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Antimicrobial efficacy of *Senna auriculata, Pongamia glabra* and *Indigofera tinctoria* against pathogenic Microorganisms

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Abstract: Different parts of medicinal plants have been used to cure specific ailments. Today, there is wide spread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacy-therapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore.All microbes show resistance to antibiotics. Also, Indian Medicinal plants contain many antimicrobial agents and properties. Hence, an attempt has been made to find out newer components from the Indian Medicinal plants, which have antimicrobial properties and also cheaper and non – toxic to the environment.

Key words :*Terminalia arjuna*, *Pongamia glabra*, *Senna auriculata and Indigofera tinctoria Escherichia coli.*

Introduction

There has been growing interest in the investigation of the natural products from plants for the discovery of new antimicrobial and antioxidant agents as well as an alternative route for the substitution of synthetic chemicals, side effects of which are always in question. For this, the essential oils and the extracts of many plants have been prepared and screened for their antimicrobial and antioxidant activities, leading to the accumulation of a large number of reports in the literature concerning the above mentioned properties of plants (1) Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (2). Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (3).

In natural products drug discovery is important to follow systems theory and system biology applications to facilitate the process (4). Routine random efforts are not likely to increase the desired success rate of discovery, while experience indicates that a modified collection policy offered better chances for the discovery and development of agents for treatment of AIDS and cancer (5). Numerous drugs have entered the international pharmacopoeia through ethnobotany and traditional medicine (6,7). There are many similarities in traditional systems of medicine such as Siddha and Ayurveda as well as ethnomedicines being connected to each other as ‘great traditions and little traditions’. All botanical drugswill have to fulfill the international requirements on quality, safety and efficacy (8).

The development of novel,efficient and inexpensive drugs is thus of great importance. In a constant attempt

to improve their quality of life, men have used plants as source of food, shelter, clothing, medicine, cosmetics, and for seeking relief from hardship of life. Some plants are known as medicinal because they contain active substances that cause certain reactions, from relenting to the cure of diseases, on the human pathogenic organism (9). Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups (10); and their use, especially in South America, contributes significantly to primary health care (11). For centuries, medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate. Elsewhere, many potent drugs have been purified from medicinal plants including anti-malarial, anti-cancer, anti-diabetic and antibacterial compounds (12)

Dilution tests widely used to determine the susceptibility of organisms, Kirby-Bauer test results are interpreted using a table that related to the degree of microbial resistance. The values in the table were derived by finding the MIC values and zone diameters for many different microbial strains (13). A plot of MIC (on a logarithmic scale) versus zone inhibition diameter (arithmetic scale) is prepared for each antibiotic. These plots are then used to find the zone diameters corresponding to the drug concentrations actually reached in the body. If the zone diameter of the lowest level reached in the body is smaller than that seen with the test pathogen, the pathogen should have an MIC value low enough to be destroyed by the drug. A pathogen with too high an MIC value (too small a Zone diameter) is resistant to the agent at normal body concentrations.

Materials and Methods

1.1 Plant Materials

Plant materials of *Terminalia arjuna*, *Pongamia glabra*, *Senna auriculata and Indigofera tinctoria* were collected from their authorized Ayurvedic store.

Terminalia arjuna is a medicinal plant of the genus *Terminalia*, widely used for its curative properties in organic / functional heart problems including angina, hypertension and deposits in arteries

Pongamia glabra is a deciduous legume tree that grows to about 15-25 meters in height with a large canopy which spreads equally wide. The leaves are a soft, shiny burgundy in early summer and mature to a glossy, deep green as the season progresses. Flowering starts in general after 3-4 years. Cropping of pods and single almond sized seeds can occur by 4-6 years.

The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at base on side of next petiole into a filliform point and persistent.

True indigo is a shrub one to two meters high. It may be an annual, biennial, or perennial, depending on the climate in which it is grown. It has light green pinnate leaves and sheafs of pink or violet flowers. The plant is a legume, so it is rotated into fields to improve the soil.

Aqueous and Alcoholic extraction of Plants

- The plant materials were dried in shade and powdered in a mechanical grinder.
- The powder of the plant materials were initially de-fatted with petroleum Benzene (60-80°C), followed by 1000 ml of ethanol by using a Soxhlet extractor for 72 hrs at a temp not exceeding the boiling point of the solvent (Lin et.al.,1999).
- The extract was filtered using Whattman filter paper (No.1) and then was concentrated in vacuum and dried at 45°C for ethanol elimination, and the extracts were kept in sterile bottles, under refrigerated conditions, until further use.
- The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. the extract thus obtained was directly used in the assay of antimicrobial activity.

1.2 Antibiotics

Broad spectrum antibiotics, Penicillin were used as control drug.

1.3 Bacterial Strains

The strains of microorganisms (*Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus*, and *Bacillus subtilis*) were used. Pure slant cultures were ordered.

1.4 Determination of Antimicrobial Activity

Antimicrobial activity was measured using the standard method of diffusion disc plates on agar and the MIC was calculated using dilution method (Kirby-Bauer method).

Dilution Methods

Dilution susceptibility testing methods were used to determine the minimal concentration of antimicrobial

to inhibit or kill the microorganisms. This was achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are generally tested in $\log_2$ serial dilutions (two fold).

Broth Dilution Method

The Broth Dilution Method is a simple procedure for testing a small number of isolates, even single isolate.

Materials

- Sterile graduated pipettes of 10 ml, 5 ml, and 1 ml.
- Sterile test tubes and test tube racks.
- Overnight broth culture of test and plant extracts.
- Required antibiotics and plant extracts.
- Sterile Distilled Water – 500 ml and freshly prepared nutrient broth medium.

Preparation of microorganisms for experiment

The pure cultures of organisms (*Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus*, and *Bacillus subtilis*) were sub-cultured in nutrient broth. They were inoculated, separately, into nutrient broth and kept at 37°C for 24 hours. Then, they were kept at 4°C until use.

Growth Method

- At least three to five well-isolated colonies, of the same morphological type, were selected from an agar plate culture of a particular microorganism. The top of each colony was touched with a loop, and the growth was transferred into a tube, containing 4 to 5 ml of nutrient broth medium.
- The broth culture was incubated at 35°C for 8 hours.
- After the incubation period broth culture became turbid.

1.5 Disc Diffusion Method: Reagents

(a) Mueller-Hinton Agar Medium

Mueller-Hinton Agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons.

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- Medium is transparent, so that the inhibition zone can be visualized clearly.
- It gives satisfactory growth of most non fastidious pathogens.
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Preparation of Mueller-Hinton Agar

- Mueller-Hinton Agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions.
- Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath.
- he freshly prepared and cooled medium was poured into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
- The agar medium should be allowed to cool to room temp. and, unless the plate is used the same day, stored in a refrigerator.
- Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
- A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hrs or longer.

(b) Preparation of antibiotic stock solutions

Powders of those two antibiotics (Penicillin and Tetracycline) were brought from authorized medical shop. They were accurately weighted and dissolved in sterile distilled water in appropriate dilutions to yield the required concentrations. The stocks were kept in aliquotes of 5 ml volumes and frozen at -20°C.

(c) Preparation of plant extracts solutions for the experiment

The dried plant extracts were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations (1.0mg/ ml, 1.5mg/ ml, 2.0 mg/ ml, and 2.5 mg/ ml). They are kept under refrigeration.

(d) Preparation of dried filter paper discs

- Whattman filter paper (No.1) was used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.
- After the sterilization, the discs were poured into the different concentration of broad spectrum antibiotics and into the prepared plant extract solutions and again kept under refrigeration for 24 hrs.

Results and Discussion

Table-1 shows that Minimum Inhibitory Concentration of test material *Pongamia glabra* with test cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis*

S.No	Name of the Cultures	Drug	Concentration of *Pongamia glabra*	Minimum Inhibitory Concentration
1	*Escherichia coli*	*Pongamia glabra*	0.5 mg/ml	6.2 mm
			1.0 mg/ml	11.9 mm
			1.5 mg/ml	16.3 mm
			2.0 mg/ml	15.4 mm
			2.5 mg/ml	18.9 mm
2	*Pseudomonas aeruginosa*	*Pongamia glabra*	0.5 mg/ml	10.2 mm
			1.0 mg/ml	8.3 mm
			1.5 mg/ml	11.2 mm
			2.0 mg/ml	12.5 mm
			2.5 mg/ml	14.6 mm
3	*Staphylococcus aureus*	*Pongamia glabra*	0.5 mg/ml	9.4 mm
			1.0 mg/ml	12.9 mm
			1.5 mg/ml	14.5 mm
			2.0 mg/ml	15.5 mm
			2.5 mg/ml	17.9 mm
4	*Bacillus subtilis*	*Pongamia glabra*	0.5 mg/ml	19.1 mm
			1.0 mg/ml	13.2 mm
			1.5 mg/ml	11.1 mm
			2.0 mg/ml	13.7 mm
			2.5 mg/ml	11.8 mm

Table -2 shows thatMinimum Inhibitory Concentration of test material *Senna auriculata* with test cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and acillus subtilis*

S.No	Name of the Cultures	Drug	Concentration of *Senna auriculata*	Minimum Inhibitory Concentration
1	*Escherichia coli*	*Senna auriculata*	0.5 mg/ml	7.2 mm
			1.0 mg/ml	14.3 mm
			1.5 mg/ml	15.4 mm
			2.0 mg/ml	16.2 mm
			2.5 mg/ml	20.1 mm
2	*Pseudomonas aeruginosa*	*Senna suriculata*	0.5 mg/ml	9.2 mm
			1.0 mg/ml	11.6 mm
			1.5 mg/ml	15.7 mm
			2.0 mg/ml	18.1 mm
			2.5 mg/ml	20.5 mm
3	*Staphylococcus aureus*	*Senna auriculata*	0.5 mg/ml	15.3 mm
			1.0 mg/ml	16.5 mm
			1.5 mg/ml	19.1 mm
			2.0 mg/ml	20.0 mm
			2.5 mg/ml	22.7 mm
4	*Bacillus subtilis*	*Senna auriculata*	0.5 mg/ml	11.4 mm
			1.0 mg/ml	13.4 mm
			1.5 mg/ml	16.2 mm
			2.0 mg/ml	19.5 mm
			2.5 mg/ml	20.6 mm

Table -3 shows thatMinimum Inhibitory Concentration of test material *Indigofera tinctoria* with test cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis*

S.No	Name of the Cultures	Drug	Concentration of *Indigofera tinctoria*	Minimum Inhibitory Concentration
1	*Escherichia coli*	*Indigofera tinctoria*	0.5 mg/ml	11.2 mm
			1.0 mg/ml	14.3 mm
			1.5 mg/ml	15.4 mm
			2.0 mg/ml	17.2 mm
			2.5 mg/ml	21.1 mm
2	*Pseudomonas aeruginosa*	*Indigofers tinctoria*	0.5 mg/ml	9.2 mm
			1.0 mg/ml	12.6 mm
			1.5 mg/ml	13.7 mm
			2.0 mg/ml	14.1 mm
			2.5 mg/ml	17.5 mm
3	*Staphylococcus aureus*	*Indigofera tinctoria*	0.5 mg/ml	15.3 mm
			1.0 mg/ml	17.5 mm
			1.5 mg/ml	20.1 mm
			2.0 mg/ml	21.0 mm
			2.5 mg/ml	23.7 mm
4	*Bacillus subtilis*	*Indigofera tinctoria*	0.5 mg/ml	11.4 mm
			1.0 mg/ml	13.4 mm
			1.5 mg/ml	16.2 mm
			2.0 mg/ml	19.5 mm
			2.5 mg/ml	20.6 mm

Discussions

The zones of inhibition of the test plant extracts of , *Pongamia glabra*, *Senna auriculata* and *Indigofera tinctoria* were compared with those of the control drugs – Penicillin and Tetracycline against the gram negative organisms of *Escherichia coli* & *Pseudomonas aeruginosa* and the gram positive organisms of *Staphylococcus aureus* & *Bacillus subtilis*.It is found that the zones of inhibition of , in all the concentrations, as compared to the control drugs (14).*Penicillin* and *Tetracycline* – against the same microbes. When we compare the zones of inhibition of *Pongamia glabra* against the test microbes, we find them to be to be less, at all concentrations, as compared to the control drugs.(15) In some cases, as against *Staphylococcus aureus* they are found to be closer in values to the control drugs. There is an anomaly in the values against Bacilus subtilis, where the value at 1 mg/ml is less than that at 0.5 mg/ml. Similarly, the value at 2 mg/ml is less than that at 1.5 mg/ml. This may have happened due to errors in the experimental set up and measurements.Plant extracts of *Senna auriculata* have inhibitory effects on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Their zones of inhibition are more than that of the control drugs against them. *E. coli* is found to be little resistant at lower concentrations, but at higher concentration, the antimicrobial activity is found to be at par with the control drugs.The antimicrobial activity of *Indigofera tinctoria* is high against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* is high. This can be suitably substantiated by comparing the zones of inhibition, at all concentrations, with the control drugs; where in, it was found that the zones of inhibitions of plant extract are more than the control drugs. In case of E. coli, the antimicrobial activity is slightly lesser at lower concentrations. But at higher concentrations, the antimicrobial activity is much more.(16)

So, we see that at lower concentrations of all test plant materials, E. coli shows resistance and has less zones of inhibition.*Senna auriculata* and *Indigofera tinctoria* have good microbial activities against all the test microbes at all concentrations and their zones of inhibition are also sufficiently high.These two plants could also be further tested against other microbes too(17). In the present study, the antimicrobial properties of *Pongamia glabra*, *Senna auriculata and Indigofera tinctoria* were investigated against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural sources.

Conclusion

Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options, Hence monitoring resistance is of paramount importance.Antimicrobial resistance monitoring will help to review the current status of antimicrobial resistance locally, nationally and globally and helpful in minimizing the consequence of drug resistance..Hence the present study was aimed to focus the antibacterial properties of Pongamia Glabra, Senna Auriculata, and Indigoferra Tintoria on gram positive and negative organisms.

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