



Comparative screening of antibacterial and antifungal activities of some Weeds and medicinal plants leaf extracts: An *in-vitro* study

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ABSTRACT

The aim of the study was to investigate and compare antibacterial and antifungal activity of leaves extract taken from four different plants *Quisqualis indica* Linn., *Calotropis procera* Ait., *Achyranthes aspera* Linn., and *Ocimum sanctum* Linn. against ten microorganisms comprising of five bacteria (*Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and five fungi (*Alternaria porri*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium chrysogenum*) using well diffusion method. The *in vitro* study revealed that methanol extract was more effective than aqueous extract. Leaf extracts of *Quisqualis indica* Linn. and *Achyranthes aspera* Linn. was reported to be more effective on fungal species and on contrary leaf extracts of *Calotropis procera* Ait. and *Ocimum sanctum* Linn. was found more effective on bacterial species.

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Introduction

Plants has been a source of medicinal agents for thousand of years, this has lead to an increasing interest in the investigation of different extracts obtained from plants as potential source of new antimicrobial agents (1). 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 and antifungal activity against various common pathogens is increasing. All parts of plants including stem, root, flower, bark, leaves possess antimicrobial property (2).

Antimicrobial substances are the substance that inhibits the growth and existence of microorganisms. These microorganisms could be pathogenic or non-pathogenic hence, antimicrobial substances are used in the treatment of various ailments. Quite a large number of antimicrobial substances exist and they are gotten from diverse sources such as microbes, plants, animals and chemicals (3, 4).

Quisqualis indica Linn. commonly known as Rangoon Creeper is an excellent vine for outdoor gardens belonging to family Combretaceae. Not much work has been reported on this plant. Its seeds and leaves are used for therapeutic purposes, including as antigelminthozoe tool, especially against tapeworm, as well as a sedative. It has also been reported to use successfully against stomach pain, diarrhea, colds, skin parasites, and rickettsia.

Calotropis procera Ait. is member of plant family Asclepiadaceae, a shrub of about 6m high and is widely distributed in the tropics. The plant is erect, tall, large, much branched and perennial with milky latex throughout. In India, the secretion from the root bark is traditionally used for the treatment of skin diseases and intestinal worms (5).

Achyranthes aspera Linn. belongs to family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as weed throughout India and used by traditional healers for the treatment of fever, dysentery and diabetes (6).

Ethanol crude extract showed high larvicidal activity (7) as well as extract of the leaves and stem of the plant reported to be inhibiting growth of some microorganisms (8).

Ocimum sanctum Linn. very commonly known as Tulsi belonging to family Lamiaceae is a time-tested premier medicinal herb It is a plant of Indian origin and apart from its religious value it is used in Ayurvedic medicine since ancient times. It is herb used to treat a variety of illness ranging from diabetes, arthritis, bronchitis, throat infection, skin diseases etc. (9). Its antimicrobial property has been tested against variety of microorganisms (10).

Considering the aforesaid properties of four different plants a comparative analysis of their leaf extract was done for antibacterial and antifungal properties against ten microorganisms comprising of five bacteria (*Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and five fungi (*Alternaria porri*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium chrysogenum*).

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Methods

Collection of Plant Materials

Fresh plants of *Quisqualis indica* Linn., *Calotropis procera* Ait., *Achyranthes aspera* Linn., and *Ocimum sanctum* Linn. were collected randomly from different parts of Noida & Greater Noida city. Leaves from fresh plants were taken and washed under running tap water, and rinsed with distilled water and air dried.

Extraction procedures

(a) For 20% aqueous extract preparation, 2g of air dried plant material was crushed in 10ml of sterile water in pestle and

mortar. The extract was filtered using Whatman's Filter paper No. 1. The filtrate was collected and stored at 4°C in sterile tubes.

(b) For 20% alcohol extract, 2g of air dried plant material was crushed in 10ml of methyl alcohol in pestle & mortar and incubated for 2-3 days for complete evaporation of methyl alcohol and later dried in oven. Dried mixture was mixed with 10ml of methyl alcohol and filtered using Whatman's Filter paper No. 1. The filtrate was collected and stored at 4°C in sterile tubes.

Preparation of Test Organisms

Ten microorganisms used in this study as test organisms comprising of five bacteria (*Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and five fungi (*Alternaria porri*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium chrysogenum*) were obtained from Department of Microbiology, IARI, New Delhi. The typed cultures of bacteria and fungi were sub-cultured on Nutrient agar (NA) and Potato dextrose agar (PDA) slants respectively and stored at 4°C until required for study.

Antimicrobial activity testing

The antimicrobial assay was performed by agar well diffusion method adapted according to (11). For assessing the antibacterial activity of the prepared extracts, 0.6ml of standardized bacterial stock suspension was thoroughly mixed with 60ml of sterile nutrient agar. 20ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The plates were allowed to dry for at least 15 minutes. A sterile cork borer No. 4 was used to make wells of 10mm diameter in each plate for extracts. The bottoms of the wells were sealed with one drop of the sterile nutrient agar, to prevent diffusion of the extracts under the agar. In 3 of 4 wells 100µl of extract (aqueous/alcohol) was poured, the 4th well marked as control, filled with 100µl sterile water/alcohol. Then the plates were incubated overnight at 37°C. The activity was evidenced by the presence of zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extract when compared to the controls. The same method as for bacteria was adopted for fungal species, instead of nutrient agar PDA was used.

Results & Discussion

The antibacterial and antifungal properties of the leaf extracts of four different plants on the test isolates were revealed (Table 1). The results indicate that the extracts from the plants studied showed inhibition of growth against tested microorganisms with to various degrees.

Successful prediction of extracted compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional practitioners make use of water as a primer solvent, but on the first observation Table 1 shows that methanol was a better solvent for extracting antimicrobial substance from these plants compared to water. This may be due to better solubility of the active compounds in organic solvents (12).

Alcoholic leaf extract of *Quisqualis indica* was found to be more effective in antifungal activity than antimicrobial showing highest activity (21mm) against *Alternaria porri* and *Penicillium chrysogenum* and low activity (11mm) against *Enterobacter aerogenes* whereas on contrary alcoholic leaf extracts of *Calotropis procera* was reported to be more effective against

bacterial species showing highest activity (15mm) against *Klebsiella pneumoniae* and lowest (06mm) against *Aspergillus* spp. Similar results were obtained when extracts from other than leaf was taken and worked on different microorganisms (13, 14, 15).

Aqueous and alcoholic extracts of *Achyranthus aspera* showed considerable degree of inhibition against test microorganisms. Methanolic extract reported to be more effective against fungal species showing highest inhibition (20mm) against *Alternaria porri* and *Penicillium chrysogenum* and lowest (12mm) against bacteria *E. coli*. The results obtained were supported by earlier observation reported on fungal growth (16). The aqueous and alcoholic extracts of *Osimum sanctum* shows lowest antibacterial and antifungal activities among all leaf extracts. This may be because the antimicrobial effect of leaf extract was tested at a particular concentration only; antimicrobial activity of *Osimum sanctum* at various concentration levels gives different results (17). In contrast the antibacterial activity was found to be relatively higher to some extent in comparison to antifungal activity.

According to the antibacterial assay done for screening purpose, the gram-positive bacteria *Bacillus subtilis* was reported to be more susceptible in comparison to gram-negative bacterial species. These observation are likely to be the results of the differences in cell wall structures between gram-positive and gram-negative bacteria, with gram-negative outer membrane acting as a barrier to many environmental substances including antibiotics (18).

The overall comparative studies shows that the methanol leaf extract revealed highest degree of antimicrobial activity for *Bacillus subtilis* and higher degree of antifungal activity for *Alternaria porri*, *Aspergillus oryzae* and *Penicillium chrysogenum* when compared with that of other fungal spp tested.

Comparative analyses of antimicrobial and antifungal activities of different plant leaf extract against these microorganisms is an indication that there is possibility of discovering alternative antibiotic substance in these plants for the development of newer antimicrobial agents and carry out further pharmacological evaluation.

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Table 1: In vitro Antibacterial and Antifungal activity of Aqueous and Methanolic Leaf Extracts of different plants using Well Diffusion Method

Test Organisms	<i>Quisqualis indica</i> L.		<i>Calotropis procera</i> Ait.		<i>Achyranthes aspera</i> L.		<i>Ocimum sanctum</i> L.	
	Ae	Me	Ae	Me	Ae	Me	Ae	Me
	Zone of Inhibition (mm)*							
Bacterial species								
<i>Bacillus subtilis</i>	16	18	13	15	13	15	06	08
<i>Enterobacter aerogenes</i>	09	11	10	12	12	13	05	07
<i>Escherichia coli</i>	14	17	08	10	10	12	04	06
<i>Klebsiella pneumoniae</i>	12	15	12	14	13	14	05	07
<i>Pseudomonas aeruginosa</i>	13	15	06	08	12	14	04	05
Fungal species								
<i>Alternaria porri</i>	20	21	05	07	18	20	04	05
<i>Aspergillus flavus</i>	17	19	04	06	17	19	03	05
<i>Aspergillus niger</i>	15	17	04	06	18	19	04	06
<i>Aspergillus oryzae</i>	16	18	04	06	20	21	03	04
<i>Penicillium chrysogenum</i>	18	21	06	08	18	20	04	06

Ae: Aqueous extract; Me: Methanolic extract

*Values are mean of three replicates