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Antimicrobial Assay of *Zizyphus oenoplia* (L.) Mill.

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ABSTRACT

The antimicrobial activity of acetone and methanolic leaf extracts from *Zizyphus oenoplia* was tested against three Gram-negative (*Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus* sp.) and two fungal strains (*Candida albicans* and *Cryptococcus neoformans*) by conducting a well-in-agar method. *P. putida* and *V. cholerae* exhibited better resistance against the plant extracts followed by *Shigella flexneri* and *Bacillus* sp. Other pathogens were ineffective against the plant extracts.

Keywords: antimicrobial activity, plant extracts, pathogenic microorganisms, *Zizyphus oenoplia*

INTRODUCTION

Microorganisms exist in every niche of our biosphere and occupy a peculiar place in the human view of life. The microbial population in the human body assists in its normal functioning like digestion and in enhancing resistance to infections. Without them, the pathogens can cause infections and abnormal digestion and other related problems in humans. Infectious diseases pervade human existence; they are the world's leading causes of premature death killing almost 50,000 people every day (Mulligen *et al.* 1993). In recent years, drug resistance to human pathogenic microbes has been commonly reported from all over the world due to indiscriminate use of antibiotics (Ahmed and Beg 2001).

Human infection, particularly that involving microorganisms (bacteria, fungi, viruses, parasites, nematodes etc.), can cause serious problems in the tropical and subtropical regions of the world. In recent years, multiple drug resistance in human pathogenic microorganisms is a result of indiscriminate use of commercial antimicrobial drugs commonly administered in the treatment of such diseases. Over the last four decades, the intensive efforts have been made to discover clinically useful antimicrobial drugs (Perumalsamy and Ignacimuthu 2000) which are innovative and effective (Bhavnani and Ballow 2000).

Plant-derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in plants as source of agents to fight microbial diseases and also used in the treatment of several diseases (Kinghorn 1987; Charindy *et al*. 1999; Aburjai *et al*. 2001). In order to evaluate the efficacy of these plants by scientific investigations, an inter-disciplinary program was started with the aim of screening plants for their antimicrobial activity.

Zizyphus oenoplia (Rhamnaceae) (suraimullu or nariellanthai, vernacular names) is one of the most common straggling medicinal shrubs found in the deciduous forests in India. The stem bark of this plant is used as mouthwash for sore throat, for dysentery and inflammation of the uterus (Pal and Jain 1998) and in some cases the whole plant is used for the treatment of diabetes (Wahab and Yousuf 2004). This plant is also therapeutically used against cough, cold, dysentery, diarrhoea, ulcers, diabetes, fertility problems, snake-bite and skin disorders (Singh *et al*. 2002).

Hence, the present research investigates the antimicrobial activity of the leaf extracts (acetone and ethanol) of *Z. oenoplia* against the human pathogenic microbial populations (both bacteria and fungi).

MATERIALS AND METHODS

Plant material

The healthy leaves of *Zizyphus oenoplia* were collected from the foothills of Kolli hills (Namakkal District), one of the segments of Eastern Ghats of Tamil Nadu. The identification of the plant was confirmed with the assistance of the Book of Tamilnadu Carnatic (Matthew 1983) and the herbarium specimen was deposited in the PG and Research Department of Botany, National College, Tiruchirappalli, Tamil Nadu. The plant materials were washed in tap water to remove the soil particles and shade dried for 10 days.

Microorganisms

In the present study, five bacterial strains (*Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri*, *Staphylococcus aureus* and *Bacillus* sp.) and two fungal strains (*Candida albicans* and *Cryptococcus neoformans*) were used. The microbial cultures were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Media

The media used for antimicrobial test were Nutrient Agar/Broth and Muller Hinton agar (bacteria) and Sabouraud Dextrose Agar (fungi). All the media were obtained from Himedia Pvt. Ltd., Mumbai, India.

Extract preparation

The dried plant material was crushed into fine particles (powder) using a mixer grinder. About 25 g of each powdered material was separately extracted with 100 ml of ethanol and acetone solvents respectively. The solvents with leaf-powder were kept at room temperature, for 7 days to allow the extraction of compounds from plants. Each mixture was stirred every 24 h using sterile glass rod.

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Short Communication

Table 1 Anti-microbial activity of two different extracts from the leaves of *Zizyphus oenoplia* (Diameter of Growth Inhibition zone in mm).

Solvents used	Extract conc. (µg)	Tested microorganisms						
		Pseudomonas putida	*Vibrio cholerae*	*Shigella flexneri*	*Staphylococcus aureus*	*Bacillus species*	*Candida albicans*	*Cryptococcus neoformans*
Acetone	100	10.33 ± 0.94	10.00 ± 0.00	10.33 ± 0.47	0	0	0	0
	200	11.00 ± 0.00	10.33 ± 0.47	10.00 ± 0.00	0	0	0	0
	300	11.66 ± 0.46	11.33 ± 0.47	11.00 ± 0.00	0	0	0	0
	400	13.00 ± 0.82	12.33 ± 0.47	13.66 ± 0.46	0	0	0	0
Ethanol	100	10.33 ± 0.46	10.00 ± 0.00	0	0	0	0	0
	200	11.66 ± 0.46	12.33 ± 0.46	0	0	0	0	0
	300	12.00 ± 0.00	13.00 ± 0.82	0	0	10.33 ± 0.46	0	0
	400	13.66 ± 094	14.00 ± 0.00	0	0	10.00 ± 0.00	0	0
Standard antibiotics		25.00 ± 0.00	20.33 ± 0.46	18.33 ± 0.46	15.00 ± 0.00	15.00 ± 0.00	15.33 ± 0.46	15.00 ± 0.00

Antibiotics – Tetracycline (bacteria); penicillin (fungi)

The greenish extracts obtained were passed through the Whatmann filter paper No.1 and the respective solvents were evaporated at 40°C with the help of heating mantle. The sticky black substances were obtained and stored in refrigerator and dissolved in DMSO (dimethyl sulfoxide) prior to use for the antimicrobial activity tests. Each extract was tested in triplicate and the standard deviations were calculated (Gupta 1977).

Preparation of inoculum

The test bacterial and fungal strains were inoculated onto nutrient broth/SD broth and incubated at 37°C (24 h) for bacteria and 25°C (24-72 h) for the fungal species. After the appropriate incubation period, the bacterial cultures were compared with the turbidity (opacity) standard.

Screening for antimicrobial properties

Antimicrobial properties of plant extracts were tested by well-in-Agar method with some modifications. The culture plates were prepared by pouring twenty ml of Mueller Hinton Agar medium into sterile Petri dishes. The inoculum suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform distribution of bacteria and fungal spores. Using a flamed cork borer, well of 5 mm diameter was made in the media at a distance of 1-2 cm from the periphery of the plates. These plates were labeled and 0.2 ml of each extract (at different concentration of extracts i.e. 100 µg, 200 µg, 300 µg and 400 µg) was added aseptically into the well. Then the plates were incubated for 24 h (at 37°C) for bacteria and 25°C (24-72 h) for fungi. The effectiveness of these extracts was recorded by measuring the diameter of the inhibition zone at the end of incubation periods. The standard antimicrobial agents and respective solvents were used as positive and negative controls. All the antimicrobial assays were carried out in triplicate. The antimicrobial activity was assessed by measuring the diameter of growth inhibition zone (including disc) of each organism after incubation periods.

RESULTS AND DISCUSSION

The results of antimicrobial screening of the acetone and ethanol leaf extracts of *Zizyphus oenoplia* were tested against *Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri*, *Staphylococcus aureus*, *Bacillus* sp. and two important human pathogenic fungi namely, *Candida albicans* and *Cryptococcus neoformans* (**Table 1**). The acetone leaf extracts of *Z. oenoplia* showed moderate activity against the bacterial pathogens, namely *Pseudomonas putida* (10-13 mm), *Vibrio cholerae* (10 -12 mm) and *Shigella flexneri* (10 -13 mm) while the same extract was inactive against all other bacterial and fungal species.

Similarly, the ethanolic leaf extracts of *Z. oenoplia* exhibited moderate to mild antibacterial activity against *Vibrio cholerae* (10-14 mm) and *Pseudomonas putida* (10-13 mm) and the level of inhibition increased with the concentration of the extracts. The ethanolic leaf extract showed very poor activity even at higher concentrations (10 mm for *Bacillus* species at 400 µg) and other species (including fungal pathogens) exhibited resistance (no zone of inhibition) to the extracts used. Similar studies using leaves and bark extracts (chloroform and methanol) of *Z. oenoplia* too had better activity against bacterial strains like *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* (Shoeb *et al*. 2005). On the other hand, Schuhly *et al*. (1999), while studying the stem bark extracts of *Zizyphus joazerio* reported considerable antibacterial activity against Gram-positive bacteria. These reports, including ours, are in agreement with the studies of Nazif (2002) who isolated phytochemicals from the fruit extracts of *Zizyphus spina-christi* and studied their antibacterial properties and Shah (2006) who investigated the biological activities of fruit extracts from *Zizyphus sativa*. Similarly other plants belonging to Rhamnaceae have also been reported to have some antimicrobial properties e.g., *Ventilago madaraspatana* (Subhalakshmi *et al*. 2005), *Condalia buxifolia* (Morel *et al*. 2002), *Scutia buxifolia* (Morel *et al*. 2005), etc.

Based on our findings, most of the pathogens studied have considerable resistance against the various concentrations of leaf extracts, which is supported by Rajakaruna *et al.* (2002), who used the same plant. In future the phytochemical constituents of *Z. oenoplia* leaves, which is more desirable to provide authentic support of the effectiveness of the extracts tested will help in formulating drugs against the pathogens.

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