extracts

	Zone of inhibition (mm)			
	Disc method		Well method	
Treatments	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Streptomycin (1000 ppm)	17.0^{a}	16.0^{ab}	16.5^{a}	14.5^{b}
Acetone	0^{e}	0^{e}	0^{g}	0^{g}
Moringa leaf	9^{cd}	16.0^{ab}	8^{d}	13.5^{b}
Fragrant Rice leaf	8^{d}	5^{e}	9^{cd}	3^{f}
Jack leaf	5^{e}	9.5^{cd}	6^{e}	10^{c}
Madras betel	15.0^{b}	10^{c}	13.5^{b}	8^{d}
	SD	CD (0.5%)	SD	CD (0.5%)
OxL	0.88052	1.81732	0.81684	1.68587

Same alphabets are on par with each other, different alphabets are significantly differ with each other

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria[21]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure[22]. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. In our presen study we report that moringa and jack leaf controlled *S. aureus* Gram positive bacteria than *E. coli.* It was reverse in case of fragrant rice leaf and madras betel. This variation may due to the doses used in the study and microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains[7].

In Argentina, a researchers tested 122 known plant species used for therapeutic treatments[23]. It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger*.

Antimicrobial properties of substances are desirable tools in the control of harmful microorganisms especially in the treatment of infectious diseases. The active components usually interfere with growth and metabolism of microorganisms[24,25].

Comparisons with pertinent data from literature indicate that, according to the methodology adopted in studies on antimicrobial activity, the most diverse results can be obtained. Plant extracts have shown inhibitory effect on the growth of the bacteria studied, although of distinct forms. It is therefore, recommended that the nature and the number of the active antibacterial principles involved in each plant extract be studied in detail. Once extracted and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Bioassays[26] have demonstrated the toxicity of extracts from different plants. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds.

CONCLUSION

The results of present study supports the traditional usage of the studied edible plants and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The studied plants were used for culinary and other purposes by people for long period, without knowing the therapeutic value in it. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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