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Evaluation of antibacterial activity of plant extracts against bacterial pathogen

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ABSTRACT

The aim of the study was to evaluate the antibacterial activities of twenty-five plant extracts against five bacteria strains. The tested plant extracts showed variation in zone of inhibition. The highest antibacterial potentials were observed from the extracts of Acacia catechu which showed highest zone of inhibition in all bacteria followed by Terminalia bellerica, Boswellia serrata, Aloe vera and Mimosa pudica. The antibacterial activity observed in plants are indicative of the methanol plant extracts of these plants could be a possible source to obtain new and effective herbal medicines to treat infections, hence justified the ethic use of species against various infectious diseases.

Keywords: Evaluation, antibacterial activity, zone of inhibition, plant extracts.

INTRODUCTION

The most of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant. In India, almost 95% of the medicines are plant based in the traditional systems of medicine. Around 80% of medicinal drugs are of plant origin. The plant compounds reflect the plants as a whole as a safety and efficacy much superior to that of its isolated and pure active compounds [1].

In recent time, the research for potential antibacterial agents has been shifted to plants. Most plants are medicinally useful in treating diseases in the human. It is suggested that about 10% of flowering plants on earth have at one time been used by local communities throughout world but only 1% have recognized by modern science. Hence there is need to evaluated the plants for the use of medicine. The traditional medicinal methods, specially the use of medicinal plants still play a vital role to convert basic health needs in the developing countries.

Plant products have been part of medicines since time immemorial. There are about 120 plant based drugs prescribed worldwide and they come from 95 plant species. Approximately, 2.5 lakh species of flowering plants and only 5000 have had their pharmaceutical potential assessed. Several studies have identified compounds in the plants that are effective antibiotics [2,3,4,5]. The need of search is to screen a number of plants for promising potentials of biological activity. The evaluation of plant constituents for antimicrobial activities has shown that higher plant represent a potential source of better antibiotic properties [6]. Since ancient time the plant and their ingredients have been known for their useful degree of antimicrobial activities [7,8,9,10].

The plant compound showed photochemical, antibacterial, antifungal, etc. activities. The antibiotic resistance has been become a global importance [11]. Hence, there is need to use plant based antibacterial. There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms and as a result plants are still recognized as the bedrock for modern medicine. In the present study twenty-five different local plant species each belonging to different families was evaluated for their antibacterial potentials.

MATERIALS AND METHODS

Collection of plant materials: The plant material was collected from P.V. P. College campus, Pravaranagar (located $19^0$34'N to $74^0$28' E), Maharashtra during August-2013. The fresh plant material was collected and washed under running tap water, air dried and then homogenized for fine powder and stored in airtight bottles. The plants materials were identified with the help standard literature.

Preparation of extraction: The leaves were washed thoroughly 2-3 times with running tap water. Then air dried under shade and followed complete shade drying. The plant material was crushed in mixer; the powder was kept in small plastic bags with paper labeling. The leaves of 5gm weighed and were crushed in 25 ml of solvent and kept on starrier for overnight and it was filtered through Whatman No.1 filter paper. Then the filtrate was stored at 4^0C.

Microorganisms: The bacterial strains studied were Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus)* and Gram-negative *(Pseudomonas aeroginosa*, *Proteus vulgaris* and *Salmonella typhi)* bacterium (Table 1).

Antibacterial Assay: The antibacterial activity assay was performed for aqueous extract and agar well diffusion method for solvent extracts [12]. The molten Muller Hinton Agar (HiMedia) was inoculated with the 100 µl of inoculums (1x 10^8Cfu) and poured into the sterilized petri plate. For agar disc diffusion method, the disc (0.7 cm) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated over night at 37^OC. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain control was maintained in pure solvent were used instead of extract. The result was observed by measuring of zone of inhibition in a diameter. The experiment was repeated three times and means values are reported (Table 2). The obtained results were compared with the standard antibiotics penicillium (100 µg/disc) and Gentamicin (10 µg/disc).

RESULTS AND DISCUSSION

The results of screening plant extracts against antibacterial activities are presented in Table 2. The methanol extracts of twenty-five plants belonging to fifteen families were tested against two Gram-positive and three Gram-negative bacteria using agar well diffusion. The plants exhibited antibacterial activity to a certain degree. *Acacia catechu* showed highest zone of inhibition in all bacteria followed by *Terminalia bellerica*, *Boswellia serrata*, *Aloe vera* and *Mimosa pudica*.

Bougainvellia spectabilis extracts was totally inactive against all the Gram-negative strains tested. *Terminalia arjuna*, *Plumbago zeylanica*, *Withaniam somnifera*, and *Clitoriea ternatea* plant material were inactive to the strains of Gram-negative bacteria while the plant species *Asparagus racemosus*and *Mirabilis jalapa* were inactive *to Pseudomonas aeroginosa* and *Salmonella typhi*. The plant species as *Butea monospermous*, *Adathoda vasica*, *Gymnema sylvestre*, and *Plumbago zeylanica* revealed inactive to the Gram-positive bacteria as *Bacillus subtilis*.

Out of twenty-five plant species, twelve species showed significant antibacterial activity in both the strains. The plant extracts *Adathoda vasica*, *Gymnema sylvestre*, *Barleria pronitis*, *Saraca indica* and *Annona squamosa* were more active in the Gram-negative strains compared to those of Gram-positive strains. The bacterial strain *Bacillus subtilis* was not developed zone of inhibition in four plant extracts, whereas *Proteus vulgaris* in seven, *Pseudomonas aureus* in eight and *Salmonella typhi* in four plant extracts reported nil zone of inhibition.

Acacia catechu showed 25mmand 24 mm zone of inhibition in *Staphylococcus aureus* and *Bacillus subtilis* strains and 25 mm, 21 mm and 13 mm in *Proteus vulgaris*, *Salmonella typhi* and *Pseudomonas aeroginosa* strains of bacteria respectively. *Terminalia bellerica* reported 21mm and 16 mm zone of inhibition in *Staphylococcus aureus* and *Bacillus subtilis* strains and 19 mm, 20 mm and 10 mm zone of inhibition in *Proteus vulgaris*, *Pseudomonas aeroginosa* and *Salmonella typhi* strains of bacteria respectively. *Boswellia serrata* revealed 19 mm and 10 mm

zone of inhibition to *Bacillus subtilis* and *Staphylococcus aureus* respectively, while13 mm, 19 mm and 15 mm zone of inhibition in *Proteus vulgeris, Salmonella typhi* and *Pseudomonas aeroginosa* strains of bacteria respectively.

Bacillus subtilis was the most susceptible bacteria amongst all the bacterial strains investigated in the present work. The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

The antibacterial substances in the higher plants are well established. The successful evaluation of plant substances from plant material is largely dependent on the type of solvent used in the extraction procedure [13]. Workers commonly performed water extracts, water as the solvent but, plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. The compounds being extracted in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the development of medicine, a natural blueprint for the development of drug [14].

Esteemed workers have identified plant compounds that are effective antibiotics [15]. Traditional systems around the world which utilize herbal remedies are an important source for the discovery of new antibiotics [16]. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria [2]. The antibiotics property of the herbal compounds that indicates the need for further research in to traditional healing system [3]. It is also important pharmacological studied leading to synthesis of more potent drug with reduced toxicity [17].

The present investigated some plant extracts did not show any antibacterial activity but, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed [18]. Lack of activity cans thus only be proven by using large doses [19]. Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents [20]. With no antibacterial activity, extracts may be active against other bacterial species or viruses which were not tested [21]. It is also showed that Gram-positive bacteria are more susceptible as compared to Gram-negative bacteria. These differences may be attributed to fact that the cell wall in Gram-positive bacteria is of single layer, whereas, the Gram-negative bacteria cell wall is multi-layered structure. The passage of the active compound through the Gram-negative cell wall may be inhibited. It is though that observed differences may result from the dose level in the study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance level between strains [22,23]. There are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species.

The present study was initiated because of increasing resistance of antibiotics including bacteria. Plant extracts (compounds) are most important in the field of antiseptic and antimicrobial agents. As a result, the microbial activity of twenty-five local plants was screened against common pathogens. The methanol plant extracts of *Acacia catechu, Terminalia bellerica, Boswellia serrata, Aloe vera* and *Mimosa pudica* showed the most remarkable activity. These plants can be further subjected to isolation of the therapeutic antimicrobial and carry out further pharmacological evaluation.

Table 1: Showing used bacteria for antibiotic screening.

Sr. no.	Bacteria used	Grown on media	Gram stain
1	*Bacillus subtilis*	Nutrient agar	G+ve
2	*Proteus vulgaris*	Nutrient agar	G-ve
3	*Pseudomonas aeroginosa*	Nutrient agar	G-ve
4	*Salmonella typhi*	Nutrient agar	G-ve
5	*Staphylococcus aureus*	Nutrient agar	G+ve

Table 2: Showing antibiotic activities of methanol plant extracts

Sr. no.	Plant species	Zone of inhibition(in mm diameter)				
		Penicillium (Gram-positive)		Gentamycin (Gram-negative)		
		Bacillus subtilis	*Staphylococcus aureus*	*Proteus vulgaris*	*Pseudomonas aeroginosa*	*Salmonella typhi*
1.	*Abruspre catorius* L.	8	11	15	-	15
2.	*Acacia catechu* L.	24	25	25	13	21
3.	*Acacia nilotica* L	9	10	8	15	-
4.	*Adathoda vasica*L.	-	7	10	15	11
5.	*Aloe vera* L.	11	17	13	15	16
6.	*Annona squamosa* L.	9	9	11	13	10
7.	*Asparagus racemosus* Willd	12	9	10	-	-
8.	*Barleria pronitis* L.	8	9	8	15	10
9.	*Boswellia serrata* L.	19	10	13	19	15
10.	*Bougainvillea spectabilis* Willd.	6	8	-	-	-
11.	*Curcuma longa* L.	-	12	-	10	8
12.	*Butea monosperma* Faub.	8	12	19	9	10
13.	*Clitoriea ternatea* L.	15	13	-	-	9
14.	*Ficus racemosa* L.	9	8	13	15	-
15.	*Gymnema sylvestre* R.Br.	-	12	12	10	12
16.	*Mimosa pudica* L.	14	12	11	15	12
17.	*Mirabilis jalapa* L.	10	12	14	-	-
18.	*Murraya koenigii* L.	8	17	15	*10*	6
19.	*Plumbago zeylanica* L.	-	11	-	-	12
20.	*Santalum album* L.	9	8	11	13	9
21.	*Saraca indica* L.	1	11	10	15	13
22.	*Terminalia arjuna* L.	10	10	-	-	9
23.	*Terminalia bellerica* L.	16	21	19	20	10
24.	*Vitis vinifera* L.	11	11	-	15	6
25	*Withania somnifera* L.	18	19	-	-	21

+ indicates presence and – indicates absence of activity.

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