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Research Note

In vitro Antimicrobial activity of Acacia nilotica, Ziziphus mauritiana, Bauhinia variegate and Lantana camara against some clinical isolated strains

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Abstract

Plants are potent biochemists; biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. Thus, the aim of the current study was to screen the antimicrobial activity of Acacia nilotica, Ziziphus mauritiana, Bauhinia variegate and Lantana camara against some selected clinical isolated strains. Although previous studies have documented the antimicrobial properties of these plants, this work is designed to evaluate the specific antibacterial activity of different extracts of these plants against tested microorganisms, in order to know the best extract against specific microorganisms. In this study the fresh parts (leaves, barks & pods) of the test medicinal plant were collected and methanol, ethanol and ethyl acetate extracts were prepared. Antibacterial susceptibility test was done by using Agar diffusion assay method. Statistical analysis was carried out with SPSS 17.0 Windows version. The results of the current study showed that a total of 8 extracts from 4 different plant species were investigated including pods of ethyl acetate extracts of Lantana camara, which showed the highest antimicrobial activity against tested clinical isolates (*Bacillus subtilus* 2±0.1mm, Bacillus circulans 2.6±0.2mm, *Bacillus sphaericius* 2±0.1mm, Staphylococcus aureus 2.5±0.1, and *Serratia liquefaciens* 2.2±0.1mm), followed by its ethyl acetate extracts of leaves. Bark extracts of four tested medicinal plants possess a lower zone on inhibitory activity as compared to the leaves extracts of these plants. Noticeably no antimicrobial activity was found in the methonolic bark extract of Acacia nilotica against the tested bacteria except *Bacillu ciurlans.* The results of the present investigation clearly indicate that the antibacterial activity varies with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Studies are in progress to further evaluate the mechanisms of action of these *active test* extracts on study organisms associated with certain human diseases.

Keywords*:* Antimicrobial susceptibility; medicinal plants; Agar diffusion; clinical isolates

1. Introduction

Plants are potent biochemists and have been components of phytomedicine since time immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [1] i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing [2]. For instance, in developed countries 25% of the medical drugs are based on plants and their derivatives [3]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased [4].

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Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations [5]. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [5]. The increase in resistance to microorganisms due to indiscriminate use of antimicrobial drugs forced scientists to search for new antimicrobial substances from various sources including medicinal plants [6]. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists [7]. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent [8].

Acacia nilotica (Mimosaceae) is commonly called Gum Arabica and locally known as Nalla Tumma (Telugu). The powdered bark of the plant with a little salt is used for treating acute diarrhea [9]. Ziziphus mauritiana (Rhamnaceae) is locally called Regu (Telugu). Previous studies reveal that the whole plant and leaves are used in the traditional system of medicine as a tonic [10]. Bauhinia variegata L. (Caesalpiniaceae), having Kachnar as the common Indian name and locally called Daevakanchanamu(Telugu), is traditionally used in bronchitis, leprosy, tumours and ulcer. Lantana camara (Verbanaceae), commonly known as wild or red sage is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant [11]. However, it is listed as one of the important medicinal plants of the world [12]. L. camara contains lantadenes, the pentacyclic triterpenes which is reported to possess a number of useful biological activities. Several previous reports have described it as antifungal [13] anti proliferative [14]. Moreover, the hydroalcoholic extracts of the leaves have shown an effect on fertility, general reproductive performance, and teratology in rats [15].

Thus, the aim of the current study was to screen the antimicrobial activity of Acacia nilotica, Ziziphus mauritiana, Bauhinia variegate and Lantana camara against some selected clinical isolated strains. Although previous studies have been documented on the antimicrobial properties of these plants, this work is designed to evaluate the specific antibacterial activity of different extracts of these plants against tested microorganisms, in order to know the best extract against a specific microorganism.

2. Materials and methods

Plant material

The fresh parts (leaves, barks and pods) of the plant were collected from a nearby medicinal plant nursery. The collection was under specialist supervision and these plants are commonly known to everyone. These plants were authenticated by a Botanist. The various plant parts were thoroughly washed, sun-dried for 7-10 days and ground into powder using a laboratory mill prior to analysis.

Extraction

25 g of shade dried powder of plant materials was filled separately in the thimble and extracted successively with 150 ml each of methanol, ethanol and ethyl acetate using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved at 4^oC in airtight bottles until further use.

Microorganisms

The clinical isolate cultures of *Bacillus subtilus*, *Bacillus circulans, Bacillus sphaericius, Staphylococcus aureus and Serratia liquefaciens* were obtained from the Microbiology department at BDRC (Bioaxis DNA Reseach Center), Hyderabad, India. The isolates were identified by conventional tests [16, 17]. All the strains were maintained on nutrient agar at 4°C and were sub cultured every month.

Determination of antibacterial activity by agar diffusion method

Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay (ADA) [18]. The plates containing Mueller-Hinton agar were spread with 0.2 ml of the inoculum. Wells (8 mm diameter) were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml of the extract. The plates inoculated with different bacteria were incubated at 37°C up to 48 h and diameter of any resultant zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in duplicate and repeated thrice.

Statistical analysis

All the tests were conducted in triplicate. The data of all the parameters were statistically analyzed and expressed as mean ± S.D with the aid of SPSS 17.0 Windows version.

3. Result and Discussion

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [19]. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [20]. Continued further exploration of plant-derived antimicrobials is needed today.

A total of 8 extracts from 4 different plant species were investigated. Extracts of the different parts of the tested medicinal plants used in this study were shown in Table 1. The Antibacterial susceptibility by means of disk diffusion method showed that the 4 plant extracts tested exhibited an antimicrobial effect against *Bacillus circulans, Bacillus circulans, Bacillus circulans, Staphylococcus aureus and Serratia liquefaciens* (Table 2). Barks extracts of the four tested medicinal plants possess a lower zone on inhibitory activity as compared to the leaf extracts of these plants.

A total of 8 extracts from 4 different plant species were investigated; pods ethyl acetate extracts of Lantana camara showed the highest antimicrobial activity against Acacia nilotica and was found to give the most potent antimicrobial extract (Table 2). It is reported to have antimicrobial, antihyperglycemic and antiplasmodial properties [21-23]. Noticeably no antimicrobial activity was found in methonolic bark extract of Acacia nilotica against the tested bacteria except *Bacillu ciurlans* (Table 2).

When tested with disk diffusion method, Lantana camara ethyl acetate extracts of pods possess significant antimicrobial activity against tested clinical isolates (*Bacillus subtilus* 2±0.1mm, Bacillus circulans 2.6±0.2mm, *Bacillus sphaericius* 2±0.1mm, Staphylococcus aureus 2.5±0.1, and *Serratia liquefaciens* 2.2±0.1mm), followed by its ethyl acetate extracts of leaves. *L. camara* has been studied extensively for their antibacterial properties [24-26]. L. camara possess many important biological activities viz., antipyretic, antimicrobial, antimutagenic, antimicrobial, fungicidal, insecticidal, nematicidal, and others [24-26]. Lantadenes present in all L. camara is believed to be responsible for almost all the biological activities [27].

Therefore, antibacterial activities of L. camara leaf and flower extracts reported here might be due to the presence of some of these chemical constituents, particularly lantadenes and theveside in the extracts. Bhakta and Ganjewala [28] have recently confirmed the presence of phenolics, anthocyanins and roanthocyanidins in *L. camara* leaves which could also be responsible for the antibacterial properties of the L. camara reported here. Though, the mechanism of the action of these chemical constituents is not yet fully known, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. Perhaps it is one of the reasons behind the differences in the antibacterial activities of the plants. Moreover, the effectiveness of the extracts varies with its concentration and the kind of bacteria used in the study. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures [29, 30]. Thus, *S. aureus* was found to be resistant to all the extracts, which is most probably due to its outer membrane. Nevertheless, it is the ability of the active principle of the extracts that disrupt the permeability barrier of cell membrane structures and thus inhibit the bacterial growth [29, 30].

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [31]. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally, ion leakage from the cells [32]. In our study *B.subtillis* was found to be highly susceptible to L.camara ethyl acetate extracts of pods and leaves, similar to one of the early studies [33]. Z. mauritiana ethanol leaf extract was active against S. *aureus* supported by a previous study [34]. The ascending sequence of maximum antimicrobial activity against test microorganisms were as follows: Lantana camara, Bauhinia varigata, Acacia niloticaand, Ziziphus mauritiana (Table 2).

Medicinal plants can be poisonous if wrong plant parts or wrong concentrations are used [35]. Herbal medicines are assumed to be harmless. Although herbal extracts need to be assured for quality control and efficacy for a particular dose, the results of present study clearly indicate that the antibacterial activity varyies with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Studies are in progress to further evaluate the mechanisms of action of these *active test* extracts on study organisms associated with certain human diseases

Table 1. Extracting solvents and parts of the medicinal plant used for antimicrobial activity

Medicinal plant	Family	Part used	Extracting Solvent
Acacia nilotica	Mimosaceae	Leaves & Barks	Methanol & Ethanol
Ziziphus mauritiana	Rhamnaceae	Leaves & Barks	Methanol & Ethanol
Bauhinia variegata	Caesalpiniaceae	Leaves & Barks	Ethanol
Lantana camara	Verbanaceae	Leaves & Pods	Ethyl acetate

Table 2. Zone of inhibitory activity (in millimeter) of different plant extracts against clinical isolates/Microoranisms

Microorganism/Clinical isolate strain	*Zone of Inhibition (mm diameter)*											
	Acacia nilotica				*Ziziphus mauritiana*				*Bauhinia variegata*		*Lantana camara*	
	Leaves		Bark		Leaves		Bark		Leaves	Bark	Leaves	Pods
	E	M	E	M	E	M	E	M	E	E	EA	EA
Bacillus circulans	0.7±00	1±0.1	1.5±0.2	0.6±00	0.6±0.1	Nil	Nil	Nil	1.3±0.2	1.1±00	2.3±0.2	2±0.1
Bacillus subtilis	1.1±0.1	1.3±0.2	1±00	Nil	1.7±0.3	0.6±00	0.9±00	1±0.1	0.7±0.1	0.4±00	2.4±0.1	2.6±0.2
Bacillus sphericius	1.1±00	0.8±00	1.7±0.1	Nil	0.9±00	1.1±0.1	Nil	Nil	1.1±0.1	Nil	1.8±0.1	2±0.1
Staphylococcus aureus	0.4±00	1±0.1	1±0.1	Nil	0.6±00	1±00	Nil	Nil	1.3±0.1	0.5±0.1	1.5±0.2	2.5±0.1
Serratia liquefaciens	1.3±0.1	0.9±0.1	1±00	Nil	1±0.1	Nil	0.6±00	0.8±0.1	1.1±00	0.6±00	2.4±0.1	2.2±0.1

E: Ethanol extract; M: Methanol extract; EA: Ethyl acetate extract

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