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Antimicrobial properties of extracts of Indian antidiabetic plants: *Syzygium cumini, Azadirachta indica* and *Bougainvillea spectabilis*

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ABSTRACT

The antimicrobial properties of Indian antidiabetic plant extracts of *Syzygium cumini, Azadirachta indica* and *Bougainvillea spectabilis*, against six bacterial strains *(Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus vulgaris*). The collected antidiabetic plants *Syzygium cumini*, *Azadirachta indica*, and *Bougainvillea spectabilis* were used as hypoglycemic plants in West Indies and some parts of Asia. Chloroform, ethanol and aqueous extracts were prepared sequentially from leaves of these plants. It was observed that methanol and ethanolic extracts of *Syzygium cumini* have significant activity against gram positive bacteria. *Klebsiella pneumonia* and *Proteus vulgaris* were more sensitive to the extracts of *Azadirachta indica* and *Bougainvillea spectabilis* respectively.

Key words: *Syzygium cumini*. *Azadirachta indica*. *Bougainvillea spectabilis*. ethanol Gram positive and negative bacteria.

INTRODUCTION

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. Numerous studies have identified compounds within herbal plants that are effective antibiotics. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics. some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria .The results of this indicate the need for further research into traditional health systems. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. The need of the hour is to screen a number of medicinal plants for promising biological activity.

Ayurveda, the traditional Indian herbal medicinal system practiced for over thousands of years have reports of antidiabetic plant with no known side effects. Such plants and their products have been widely prescribed for diabetic treatment all around the world with less known mechanistic basis of their functioning. Here these natural products need to be evaluated significantly and methodically in order to check for their properties[1, 2].

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use plants against different illness may be expected to have accumulated in areas were the use of plants is still of great importance[3]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavanoids, tannins and phenolic compounds[4]. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their

primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment.

Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds of therapeutic use. *Syzygium cumini has been introduced to many different places where it has been utilized as a fruit producer, as an ornamental and also for its timber.* Bark, leaves and fruits are used in medicine. The leaves are antibacterial and are used for strengthening the teeth and gums. The tender leaves are used for vomiting. The fruits and seeds are sweet, acrid, sour, tonic and cooling, and are used in diabetes, diarrhoea, pharyngitis, splenopathy, urethrorrhea.

MATERIAL AND METHODS

Collection of plant materials and preparation of extracts

Plants were collected in and around Tirupati of Chittoor dist. of A.P. The leaves were washed and air dried at room temperature. The chloroform, ethanol and aqueous extracts were prepared sequentially in soxhlet extractor using 30 gms of dried plant tissue mixed with 150 ml of respective solvents (100% v/v) for 24 hours[5, 6]. Chloroform and ethanol extracts were evaporated to dryness in rotary evaporator, where as aqueous extracts are lyophilized[7]. 25 mg of dry weight of each crude extract was further reconstituted in 2.5ml of distilled water and 1:15 dilutions of all these extracts were used for further studies.

Antimicrobial screening

The chloroform and ethanolic extracts of three plants were screened against six bacterial strains. The test organisms were *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus vulgaris* (MTCC 1771) obtained from IMTECH, Chandigarh.

Preparation of inoculum

Stock cultures were maintained at 4^{0}C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated with out agitation for 24 hours for 37^{0}C. The cultures were diluted with fresh Mueller-Hinton broth to achieve optical densities corresponding to 2.0 x 10^6 Colony Forming Units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method Bauer et al[8] was used to screen the antimicrobial activity. Invitro antimicrobial activity was screened by Mueller-Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 20 ml of molten media in to sterile Petri plates. The plates were allowed to solidify for 10 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different concentrations of extracts were loaded on 6mm sterile discs. The loaded discs were placed on the surface of the medium and the compounds were allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{0}C for 24 hours. At the end of incubation, inhibition zones were formed around the discs were measured with transparent ruler in millimeters. These studies were performed in triplicate.

RESULTS AND DISCUSSION

The results of the antibacterial properties of extracts on the microorganisms are tabulated as follows.

Isolation of chemical compounds from plant material is largely dependent on the type of solvent used in extraction procedure. In this assay the extracts were prepared using sterile distilled water and solvents like chloroform and Ethanol. We found in this study that none of the aqueous extracts produced any zones of inhibition. The ethanolic and chloroform extracts were found to show consistent antimicrobial activity in comparison to the aqueous extracts. This might have resulted from the lack of solubility of active constituent in aqueous solutions while the extracts showed increased solubility in solvents like chloroform and ethanol.

The tested plant extracts were most active against gram positive microorganisms than gram negative microorganisms. This is in agreement with previous reports by several workers[9, 10, 11, 12, 13, 14]. The antimicrobial assay was carried out on gram positive bacteria i.e., *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria i.e., *Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa.*

Bacillus subtilis and *Staphylococcus aureus* were *found* to be susceptible to ethanolic extracts of *Syzygium cumini. Klebsiella pneumonia* and *Proteus vulgaris* were found to be more

Table 1: Sensitivity Pattern of *Syzygium cumini* on test organisms

Organism	Type of Organism	Concentration of Extract (in µl) Zone of Inhibition(in mm)			
		20	30	80	110
Bacillus subtilis	Gram positive	2.5	3.1	3.5	3.8
Pseudomonasaeruginosa	Gram negative	2.3	2.6	3	3.1
Staphylococcus aureus	Gram positive	2.4	2.9	3.2	3.7
Proteus vulgaris	Gram negative	1.9	2.3	2.5	3
Klebsiella pneumonia	Gram negative	1.3	1.6	2	2.2
Escherichia coli	Gram negative	1.8	2	2.5	2.7

Table 2: Sensitivity Pattern of *Azadirachta indica* on test organisms

Organism	Type of Organism	Concentration of Extract (in µl) Zone of Inhibition(in mm)			
		20	30	80	110
Bacillus subtilis	Gram positive	0.7	0.9	1.1	1.3
Pseudomonasaeruginosa	Gram negative	0.7	0.8	1	1.2
Staphylococcus aureus	Gram positive	0.7	1	1.3	1.6
Proteus vulgaris	Gram negative	0.6	0.9	1.1	1.2
Klebsiella pneumonia	Gram negative	1.2	1.5	1.8	2.5
Staphylococcus aureus	Gram negative	1	1.4	1.9	2
Escherichia coli	Gram negative	0.7	1	1.7	1.8

Table 3: Sensitivity Pattern of *Syzygium cumini* on test organisms

Organism	Type of Organism	Concentration of Extract (in µl) Zone of Inhibition(in mm)			
		20	30	80	110
Bacillus subtilis	Gram positive	0.7	0.9	1.5	1.7
Pseudomonasaeruginosa	Gram negative	-	1	1.1	1.3
Proteus vulgaris	Gram positive	1.4	1.5	1.9	2.6
Klebsiella pneumonia	Gram negative	0.6	0.8	1.4	1.8
Staphylococcus aureus	Gram negative	1.2	1.3	1.5	1.8
Escherichia coli	Gram negative	1.1	1.2	2	2.1

sensitive to the extracts of *Azadirachta indica* and *Bougainvillea spectabilis* respectively. It was found that methanolic extracts of *Syzygium cumini, Azadirachta indica* and *Bougainvillea spectabilis* inhibited the growth.

Prasanth *et al.*[15] reported that, different extracts of *Azadirachta indica* showed some antibacterial activity against some gram positive and negative bacteria such as *P.vulgaris* and *B.subtilis*. Rajakaruna *et al.*[16] reported that *Syzygium cumini* showed good activity against *Bacillus subtilis* and *Staphylococcus aureus.* The essential oils from the leaves of *Syzygium cumini* were most active against *Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*[17].

These differences in the bacterial susceptibility to different extracts may be attributed to the fact that cell wall in gram positive bacteria are of single layer whereas gram negative cell wall is multilayered. Alternatively the passage of the active compound through the gram negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains. The microorganism susceptibility to different extracts did not correlate with the susceptibility or resistance to a particular antibiotic within same species. Further the results obtained by this method may vary as many factors such as microbial growth, exposure of microorganisms to different chemical substances and the quantity of the substance. The factors responsible for this high susceptibility of gram positive and negative organisms to the extracts are not exactly known but may be attributed to the presence of secondary metabolites[18]. It is worthy to note that the antimicrobial activities of these plants extracts were dependent on the concentration of the extracts as reported by Ekwenye et al. [19] also, if the extract has high molecular weight, the rate of diffusion is always slow, reduced and also takes longer time, whereas an extract of low molecular weight diffuses faster and at a quicker rate.

CONCLUSION

In conclusion the negative result does not mean the absence of bioactive compounds nor is the plant inactive. Active compounds may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of Activity can thus only be proven using higher doses. 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 bioactive agents.

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