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RESEARCH ARTICLE

EVALUATING THE ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST BACTERIAL PATHOGENS

***Kumar Sudhir[1], Nancy[2], Singh Devendra[3], Kumar Vijay[4]**

[1]Lecturer, Department of Food Technology, BFIT Institute of Science and Research, Dehradun, India

[2]M.Sc student, Department of Microbiology, BFIT, Dehradun

[3]Assoc. Prof., Department of Microbiology, BFIT, Dehradun

[4]Lecturer, Department of Microbiology, BFIT, Dehradun

*****Corresponding Author's Email: sudhirkumarjakh@gmail.com*

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ABSTRACT

Four plants were screened for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella* species by the disc diffusion assay. The aqueous extracts of *Psidium guajava, Citrus limonium, Allium sativum* and *Zingiber officinale* were found active against both Gram-positive and Gram-negative bacteria. Guava leaves were found more effective against *B. subtilis* while Garlic cloves inhibited the growth of *S. aureus* to greater extent. On the other hand lemon juice and leaves extract inhibited the growth of *P. aeruginosa* and *E. coli* respectively to high level. These data support the use of such plants based medicines in treatment of infectious diseases where access to commercial antibiotics is restricted. The plants extracts are active against human microbial pathogens thus emerging as potential sources of new antimicrobial compounds. The present investigation expresses that plants have great potential as antimicrobial compounds against microorganisms. These findings provide scientific evidence to support the traditional medicinal uses of these extracts and indicate a promising potential of these plants for medicinal purposes. Thus they can be used in the treatment of infectious diseases caused by pathogenic bacteria. Further *in vivo* studies are necessary to substantiate our findings. More importantly there is need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of plants is preserved and also to provide scientific evidence for their efficacy.

Key-words: Plant extracts, Guava, Ginger, Garlic, Lemon, antibacterial activity

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. There are many medicinal herbs and spices, which find place in day-to-day uses, many of these, are used as herbal remedies. It is estimated that about 80 percent of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas[1]. The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases[2,3]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [4,5].

With increase in awareness and people becoming more health conscious, their attitude towards diet has undergone a dramatic transformation. They want their diet derived nutrients to possess and provide pharmaceutical action. From the time of Hippocrates, diet has been a primary part of management of sick people. The tenet "let food be thy medicine and medicine be thy food", espoused by Hippocrates nearly 25 decades ago, is receiving renewed interest. It is well known that every molecule in the human body originates from the diet, and that the absence of certain essentials nutrients can create a predisposition to illness. However, we are only beginning to understand that many previously unappreciated components of our diets may prove important in preventing many chronic diseases such as cancer, cardiovascular disease coronary disease and in promoting bone health, gut health, immune function, performance and enhancing human health. The omnivorous diet of human can provide the diverse blends of nutrients needed for growth, maintenance, and overall health. What people eat is one of the major environmental influences that affects health and can sooner or later contribute to disease. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[6]. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[7]. Microbiologists and food-product developers have conducted laboratory experiments that involve numerous challenging food-borne

bacteria, fungi, and yeasts with phytochemicals extracted from spice plants. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated.

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the local flora for antibacterial activity from *Psidium guajava*, *Citrus limonium*, *Allium sativum* and *Zingiber officinale*. Their extracts were evaluated for antibacterial properties human bacterial pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella species*. This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine.

MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected from Garhwal region of Uttarakhand, India in April–May 2010. Their identity was confirmed by Dr. A.S. Bisht, Assistant professor, Department of Botany, BFIT and voucher specimens were deposited in the Institute's herbarium. Table 1 shows the botanical name, local name and plant part used of the plants under study.

Table 1: List of medicinal plants used in the present study

Specimen voucher	Botanical name	Family	Common name	Plant part used
MB001	*Psidium guajava*	Myrtaceac	Guava	Leaves with and without midribs
MBOO2	*Citrus limonium*	Rutaceae	Lemon	Leaves and juices
MBOO3	*Allium sativum*	Liliaceae	Garlic	Clove
MBOO4	*Zingiber officinale*	Zingiberaceae	Ginger	Rhizome

Preparation of crude extracts

The plant materials were properly cleaned by washing with tape water followed by distilled water dried under shade. The samples of leaves with and without mid ribs, Clove and rhizome were placed in the hood for twenty four hours to evaporate the water. Approximately 100 g of each plant material was grinded with lab grinder (Bajaj, India) and the resultant liquid was filtered (Whatman filter paper no. 1; Millipore, Bangalore, India) and lyophilized under reduced pressure. The lemon juice was extracted with juicer.

Test microorganisms

The bacterial cultures, *Staphylococcus aureus* MTCC96, *Bacillus subtilis* MTCC441, *Escherichia coli* MTCC739 and *Pseudomonas aeruginosa* MTCC424 were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India while clinical isolates of *Salmonella* sp. was obtained from Departmental culture collection, Department of Microbiology, Baba Farid Institute of Technology, Sudhowala, Dehradun, India.

Antibacterial activity of crude extracts

The extracts were dissolved in DMSO (1% v/v) to yield the final concentration of 2 mg/ml. The juice was directly used for antibacterial testing. The anti-bacterial assay was carried out on Mueller Hinton agar (Difco) plates by agar well diffusion method. The bacterial inoculum was prepared from overnight-grown cultures (24 h) in nutrient broth (Difco) containing tween-80 (0.1% v / v; Merck), and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 1.2×10^8 CFU ml^{-1}). Aliquots (100 µl) of inoculums were spread over the surface of agar plates with a sterile glass spreader. 50 µl of crude extract was poured in to wells. Rifampicin (5µg $well^{-1}$; Sigma, St Louis, MO) was used as positive control. The plates were then incubated for 24 h at 37°C, and the zone of bacterial growth inhibition around the well was measured. The assay was repeated twice, and mean of the three experiments was recorded.

RESULT AND DISCUSSION

The emergence of multiple drug resistant bacteria has become a major cause of failure of the treatment of infectious diseases[8,9]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some cases all, effective antibiotics[10]. In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. Considering these, four plants were screened *in vitro* for antibacterial activity against five human pathogenic bacteria known to cause diseases in humans. These plants were selected based on traditional medicine knowledge. On the basis of zone of inhibition, the antibacterial activity of various plant extracts was evaluated. The zone of inhibition indicates the effectiveness of a plant extracts in inhibiting the growth of a bacteria. Generally the larger the zone, the more sensitive the bacterium is to the plant extracts. The bacterial pathogens namely *B.subtilis*, *E.coli*, *P.aeruginosa*, *S.aureus* and *Salmonella* sp. expressed differential sensitivity to the plant extracts as indicated by their zone of inhibitions.

The antibacterial activity of lemon extract and Guava extract is given in table 2. It was observed that leaves with mid ribs showed more antibacterial activity as compared to leaves without mid ribs (Table 2) which may be due to oils present in the mid ribs of Leaves.[11] The results of this study are comparable with studies carried out by previous authors on mid ribs of sugar beets.[12] The leaf extract of lemon was more effective as compared to juice (Table 2) which may be due to the presence of polyphenolic compounds, caffeic acid and rosmarinic acid[13].

Table 2: Antibacterial effect of lemon and guava extracts against some bacterial pathogens

Bacterial Culture	*Diameter of zone of inhibition (mm)				
		Lemon extract		Guava leaves	Rifampicin (5 µg/disc)
B. subtilis	L	21.0 ± 0.81	MR	20.33 ± 0.47	28.00 ± 0.47
	J	18.33 ± 0.94	WMR	19.33 ± 1.24	
E. coli	L	21.0 ± 0.0	MR	18.0 ± 0.81	26.00 ± 0.47
	J	20.0 ± 0.81	WMR	18.66 ± 0.47	
P. aeruginosa	L	22.0 ± 0.81	MR	16.0 ± 0.81	24.00 ± 0.81
	J	-	WMR	14.0 ± 0.81	
S. aureus	L	19.66 ± 1.24	MR	-	32.00 ± 1.2
	J	19.33 ± 1.24	WMR	-	
Salmonella sp.	L	-	MR	17.66 ± 0.94	24.00 ± 0.94
	J	21.66 ± 0.47	WMR	17.0 ± 1.41	

All values are mean of triplicate readings; L, lemon leaves; J, Juice; MR, mid-ribs; WMR, without mid-ribs - Absent; values ± Standard deviation

Other researchers have also reported the antibacterial effect of guava leaves against numerous bacteria[14]. Previously it was reported that guaijverin extract from guava leaves inhibited the growth of *Streptococcus mutans*[15]. The health benefit of guava may be attributed to natural antioxidants and other types of vitamins that can be found in it. Guava is rich in ascorbic acid [16]. Guava possess significant antibacterial activity against common diarrhea causing bacteria such as *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *E. coli*, *Clostridium* and *Pseudomonas*. Lectin chemicals in guava were shown to bind to *E. coli* (a common diarrhea-causing organism), preventing its adhesion to the intestinal wall and thus preventing infection and resulting diarrhea[17]. Lemon leaves extracts inhibited *Bacillus subtilis*, *Staphylococcus aureus*. *Salmonella* species was resistant to most of the decoctions. This finding agrees with that of other authors [18,19]. They reported the antibacterial effect of lemon leaves on some bacterial isolates. *Salmonella* species was resistant to most of the decoctions. The effect of lemon on these organisms *in vivo* cannot be predicted from this study.

The crude extracts of garlic and ginger showed variable antibacterial activities as is evident from Table 3.

Table 3: Antibacterial effect of garlic cloves and ginger rhizome extract against some bacterial pathogens

Bacterial Cultures	Inhibition Zone diameter (mm)		
	Garlic cloves extract	Ginger rhizome extract	Rifampicin (5µg/disc)
B. subtilis	17.66 ± 0.47	18.33± 0.47	28.00 ± 0.47
E. coli	20.33 ± 0.94	19.33 ±1.69	26.00 ± 0.47
P. aeruginosa	18.0 ± 0.81	19.66 ± 2.35	24.00 ± 0.81
S. aureus	20.33 ± 0.47	21.66 ± 0.94	32.00 ± 1.2
Salmonella sp.	15.33 ± 1.24	19.33 ± 0.47	24.00 ± 0.94

All values are mean of triplicate readings; - Absent; values ± Standard deviation

Against *B. subtilis*, the antibacterial effect of ginger extract was high (zone of inhibition:18.33±0.47 mm) in comparison to extract of garlic (zone of inhibition:17.66 ± 0.47mm). Against *E. coli*, the effect of garlic extract (20.33 ± 0.94 mm) was more pronounced in comparison to that by ginger extract (19.33 ±1.69 mm). The antibacterial effect against *P. aeruginosa* was more by ginger extract (19.66 ± 2.35 mm) in comparison to that of garlic extract (18.0 ± 0.81 mm). Same effect was observed against *S. aureus* and *Salmonella* sp.

The antibacterial activity of ginger extracts could be attributed to the chemical properties of ginger. The main constituents of ginger are sesquiterpenoids with zingiberene as the main component. Other components include β- sesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids, and monoterpenoid (β-sesquiphellandrene, cineol and citral) fractions in traces[20]. The terpenoids are of important in pharmacy due to their relationship with such compounds as vitamin A and could be of immense medical applications. Ginger has a sialagogue action, which stimulate the production of saliva, and can be used to disguise the taste of medicines[20]. The presence of gingerols make viable the treatment of stomach acidity with ginger juice and it may have analgesic and sedative properties [20].

CONCLUSIONS

The present investigation expresses that plants have great potential as antimicrobial compounds against microorganisms. Thus they can be used in the treatment of infectious diseases caused by pathogenic bacteria. Guava leaves inhibited the growth of *B. subtilis* to maximum extent. Garlic cloves inhibited the growth of *S. aureus* to greater extent. On the other hand lemon juice and leaves extract inhibited the growth of *P. aeruginosa* and *E. coli* respectively to high level. These data support the use of such plants based medicines in treatment of infectious diseases where access to commercial antibiotics is restricted. In conclusion plants extracts are active against human microbial pathogens thus emerging as potential sources of new antimicrobial compounds. Further in vivo studies are necessary. More importantly there is need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of plants is preserved and also to provide scientific evidence for their efficacy.

CONFLICT OF INTEREST: The authors confirm there is no conflict of interest.

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