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Antibacterial activity of selected Medicinal plants from South India

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Abstract

Plan: *Antibacterial activity of leaves and seed of Pongamia pinnata and Lawsonia innermis have been studied for their antimicrobial activities using agar well diffusion method.*

Methodology: *The selected bacterial strains, namely Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus and Pseudomonas aeroginosa isolated from PSG Hospital, Coimbatore, Tamilnadu were selected for the study. Maximum zone of inhibition of the active compounds was evaluated by using NCCLS Method. The plant extracts were tested with the standard positive control chloramphenicol.*

Outcome: *Among the three solvents analysed ethanolic leaf extract of Pongamia pinnata and Lowsonia innermis exhibited a maximum resistance against Bacillus subtilus and possessed a highest inhibitory zone against Pseudomonas aeroginosa and Micrococcus luteus respectively when compared to other bacterial strains.*

Keywords: *Pongamia pinnata, Lawsonia innermis antibacterial activity, plant extracts, agar well diffusion method.*

1. Introduction

Plants have the major advantage of being the most effective and cheaper alternative source of drugs[1]. The local use of natural plants as primary health remedies, due to their pharmacological properties is quiet common in Asia, Latin America and Africa[2]. Medicinal plants contain substances that can be used for therapeutic purposes or which are used as precursors for the synthesis of useful drugs[3]. Researches on use of plants as the source of drugs and dietary supplements are increasing in recent years. Plants have been found *in vitro* to have antimicrobial property as they are rich in a wide variety of secondary metabolites[4].

Potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000 – 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological and pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its instituents.

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Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics[5, 6]

Researchers are increasingly turning their attention to natural products looking for leads to develop better drugs against many microbial infections [7, 8, 9]. More than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importance[10]. The medicinal value of plant lies in some chemical substances present in them. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds[11].

Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. A number of medicinal plants have been documented as important source of bioactive compounds[12].

The expanding bacterial resistance to antibiotics has become a growing concern worldwide[13]. Intensive care physicians consider antibiotic resistance bacterial a significant problem in the treatment of patients[14]. Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains [15, 16]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [17]. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [18.]

The indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms[19]. Plants containing active compounds are able to inhibit the microbial growth. Studying plant based antimicrobial properties provides additional information in developing natural antibiotics and discovering the alternative of antimicrobial drugs for the treatment of infectious diseases[20].

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs[21]. In developing countries were medicines are quite expensive, the investigation on antimicrobial activities from ethnomedicinal plants may still be needed.

2. Materials and Methods

2.1. Collection of test materials

Leaves and seeds of *Pongamia pinnata (L) Pierre and Lawsonia innermis (L)* were collected from Tamil Nadu district, India located at 11°N Latitude and 77°E longitude.

2.2. Preparation of leaf and seed powder

Fresh leaves were collected, washed in water and air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. Ripe fruits that had fallen on the ground were collected. The seeds were separated, washed in water and dried (under shade). After drying for four weeks, the seeds were ground in an electric pulverizer to get the powder.

2.3. Preparation of extracts

10 g of each of the leaf powder or the seed powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zerohaze filter paper (A Grade, SD's). These powders were subjected to extraction[22, 23]. Petroleum ether (60 – 80°C) extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity.

2.4. Test microorganism

The five bacterial strains used in the present study were the clinical isolates obtained from P.S.G. Hospitals, Coimbatore. The bacterial strains used were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus and Pseudomonas aeroginosa.* The effects of various plant extracts on the several bacterial strains were assayed by agar well diffusion method.

2.4.1. Procedure

Petri plates containing 20 ml Muller Hinton medium were seeded with 24 hr culture of bacterial strains. Well were cut and 20 µl of the plant extracts (namely petroleum ether, chloroform and ethanol extracts) were added. The plates were then incubated at 37°C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well[24]. Chloramphenicol was used as positive control.

3. Results and Discussion

The antibacterial activity of leaf and seed extracts of *Pongamia pinnata and Lawsonia innermis* was screened *in vitro* by agar well diffusion method using chloramphenicol as the standard positive control against selected bacterial strains. The results of leaf and seed extracts of *P. pinnata and L. innermis* using solvents of different polarity were shown in Table 1 and Table 2 respectively.

Among the three solvents analyzed maximum antibacterial activity was exhibited by the ethanolic leaf extract against *B. subtilis* (24.66 ± 0.58) and minimum activity was observed in petroleum ether leaf extract against the species *E. coli* (7.66 ± 2.08). Similar observations were recorded by[25] in which the extract of *Paris polyphylla and Zanthoxylum armatum* controlled the growth of *E. coli,* which indicated that the plant could inhibit the activity of bacteria, which can cause diarrhea and dysentery.

Table 1: Antibacterial activity of leaf and seed extracts against test organisms

Micro organisms	Extracts (Diameter of the zone of inhibition in mm)							
	Pongamia pinnata leaf				Pongamia pinnata seed			
	Cont.	PE	CH	E	Cont.	PE	CH	E
B. Subitilis	20.66±1.15	17.33 ± 1.15	20.66 ± 0.58	24.66± 0.58	22.33± 0.58	26.66 ± 1.57	11 ± 1	15 ± 1
E. Coli	22.66± 1.15	7.66 ± 2.08	12 ± 2	17 ± 1	18.66± 0.58	17 ± 1	16.33 ± 1.52	10 ± 2
E. aerogenes	33 ± 1.73	9±1	11 ± 1	11 ± 1	25.33± 0.58	11 ± 1	11 ± 1	11 ± 1
M. luteus	32.33± 0.58	11.33 ± 1.52	12.66 ± 1.52	13.33 ± 1.52	26.66± 1.15	10 ± 2	21 ± 1	20.66 ± 3.05
P. aeroginosa	29.66± 0.58	16.33 ± 1.52	12.33 ± 1.52	10 ± 2	12.33± 0.57	8 ± 1	14.66 ± 1.52	27.33 ± 2.08

Values are mean inhibition zone (mm) ± S.D, Cont. – Chloramphenicol, PE – Petroleum Ether, CH – Chloroform, E – Ethanol

In case of seed, ethanol extract possessed highest inhibitory zone against *P. aeroginosa* (27.33 ± 2.08) and minimum inhibitory zone was observed in petroleum ether extract against *P. aeroginosa* (8 ± 1). Similar findings were reported by[26] in which *Humulus lupulus* showed the strongest antibacterial activity with the maximum activity in 90% ethanol extract against *Staphylococcus aureus* and MRSA strains of *S. aureus.*

Table 2: Antibacterial activity of leaf and seed extracts against the organisms

Micro organisms	Extracts (Diameter of the zone of inhibition in mm)							
	Lawsonia innermis leaf				Lawsonia innermis seed			
	Cont.	PE	CH	E	Cont.	PE	CH	E
B. Subitilis	20.66± 1.15	18.33 ± 0.58	21.33 ± 1.15	20 ± 2	22.33± 0.57	19.33± 1.52	10.33 ± 0.57	22 ± 1
E. Coli	22.66± 1.15	15 ± 1	16.66 ± 1.52	16 ± 1	18.66± 0.58	10 ± 2	10.66 ± 1.52	11.66 ± 1.52
E. aerogenes	33 ± 1.73	14.33 ± 1.15	20.66 ± 1.15	19.66 ± 1.52	25.33± 0.58	11 ± 1	9.66 ± 0.58	8.66 ± 0.58
M. luteus	32.33± 0.58	21.66 ± 1.15	21.66 ± 1.52	23 ± 2	26.66± 1.15	21.33± 3.21	17.33 ± 2.08	21 ± 1
P. aeroginosa	29.66± 0.58	21 ± 1	15 ± 1	17.66 ± 0.58	42.33± 0.57	18 ± 1	10.66 ± 1.15	24 ± 1

Values are mean inhibition zone (mm) ± S.D. Cont. – Chloramphenicol, PE – Petroleum Ether, CH – Chloroform, E – Ethanol

L. innermis leaf extract showed a highest inhibition zone (23 ± 2) against *M. luteus* in ethanol extract and lowest inhibitory activity was recorded against *E. aerogenes* (14.33 ± 1.15) in petroleum ether extract. Rather the inhibition to the growth of the organisms by the extracts can be attributed to the potency of active principles as well as the strain and possibly, weakness in the strength of organism used. Similar results were reported by[27] in which the alcoholic extract of *Woodfordia fruitcosa* showed maximum antibacterial activity against gram-negative *Klebsiella pneumonia*

Petroleum ether seed extract of *L. innermis* showed a highest inhibitory activity against the positive bacterial strains *M. luteus* (21.33 ± 3.21) whereas the lowest inhibition zone was recorded against *E. aerogenes* (8.66 ± 0.58) in ethanolic seed extract.

Similar observations were reported by[28] in which the methanol extract of *Aloe ferox, Ptaeroxylon obliquum and Calpurnia aurea* inhibited both gram positive and gram negative bacteria but there was more inhibition on gram positive on gram positive strains.

It can be concluded that from the overall results of the antibacterial assays in the study, both the plants species form a good basis for further investigation in the potential discovery of new natural bioactive compounds. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem by continuing studies to develop new drugs naturally.

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