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ORIGINAL RESEARCH ARTICLE

Screening of *Phyllanthus amarus, Acalypha indica* and *Datura metel* for its antimicrobial activity against selected pathogens

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ABSTRACT

Several hundreds of plant genera are used medicinally and plants are vital sources for potent and powerful drugs. The present study was conducted to screen the pharmacological activity of the ethanol and acetone extract of *Phyllanthus amarus, Acalypha* and *indica Datura metel* for its antimicrobial activity against selected pathogen. The bacterial *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Bacillus subtilis.* The fungal *Aspergillus niger* and *Penicilliumsp.* The collected leaf samples were powdered and the bioactive compounds were extracted by using ethanol and acetone in a Soxhelet extractor. The antimicrobial activity was determined by using Disc diffusion method. Ethanol and acetone extracts with different concentrations (100mg/ml, 200mg/ml and 300mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO). The inhibitory effect of ethanol extract was relatively high when compared to acetone extract. The study of antimicrobial activity of herbal plant extract of *Datura metel, Acalypha indica* and *Phyllanthus amarus* showed that the ethanol extract shows promising antimicrobial activity against bacterial and fungal human pathogens when compared to acetone extract. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators and provide a support to use of the plant in traditional medicine.

Key words: Ethanol extract, Zone of inhibition, *Datura metel, Acalypha indica,* Bacteria and Fungi.

1. INTRODUCTION

Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [1]. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry [2]. A large number of these plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs which have high export potential. [3].

Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [4]. Interest in a large number of traditional natural products has increased [5] It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [6]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [7]. The aqueous and ethanolic extracts of *Azadiracha indica* have antimicrobial activity against *Microsporum canis, Aspergillus fumigatus, Candida albicans, Escherichia coli* and *Staphylococcus aureus* by disc diffusion method [8].

Tested the antimicrobial activity of ethanol extract obtained from *Thevetia peruviana* against

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bacterial species of *Escherichia coli*, *Streptococcus lactis*, *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and fungal species of *Fusarium oxysporum*, *Alternaria helianthii*, *Curvularia lunata*, *Aspergillus niger* and *Penicillium* sp.[9]. Evaluated the antimicrobial activity of *Acorus calamus* rhizome and leaf extracts obtained with different solvents *viz.*, petroleum ether, chloroform, hexane and ethyl acetate. Extracts obtained with ethyl acetate among others were found to be highly effective [10].

The *Phyllanthus* genus of the family Euphorbiaceae was first identified in Central and Southern India in 18th century. It is commonly called carry me seed, stone-breaker, windbreaker,gulf leaf flower or gala of wind [11, 12]. *Acalypha indica* are two closely related species of Meliaceae. The former is popularly known as Indian Kuppaimeni (margosa tree) or Indian lilac, and the latter as the Persian lilac. The plant is a small-to medium-sized deciduous tree. It grows to a height of 5 to 15 m tall and 30 to 60 cm in diameter. *Datura metel* (Family Labiatae) is a many branched, erect, stout and aromatic herb about 75 cms high. This small herb is found throughout India and is cultivated, worshiped in temples and houses of Hindus. This is commonly known as Vishnu-Priya, Oomathai in Sanskrit, Kala- Oomathai in Hindi and India's Holy Basil in English.

2. MATERIALS AND METHODS

Collection of plant materials

Healthy leaves of *Phyllanthus amarus, Acalypha indica* and *Datura metel* were collected the Herbal garden, Department of Microbiology, Annamalai University, Chidambaram, Tamil Nadu. The leaves of *Acalypha indica* were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

50 g of powdered leaves of *Phyllanthus amarus, Acalypha indica* and *Datura metel* with 200 ml of ethanol at 56-60°C and acetone in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [13].

Test microorganisms

Microorganisms chosen were obtained from the laboratory of Department of Microbiology, Annamalai University. The organisms used for this study were *Staphylococcus aureus, Streptococcus pyogenes,Pseudomonas aeruginosa,Bacillus subtilis, Aspergillus niger,Penicillium* sp.

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at room temperature for 3-5 hours till a moderate turbidity was developed.

Preparation of paper disc

Disc of 5 mm diameter were pretreated using Whatman filter paper No.1. These were sterilized in the hot air oven at 160°C for 1 hour. The discs were impregnated with 20µl of different solvent extracts (Ethanol and Acetone) at different concentration ranging from 100-300 mg/ml for the five different seeds to check their antimicrobial activity. Control paper discs were also prepared by using 1% DMSO.

Determination of antibacterial activity

Disc diffusion method was adopted for evaluation of antimicrobial activity of five different medicinal leaves. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled at 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The disc impregnated with respective leaf extract at different concentration (100-300 mg/ml) individually were placed on the four corners of each petridishes, control disc was also placed. The petridishes were then incubated at 37°C for 24 hours. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm [14].

3. RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [15]. The screening of plants

usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

The antimicrobial activity of ethanol and acetone extract of *Phyllanthus amarus* showed inhibitory effect on a concentration of 100, 200, 300mg/ml and the results were showed in (**Table 1 & 2**). The antimicrobial activity of ethanol and acetone extract of *Azadiracha indica* showed inhibitory effect on a concentration of 100, 200, 300mg/ml and the results were showed in (**Table 3 & 4**). The antimicrobial activity of ethanol and acetone extract of *Datura metel* showed inhibitory effect on a concentration of 100, 200, 300mg/ml and the results were showed in (**Table 5 & 6**).

The antimicrobial activity of *Datura metel* leaves against bacteria and yeast. The diameter of inhibition zone recorded in *Escherichia coli* was 18 mm for 22 µl of oil. These differences may be attributed due to presence of antibacterial component in high concentration in local variety enhancing the medicinal importance of indigenous essential oil [16]. Proposed that the aqueous and ethanolic extracts of *Azadiracha indica* have antimicrobial activity against *Microsporum canis, Aspergillus fumigatus, Candida albicans, Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. There was no zone of inhibition of *Acalypha indica* towards *Aspergillus fumigatus* and *Candida albicans*. The leaves and roots of the aqueous extract of *Azadiracha indica* inhibit the growth of *Microsporum canis* [17].

The antibacterial activity of valuable compounds from various solvent extracts of *Azadiracha indica, Blumea lacera* and *Melia azadirachta* against *Escherichia coli, Pseudomonas aeruginosa, Serratia maraceseuns* and *Staphylococcus aureus* by tube diffusion method. Acetone and methanol extracts of all plants showed strong antibacterial effect, where as petroleum ether and aqueous did not exhibit any effect. *Pseudomonas aeruginosa* and *Serratia marcesenes* were relatively more sensitive [18]. Some bacteria posses mechanism for converting substance toxic to it into non-toxic substances. *Staphylococcus aureus* and other species produce the enzyme penicillinase, which convert penicillin to penicillinic acid which could not inhibit its growth. [19] *Phyllanthus amarus* can help control infection caused by *Staphylococcus aureus* which is a major pathogen of human infections varying from food poisoning or minor skin infections [20] to severe life threatening infections, such as septicaemia [21] and disseminated abscesses in all organs and *Escherichia coli* which causes Urinary Tract Infection (UTI), diarrhea, sepsis and meningitis.

Tested the aqueous and ethanol extracts of *Acalypha indica* against *Microsporum canis, Aspergillus fumigatus, Candida albicans, Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. There was no zone of inhibition of *Acalypha indica* towards *Aspergillus fumigatus* and *Candida albicans* [22]. Tested the medicinal plant extracts of *Curcuma longa, Acalypha indica*, and *Anona sqamosa* by Cold percolation method against Dermatophytic isolates. *Curcuma lounga* showed antifungal effect against *Trichophyton rubrum* and *Microsporum gypseum*. These two organisms were found to be resistant towards *Acalypha indica* and *Anona sqamosa*. The other dermatophytes were resistant to all medicinal plants tested [23].

Table 1: Antimicrobial activity of ethanol extract of *Phyllanthus amarus*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	15mm	21mm	24mm
2	*Streptococcus pyogenes*	-	10mm	13mm	17mm
3	*Pseudomonas aeruginosa*	-	18mm	23mm	26mm
4	*Bacillus subtilis*	-	20mm	25mm	28mm
5	*Aspergillus niger*	-	5mm	8mm	12mm
6	*Penicillium* sp.	-	7mm	11mm	15mm

Table 2: Antimicrobial activities of acetone extract of *Phyllanthus amarus*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	12mm	18mm	21mm
2	*Streptococcus pyogenes*	-	7mm	10mm	14mm
3	*Pseudomonas aeruginosa*	-	15mm	20mm	23mm
4	*Bacillus subtilis*	-	17mm	22mm	26mm
5	*Aspergillus niger*	-	2mm	5mm	9mm
6	*Penicillium* sp.	-	4mm	8mm	12mm

Table 3: Antimicrobial activity of ethanol extract of *Acalypha indica*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	10 mm	12 mm	13 mm
2	*Streptococcus pyogenes*	-	8 mm	9 mm	11mm
3	*Pseudomonas aeruginosa*	-	11 mm	13 mm	16 mm
4	*Bacillus subtilis*	-	13 mm	15 mm	18 mm
5	*Aspergillus niger*	-	-	-	-
6	*Penicillium* sp.	-	2mm	6mm	10mm

Table 4: Antimicrobial activity of acetone extract of *Acalypha indica*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	15mm	17mm	18mm
2	*Streptococcus pyogenes*	-	-	-	-
3	*Pseudomonas aeruginosa*	-	9mm	12mm	15mm
4	*Bacillus subtilis*	-	18mm	22mm	28mm
5	*Aspergillus niger*	-	-	-	-
6	*Penicillium* sp.	-	7mm	9mm	10mm

Table 5: Antimicrobial activity of ethanol extract of *Datura metel*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	7mm	10mm	16mm
2	*Streptococcus pyogenes*	-	13mm	17mm	23mm
3	*Pseudomonas aeruginosa*	-	10mm	15mm	21mm
4	*Bacillus subtilis*	-	-	-	-
5	*Aspergillus niger*	-	3mm	7mm	12mm
6	*Penicillium* sp.	-	-	-	-

Table 6: Antimicrobial activity of acetone extract of *Datura metel*

S. No	Bacteria	Concentration of the extract (mg/ml) and zone of inhibition (mm)			
		Control	100 mg/ml	200 mg/ml	300 mg/ml
1	*Staphylococcus aureus*	-	11mm	13mm	15mm
2	*Streptococcus pyogenes*	-	-	-	-
3	*Pseudomonas aeruginosa*	-	5mm	9mm	11mm
4	*Bacillus subtilis*	-	14mm	18mm	24mm
5	*Aspergillus niger*	-	-	-	-
6	*Penicillium* sp.	-	3mm	5mm	6mm

4. CONCLUSION

The study of antimicrobial activity of herbal plant extract of *Phyllanthus amarus*, *Acalypha indica* and *Datura metel* showed that the ethanol extract shows promising antimicrobial activity against bacterial and fungal human pathogens when compared to acetone extract. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

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