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In-vitro Antibacterial Screening of Selected Folklore Indian Medicinal Plants with few Clinical Pathogens

V.S. Sudhanandh[1]*, J.K. Arjun[2], A. K. Faisal[1], M.V. Ani[2], V.S. Renjini[1] and K. Narendra Babu[1]

[1]Microbiology laboratory, Chemical Sciences Division, Centre for Earth Science Studies, Akkulam, Thiruvananthapuram-31, Kerala, India

[2]School of Biosciences, Mahatma Gandhi University, Priyadarsini Hills, Athirampuzha, Kottayam, Kerala, India

ABSTRACT

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Antibacterial activities of 7 plant extracts were evaluated against 5 bacterial strains using well diffusion assay at different concentration (10%, 20% and 30%) and the results were compared with therapeutically used antibiotics. Rapid formation of inhibition zones within 24 hours of incubation was obtained with ethanolic extracts (30%) of *Justicia gendarussa* against all tested strains. Ethanol extracts (30%) of the *Gymnema sylvestre* produces a maximum inhibition zone of 24mm against *Pseudomonas aeruginosa* which are known to be multi-resistant to drugs, while the standard values lies behind 17mm. Cluster analysis was applied to find out the similar groups of solvent extracts in their antibacterial action. Ethanolic plant extracts figured a separate cluster with the high antibacterial activity. Significant correlations were observed between the extract concentrations and antibacterial activity ($P > 0.01$).

Key words: Antibacterial activity, plant extracts, inhibition zone, cluster analysis

INTRODUCTION

The number of multi-drug resistant strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing due to the indiscriminate use of commercial antimicrobial treatment of infectious diseases[1,2]. In addition in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is a need to search new infection-fighting strategies to control microbial infections[3].

In concern to drawbacks of the conventional medicine, the use of natural products as an alternate to the conventional treatment in healing and treatment of various diseases still remain as one of the best reservoir of new structural types[4]. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing[5]. About 80% of individuals from developed countries use traditional medicine which has compound derived from medicinal plants[6]. Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions[7]. The first step towards this goal is the in vitro antibacterial assay[8]. Although hundreds of plant species have been tested for antimicrobial properties; the vast majority of them have not been adequately valuated[9].

The aim of this study was to evaluate the antimicrobial activity of medicinal plants in Ayurveda (ancient health care system) and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore extracts of the seven plants i.e, *Gymnema sylvestre (*Chakara kolli*), Aegle marmelos* (Koovalam)*, Adhatoda vasica* (Adalodakam)*, Wrigthia tinctoria* (Vettupala)*, Vitex negundo* (Karinochi)*, Ricinus communis (*Aavanak) *and Justicia gendarussa* (Vatham kolli) were evaluated for their antimicrobial potentials.

MATERIALS AND METHODS

Collection of plant material and drug preparation

Different plant leaves used in Ayurveda and traditional systems of medicine were collected from the local areas of Trivandrum district, Kerala, India after careful identification. 250 gm of shade dried leaves were powdered and extracted successively with 3 different solvents- ethanol, acetone and methanol to afford corresponding fractions. Solvents were evaporated under reduced pressure. The dried extracts were scraped from the plates and 10%, 20% and 30% concentrations are prepared by dissolving the powder in dimethyl sulfoxide (DMSO) as solvent. All the preparations were stored at 4°C till analyzed.

***Address for Correspondence:**

V.S. Sudhanandh, Microbiology laboratory, Chemical Sciences Division, Centre for Earth Science Studies, Akkulam, Thiruvananthapuram-31, Kerala, India

E-mail: sudhanandhvs@gmail.com

Antibacterial assay

The antibacterial effect was tested by well diffusion method, using following strains *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis* and *Vibrio cholerae,* collected from various hospitals after their purity checked by Hi-Media biochemical test kits. The antibacterial effect was tested by agar well plate diffusion method[10]. Briefly, nutrient agar plates were swabbed with the test organism by following the procedure described in antibiotic susceptibility testing. Wells of diameter 4mm were cut into the inoculated plates by using sterile cork borer. To the wells 10, 20 and 30% of the extracts were added and the plates were incubated at 37°C for overnight. After incubation, the plates were analyzed for the zones of growth inhibition. The diameter of the zones of growth inhibition including the width of the well was measured in millimeter (mm) and recorded. The results were compared with the standard antibiotic disc (Gentamicin).

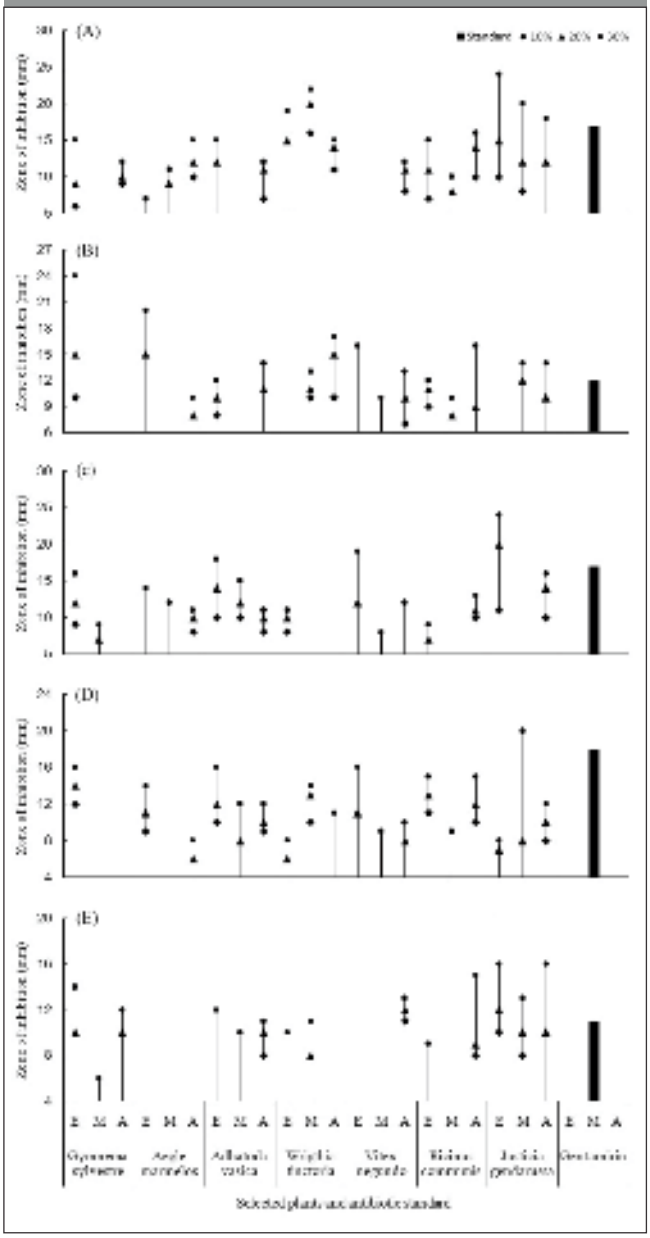
RESULTS AND DISCUSSION

The present study was designed to obtain preliminary information on the antimicrobial effect of seven Indian folk medicinal plants on certain pathogenic microorganisms. As a matter of fact, inhibition zones for the plant extracts obtained by well diffusion were equal or larger than those of the common antibiotics used in this study (Gentamicin). The well diffusion method was preferred to be used in this study since it was found to be better than the disc diffusion method. The data pertaining to the antimicrobial potential of the plant extracts and standard antibiotics are presented in fig. 1(A-E).

Among all the twenty one solvent extracts of plant leaves, 81% extracts showed antibacterial activity against one or more bacteria and the extracts showed significant differences in their efficacy. Inhibitory zones for all isolates were determined as 0–24 mm. Of the selected plants ethanol extracts of *Gymnema sylvestre* and *Justicia gendarussa* showed exceptionally prominent activity. Former showed maximum activity against *P. aeruginosa* (24mm zone diameter of inhibition) and the latter against *V. cholerae* and *E. c*oli (24mm zone diameter of inhibition) even at lower concentrations nearly equal to the standard antibacterial agent. Such results are quite interesting as *P. aeruginosa* and *E.coli* are already known to be multi-resistant to drugs[11].

The observed antibacterial activity is due to the potent bioactive phyto- constituents present in the extract. In general the plant extracts much more active against gram positive bacteria than gram negative bacteria[12,13]. The density of lipopolysaccharide layer in the outer surface of bacterial cell wall is much lower in gram positive bacteria when compared to that of gram negative bacteria[14]. So certain antibacterial compounds can easily reach the peptidoglycan layer of the cell wall of gram positive bacteria and penetrate into the cytoplasm. It causes the loose of the cells turgor pressure, with a subsequent disorganization of the internal organelles[15]. This explains the maximum sensitivity shown by *P. aeruginosa* to ethanol extract of *Gymnema sylvestre* in this study.

Fig. 1: Antibacterial activity of different solvent (E- ethanol, M- methanol, A- Acetone) plant extracts against selected pathogens, Fig. 1(A): Plant extracts against *Vibrio cholerae* Fig. 1(B): Plant extracts against *Pseudomonas aerugisosa* Fig. 1(C): Plant extracts against *Escherichia coli,* Fig. 1(D): Plant extracts against *Staphylococcus aureus,* Fig. 1(E): Plant extracts against *Streptococcus faecalis)*



The control plate representing DMSO did not exhibit inhibition on the tested bacteria where as standard antifungal drug, gentamicin, have antibacterial activity even at 5µg/ well. Extract concentration was found to have a direct effect on the efficacy of plant extracts evaluated. At 10 and 20 % concentration the activity of the extracts of *Justicia gendarussa* against *E. coli* were 11 and 20 mm (zone diameter of inhibition), respectively but this increased to 24 mm when the extracts concentration was changed by 30%. Statistical analysis also reveals the significant correlation observed between the extract concentrations and antibacterial activity ($P > 0.01$). It also supports the earlier investigation that the tannins isolated from *Justicia gendarussa* possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance[16].

Cluster analysis was applied to find out the similar plant groups based on their antibacterial activity with tested pathogens (Fig. 2). *Ricinus communis, Aegle marmelos and Adhatoda vasica* forms the first cluster with low antibacterial activity. The second cluster provides information on plants having moderate antibacterial activity and which includes *Vitex negundo and Gymnema sylvestre. Wrigthia tinctoria and Justicia gendarussa* provides the maximum activity against most of the pathogenic bacteria with all the solvent and forms the cluster 3. These differences in the antimicrobial activity of the extracts might be due to the chemical composition of the plants, the species of microorganisms used and the method of extraction[17].

Similarity groups of different solvent extracts (Fig. 3) in their antibacterial action were also revealed by cluster analysis as different solvents have various degrees of solubility for different phytoconstituents[18]. Majority of the ethanolic extracts of plants form a separate cluster as the extracts provided the maximum activity. Studies suggests that ethanolic solvent extracts may contain active fractions like tannins[19], Polyphenols[20,21], Flavonol[22], Terpenoids[23], Sterols[24] and Alkaloids[25] which are having antimicrobial properties. Among the ethanolic extracts of *Aegle marmelos Justicia gendarussa* and *Vitex negundo* have produce better results than the standard gentamicin. Study suggests that ethanolic extracts of screened plants would be more obliging in treating diseases caused by bacterial pathogens.

Fig. 2: Cluster analysis showing the similarity groups of plants in their activity.

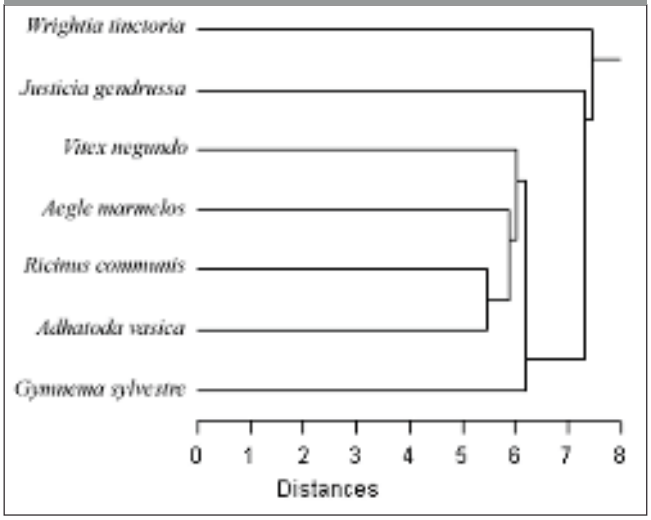
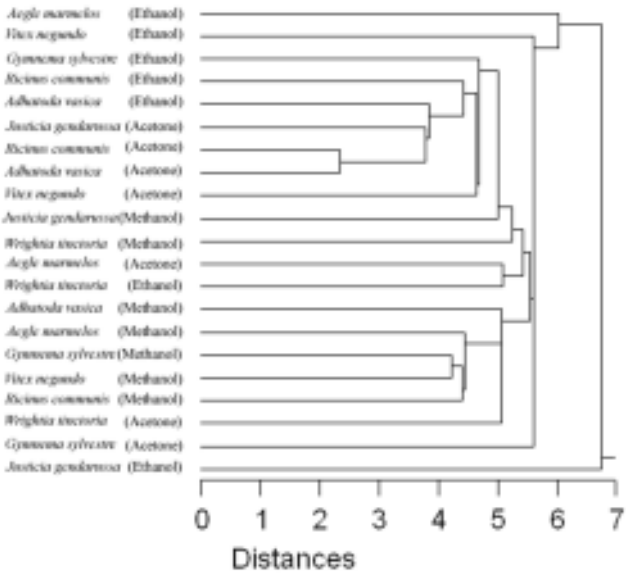


Fig. 3: Cluster analysis showing the similarity groups different solvent extracts in their activity.



In general, the plant *Justicia gendarussa* is found to be having broad spectrum of antibacterial activity. This folk medicine was active against all bacteria investigated – *P. aeruginosa, E. coli, S. aureus*, *S. faecalis* and *V. cholerae*. The demonstration of activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. In particular, the authors may recommend that the ethanolic extract of *Justicia gendarussa* to be used as a multi resistant drug to treat diseases caused by bacterial pathogens. However extensive bioprocess parameter studies should be undertaken for the ethanolic extract of Justicia gendarussa as a strong antibacterial agent against bacterial diseases especially with *E. coli*, *V. cholerae* and *S. faecalis.*

With the current spread of antibiotic resistance almost at geometrical scale and obvious challenges confronted with by medical practitioners in the treatment of infectious diseases proper attention should be given to such plants to reap the potential antimicrobial benefits inherent in them[26,27]. Actual

antimicrobial ingredients need to be extracted and identified, also its tolerable values in the human body as well as any toxic effects on human and animal tissues be investigated accordingly.

CONCLUSION

The present study ascertains the value of plants used in Ayurveda and Folk medicine, which could be of considerable interest to the development of new drugs. Scientists from the divergent fields should investigate plants with a new eye on their antimicrobial usefulness. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation and finally subjecting to clinical trials with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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