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# Antimicrobial Activity of Solvent Extracts of *Ocimum sanctum*, *Azadirachta Indica* and *Phyllanthus amarus* Against Clinical Pathogens

*T. Bharathi, K. Kolanjinathan and P. Saranraj*

Division of Microbiology, Faculty of Science, Annamalai University,
Annamalai Nagar-608 002, Tamil Nadu, India

**Abstract:** In the present study, leaves of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* were collected and the collected leaves were shade dried and powdered by hand crushing. The preparations of different leaves extract was done through modified method. Three different solvents *viz*., methanol, chloroform and ethyl acetate were used to study the antimicrobial activity of herbal plants. Disc diffusion method was adopted for evaluation of antimicrobial activity of five different medicinal leaves. The antimicrobial activity of methanol, chloroform and ethyl acetate leaf extract of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* were studied in different concentrations (100 mg/ml, 200 mg/ml, 300 mg/ml). Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. No zone of inhibition was observed in the negative DMSO control. Methanol extract showed more inhibitory effect when compared to other solvents. Among the three plants, maximum inhibition activity was exhibited by *Phyllanthus amarus* followed by *Azadirachta indica* and *Ocimum sanctum*.

**Key words:** Antimicrobial Activity · Bacteria · Fungi · *Ocimum sanctum* · *Azadirachta indica* and *phyllanthus amarus*

## INTRODUCTION

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. A country like India is very much suited for development of drugs from medicinal plants. Because of its vase and wide variations in soil and climate, the Indian sub-continent is suitable for cultivation of large number of medicinal and aromatic plant which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavour and food and agrochemical industries. 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 [1].

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rig veda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing [2].

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. 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 [3].

**Corresponding Author:** Kolanjinathan K., Division of Microbiology, Faculty of Science,
Annamalai University, Annamalai Nagar-608 002., Tamil Nadu, India.

*Ocimum sanctum* (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, Tulsi in Sanskrit, Kala- Tulsi in Hindi and India's Holy Basil in English. The leaves, seeds and root of this plant have been used in indigenous Ayurvedic medicine. The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds. These constituents significantly vary with time, cultivation process and storage. The nutritional and pharmacological properties of the whole herb in natural form, as it has been traditionally used, result from synergistic interaction of many different active phytochemicals, consequently, the overall effects of Tulsi cannot be fully duplicated with isolated compound or extracts. Due to its inherent botanical and biochemical complexity, standardization of the active components of Tulsi so far is very complex. Tulsi is traditionally taken in a variety of forms including cold, hot or dried leaf tea (Herbal teas), powdered leaf, alcohol tinctures and oil (Ghee) preparations, as well as seed, root, stem formulations, both systemically and topically. In addition to various extracts, isolated compound is also administered by injection in human clinical studies and animal experiments.

*Azadirachta indica* are two closely related species of Meliaceae. The former is popularly known as Indian Neem (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. The plant is characterized by the presence of a spreading, dense and dark green crown. Its bark is dark brown in color, relatively smooth and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green (Ventral) and relatively pale (Dorsal). Flowers are white with purple stripes and are characterized by the presence of a typical fragrance (Odor). Fruits or berries are yellow, round, smooth and fleshy. Dried fruits are hard with 4 to 5 seeds. *Azadirachta indica* Linn. is native to tropical Asia. It is widespread and naturalized in most of the tropics and subtropical countries. Leaves: leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent. Root: resolvent, deobstruent. Seeds: rheumatism. Leaves: Leaf extract has insecticidal property (Azadirachtin) that repels insects in clothing. The leaves can also serve as feed for goats. Seed oil: The oil is the most active medicinal product of the plant. It is used as antiseptic for sores and ulcers that show no tendency to heal. It is also used for rheumatism and skin diseases such as ringworm and scabies. Internally, the oil is useful in malaria fever and leprosy. Powdered dust of fruit insecticidal, crude extract from wood and bark insecticidal, oil antibacterial.

The *Phyllanthus* genus of the family Euphorbiaceae was first identified in Central and Southern India in 18$^{th}$ century. It is commonly called carry me seed, stone-breaker, wind breaker, gulf leaf flower or gala of wind [4]. There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide. The *Phyllanthus* is one of the genus that falls under this enormous family. *Phyllanthus* has about 750-800 species, found in tropical and subtropical regions worldwide. Green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [5].

Screening of compounds obtained from plants for their pharmacological activity has resulted in the isolation of innumerable therapeutic agents. *Phyllanthus amarus* is an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers. In folk medicine *Phyllanthus amarus* has reportedly been used to treat jaundice, diabetes, otitis, diarrhoea, swelling, skin ulcer, gastrointestinal disturbances and blocks DNA polymerase in the case of hepatitis B virus during reproduction [6]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant [7].

Several compounds including alkaloids, flavonoids, lignans, phenols and terpenes were isolated from this plant and some of them interact with most key enzymes. In traditional medicine, it is used for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties [8]. *Phyllanthus amarus* leaf extract as a hepatoprotective agent. The plant is also used in the treatment of stomach disorders, skin diseases and cold [9]. It has anti-diarrhea effect [10]. Its anti-viral activity against hepatitis B virus has been established [11] anti-carcinogenic [12] and antimutagenic activities [13]. It also has anti-nociceptive and anti-inflammatory activities [14] antidiabetic and antilipidemic potentials [15]. *Phyllanthus amarus* has been reported to include antioxidant, antiviral, antibacterial, hypoglycemic, cancer suppressive and anthelmintic effects.

## MATERIALS AND METHODS

**Collection of Plant Materials:** Healthy leaves of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* were collected from Annamalai University Medicinal Garden at Annamalai Nagar, Cuddalore District. The plant

materials like leaves were washed thoroughly with tap water and then with sterilized distilled water for the removal of dust and sand particles. The leaves were shade dried and powdered by hand crushing. The powdered samples were hermetically sealed in separate polythene bags until the time of the extraction. This was used as the raw material for the extraction of antimicrobial compounds against the microbes used.

**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* and *Penicillium* sp. The bacterial isolates were confirmed using Gram staining, motility test, platting on selective medium, catalase test, oxidase test and other biochemical test and also inoculating them on specific media. The fungal isolates were identified by Lactophenol cotton blue staining (LPCB) and plating of Sabouraud's Dextrose agar (SDA).

**Preparation of Leaf Extract:** The preparations of different leaves extract was done through modified method of Priya and Deepak Ganjewala [16].

**Methanol Extraction Method:** The shade dried leaf materials were used for the methanol extraction procedure; about 5 gm of leaf powder were weighed and mixed with ethanol (1:3 w/v) which was incubated for two days. After the incubation period, the slurry was filtered through Whattman No.1 filter paper in a beaker and allowed it for evaporation. The residue was dissolved with Dimethyl sulfoxide (DMSO) with different concentrations and checked it for its Antimicrobial activity.

**Ethyl Acetate Extraction Method:** The shade dried leaf materials were used for the ethyl acetate extraction procedure; about 5 gm of leaf powder were weighed and mixed with ethyl acetate (1:3 w/v) which was incubated for two days. After the incubation period, the slurry was filtered through Whattman No.1 filter paper in a beaker and allowed it for evaporation. The residue was dissolved with Dimethyl sulfoxide (DMSO) with different concentrations and checked it for its antimicrobial activity.

**Chloroform Extraction Method:** The shade dried leaf materials were used for the chloroform extraction procedure; about 5 gm of leaf powder were weighed and mixed with chloroform (1:3 w/v) which was incubated for two days. After the incubation period, the slurry was filtered through Whattman No.1 filter paper in a beaker and allowed it for evaporation. The residue was dissolved with Dimethyl sulfoxide (DMSO) with different concentrations and checked it for its antimicrobial activity.

**Evaluation of Antimicrobial Activity of Medicinal Plants:** Antimicrobial activity of medicinal plant was tested through several methods like Tube dilution method, well plate method and Disc diffusion method. Disc diffusion method is most commonly employed method to evaluate the antimicrobial activity. In the present study, Disc diffusion method was used to test the antimicrobial activity of leaves of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus*. The Disc diffusion technique was introduced by Kirby-Bauer.

**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. The turbidity was matched with 0.5 Mc Farland standards.

**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 (Methanol, Ethyl acetate and Chloroform) 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.

**Antimicrobial Susceptibility Test:** Disc diffusion method was adopted for the 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 extracts 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.

## RESULTS

**Antimicrobial Activity of *Ocimum Sanctum:*** The antimicrobial activity of methanol extract of *Ocimum sanctum* was analyzed in the present study and the results were furnished in Table-1. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (24 mm, 28 mm and 34 mm) followed by *Pseudomonas aeruginosa* (25 mm, 30 mm and 31 mm), *Staphylococcus aureus* (21 mm, 23 mm and 25 mm) and *Streptococcus pyogenes* (8 mm, 13 mm and 15 mm). The fungi *Penicillium* sp. (13 mm, 15 mm and 16 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 12 mm). No zone of inhibition was observed in the negative DMSO control. The antimicrobial activity of chloroform extract of *Ocimum sanctum* was determined in the present investigation and the results were given in Table-2. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was noticed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml.

Table 1: Antimicrobial activity of methanol extract of *Ocimum sanctum*

| | | Concentration of the extract (mg/ml) and zone of inhibition (mm in dm) | | | |
|---|---|---|---|---|---|
| S. No | Bacteria | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 21 mm | 23 mm | 25 mm |
| 2 | *Streptococcus pyogenes* | NZ | 8 mm | 13 mm | 15 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 25 mm | 30 mm | 31 mm |
| 4 | *Bacillus subtilis* | NZ | 24 mm | 28 mm | 34 mm |
| 5 | *Aspergillus niger* | NZ | NZ | 10 mm | 12 mm |
| 6 | *Penicillium* sp. | NZ | 13 mm | 15 mm | 16 mm |

NZ-No Zone

Table 2: Antimicrobial activity of chloroform extract of *Ocimum sanctum*

| | | Concentration of the extract (mg/ml) and zone of inhibition (mm in dm) | | | |
|---|---|---|---|---|---|
| S.No | Bacteria | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 16 mm | 18 mm | 20 mm |
| 2 | *Streptococcus pyogenes* | NZ | NZ | 8 mm | 10 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 20 mm | 25 mm | 26 mm |
| 4 | *Bacillus subtilis* | NZ | 19 mm | 23 mm | 29 mm |
| 5 | *Aspergillus niger* | NZ | NZ | NZ | NZ |
| 6 | *Penicillium* sp. | NZ | 8 mm | 10 mm | 11 mm |

NZ-No Zone

Table 3: Antimicrobial activity of ethyl acetate extract of *Ocimum sanctum*

| | | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| S.No | Bacteria | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 13 mm | 15 mm | 17 mm |
| 2 | *Streptococcus pyogenes* | NZ | NZ | NZ | 8 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 17 mm | 22 mm | 23 mm |
| 4 | *Bacillus subtilis* | NZ | 16 mm | 20 mm | 26 mm |
| 5 | *Aspergillus niger* | NZ | NZ | NZ | NZ |
| 6 | *Penicillium* sp. | NZ | NZ | NZ | NZ |

NZ-No Zone

Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (19 mm, 23 mm and 29 mm) followed by *Pseudomonas aeruginosa* (20 mm, 25 mm and 26 mm), *Staphylococcus aureus* (16 mm, 18 mm and 20 mm) and *Streptococcus pyogenes* (No zone, 8 mm and 10 mm). The fungi *Penicillium* sp. (8 mm, 10 mm and 11 mm) showed more inhibitory activity than *Aspergillus niger*. No zone of inhibition was observed against *Aspergillus niger* and negative DMSO control.

The antimicrobial activity of ethyl acetate extract of *Ocimum sanctum* was evaluated in the present research and the results were presented in Table-3. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was recorded at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (16 mm, 20 mm and 26 mm) followed by *Pseudomonas aeruginosa* (17 mm, 22 mm and 23 mm), *Staphylococcus aureus* (13 mm, 15 mm and 17 mm) and *Streptococcus pyogenes* (No zone, No zone and 8 mm). The ethyl acetate extract of *Ocimum sanctum* showed resistance against *Penicillium* sp. and *Aspergillus niger*. No zone of inhibition was observed against *Penicillium* sp., *Aspergillus niger* and negative DMSO control.

**Antimicrobial Activity of *Azadirachta Indica:*** The antimicrobial activity of methanol extract of *Azadirachta indica* was studied and the results were showed in Table-4. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (28 mm, 32 mm and 35 mm) followed by *Staphylococcus aureus* (25 mm, 26 mm and 28 mm), *Pseudomonas aeruginosa* (19 mm, 22 mm and 25 mm) and *Streptococcus pyogenes* (17 mm, 20 mm and 23 mm). The fungi *Penicillium* sp. (15 mm, 17 mm and 20 mm) showed more inhibitory activity when compared to *Aspergillus niger* (12 mm, 14 mm and 18 mm). No zone of inhibition was observed in the negative DMSO control.

The antimicrobial activity of chloroform extract of *Azadirachta indica* was tested and the results were tabulated in Table-5. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was noticed in the bacteria *Bacillus subtilis* (23 mm, 27 mm and 33 mm) followed by *Staphylococcus aureus* (20 mm, 21 mm and 23 mm), *Pseudomonas aeruginosa* (14 mm, 17 mm and 20 mm) and *Streptococcus pyogenes* (12 mm, 15 mm and 18 mm). The fungi *Penicillium* sp. (10 mm, 12 mm and 15 mm) showed more inhibitory activity when compared to *Aspergillus niger* (7 mm, 9 mm and 11 mm). No zone of inhibition was observed in the negative DMSO control.

The antimicrobial activity of ethyl acetate extract of *Azadirachta indica* was investigated and the results were furnished in Table-6. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml. Maximum antibacterial activity was recorded in the bacteria *Bacillus subtilis*

Table 4: Antimicrobial activity of methanol extract of *Azadirachta indica*

| | | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| S.No | Bacteria | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 25 mm | 26 mm | 28 mm |
| 2 | *Streptococcus pyogenes* | NZ | 17 mm | 20 mm | 23 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 19 mm | 22 mm | 25 mm |
| 4 | *Bacillus subtilis* | NZ | 28 mm | 32 mm | 35 mm |
| 5 | *Aspergillus niger* | NZ | 15 mm | 17 mm | 20 mm |
| 6 | *Penicillium* sp. | NZ | 12 mm | 14 mm | 18 mm |

NZ-No Zone

Table 5: Antimicrobial activity of chloroform extract of *Azadirachta indica*

| | | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| S.No | Bacteria | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 20 mm | 21 mm | 23 mm |
| 2 | *Streptococcus pyogenes* | NZ | 12 mm | 15 mm | 18 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 14 mm | 17 mm | 20 mm |
| 4 | *Bacillus subtilis* | NZ | 23 mm | 27 mm | 33 mm |
| 5 | *Aspergillus niger* | NZ | 10 mm | 12 mm | 15 mm |
| 6 | *Penicillium* sp. | NZ | 7mm | 9mm | 10mm |

NZ-No Zone

Table 6: Antimicrobial activity of ethyl acetate extract of *Azadirachta indica*

| S.No | Bacteria | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 17 mm | 18 mm | 20 mm |
| 2 | *Streptococcus pyogenes* | NZ | 9 mm | 12 mm | 15 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 11 mm | 14 mm | 17 mm |
| 4 | *Bacillus subtilis* | NZ | 20 mm | 24 mm | 30 mm |
| 5 | *Aspergillus niger* | NZ | NZ | 9 mm | 12 mm |
| 6 | *Penicillium* sp. | NZ | NZ | NZ | 8 mm |

NZ-No Zone

Table 7: Antimicrobial activities of methanol extract of *Phyllanthus amarus*

| S.No | Bacteria | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 22 mm | 28 mm | 31 mm |
| 2 | *Streptococcus pyogenes* | NZ | 17 mm | 20 mm | 24 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 25 mm | 29 mm | 32 mm |
| 4 | *Bacillus subtilis* | NZ | 27 mm | 30 mm | 35 mm |
| 5 | *Aspergillus niger* | NZ | 12 mm | 15 mm | 19 mm |
| 6 | *Penicillium* sp. | NZ | 15 mm | 18 mm | 21 mm |

NZ-No Zone

Table 8: Antimicrobial activities of chloroform extract of *Phyllanthus amarus*

| S.No | Bacteria | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 17 mm | 23 mm | 26 mm |
| 2 | *Streptococcus pyogenes* | NZ | 12 mm | 15 mm | 19 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 20 mm | 25 mm | 28 mm |
| 4 | *Bacillus subtilis* | NZ | 22 mm | 27 mm | 31 mm |
| 5 | *Aspergillus niger* | NZ | NZ | 10 mm | 14 mm |
| 6 | *Penicillium* sp. | NZ | 10 mm | 13 mm | 16 mm |

NZ-No Zone

Table 9: Antimicrobial activity of ethyl acetate extract of *Phyllanthus amarus*

| S.No | Bacteria | Concentration of the extract (mg/ml) and zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | DMSO Control | 100 mg/ml | 200 mg/ml | 300 mg/ml |
| 1 | *Staphylococcus aureus* | NZ | 14 mm | 20 mm | 23 mm |
| 2 | *Streptococcus pyogenes* | NZ | 9 mm | 12 mm | 16 mm |
| 3 | *Pseudomonas aeruginosa* | NZ | 17 mm | 22 mm | 25 mm |
| 4 | *Bacillus subtilis* | NZ | 19 mm | 24 mm | 28 mm |
| 5 | *Aspergillus niger* | NZ | NZ | 9 mm | 11 mm |
| 6 | *Penicillium* sp. | NZ | NZ | 10 mm | 13 mm |

NZ-No Zone

(20 mm, 24 mm and 30 mm) followed by *Staphylococcus aureus* (17 mm, 18 mm and 20 mm), *Pseudomonas aeruginosa* (11 mm, 14 mm and 17 mm) and *Streptococcus pyogenes* (9 mm, 12 mm and 15 mm). The fungi *Penicillium* sp. (No zone, 9 mm and 12 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, No zone and 8 mm). No zone of inhibition was observed in the negative DMSO control.

**Antimicrobial Activity of *Phyllanthus Amarus:*** The antimicrobial activity of methanol extract of *Phyllanthus amarus* was determined and the results were given in Table-7. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was noticed in the bacteria *Bacillus subtilis* (27 mm, 30 mm and 35 mm) followed by *Pseudomonas aeruginosa* (25 mm, 29 mm and 32 mm), *Staphylococcus aureus* (22 mm, 28 mm and 31 mm) and *Streptococcus pyogenes* (17 mm, 20 mm and 24 mm). The fungi *Penicillium* sp. (15 mm, 18 mm and 21 mm) showed more inhibitory activity when compared to *Aspergillus niger* (12 mm, 15 mm and 19 mm). No zone of inhibition was observed in the negative DMSO control.

The antimicrobial activity of chloroform extract of *Phyllanthus amarus* was evaluated and the results were presented in Table-8. Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (22 mm, 27 mm and 31 mm) followed by *Pseudomonas aeruginosa* (20 mm, 25 mm and 28 mm), *Staphylococcus aureus* (17 mm, 23 mm and 26 mm) and *Streptococcus pyogenes* (12 mm, 15 mm and 19 mm). The fungi *Penicillium* sp. (10 mm, 13 mm and 16 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 14 mm). No zone of inhibition was observed in the negative DMSO control.

The antimicrobial activity of ethyl acetate extract of *Phyllanthus amarus* was investigated and the results were given in Table-9. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (19 mm, 24 mm and 28 mm) followed by *Pseudomonas aeruginosa* (17 mm, 22 mm and 25 mm), *Staphylococcus aureus* (14 mm, 20 mm and 23 mm) and *Streptococcus pyogenes* (9 mm, 12 mm and 16 mm). The fungi *Penicillium* sp. (No zone, 9 mm and 11 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 13 mm). No zone of inhibition was observed in the negative DMSO control.

## DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. 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 [17]. 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 [18].

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [19]. Much work has been done on ethnomedicinal plants in India [20]. In the present research, antimicrobial activity of methanol, chloroform and ethyl acetate leaf extract of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* were studied in different concentrations (100 mg/ml, 200 mg/ml, 300 mg/ml). Among the three concentrations (100 mg/ml, 200, 300 mg/ml) used, maximum inhibitory zone was observed at 300 mg/ml followed by 200 mg/ml and 100 mg/ml. No zone of inhibition was observed in the negative DMSO control. Among the three plants, maximum inhibition activity was exhibited by *Phyllanthus amarus* followed by *Azadirachta indica* and *Ocimum sanctum*. The results of the present study coincide with the findings of Saranraj and Sivasakthivelan [21] and Sekar *et al*. [22].

Interest in a large number of traditional natural products has increased [23]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [24]. 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 [25]. The antimicrobial activity of methanol extract of *Ocimum sanctum* was analyzed in the present study. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (24 mm, 28 mm and 34 mm) followed by *Pseudomonas aeruginosa* (25 mm, 30 mm and 31 mm), *Staphylococcus aureus* (21 mm, 23 mm and 25 mm) and *Streptococcus pyogenes* (8 mm, 13 mm and 15 mm). The fungi *Penicillium* sp. (13 mm, 15 mm and 16 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 12 mm).

Sattar and Shahid [26] studied the composition of Tulasi leaves and indicated that the essential oil is an important one as it has a sweet fragrance, high % of eugenol (61.2%) considered as antibacterial component [27] and other phenolic substances e.g. methyl eugenol (1.8%), carvacrol (30.4%) and the oil has a potential of becoming a commercial commodity. The antimicrobial activity of chloroform extract of *Ocimum sanctum* was determined in the present investigation. Maximum antibacterial activity was observed in the bacteria

*Bacillus subtilis* (19 mm, 23 mm and 29 mm) followed by *Pseudomonas aeruginosa* (20 mm, 25 mm and 26 mm), *Staphylococcus aureus* (16 mm, 18 mm and 20 mm) and *Streptococcus pyogenes* (No zone, 8 mm and 10 mm). The fungi *Penicillium* sp. (8 mm, 10 mm and 11 mm) showed more inhibitory activity than *Aspergillus niger*.

The antimicrobial activity of ethyl acetate extract of *Ocimum sanctum* was evaluated in the present research. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (16 mm, 20 mm and 26 mm) followed by *Pseudomonas aeruginosa* (17 mm, 22 mm and 23 mm), *Staphylococcus aureus* (13 mm, 15 mm and 17 mm) and *Streptococcus pyogenes* (No zone, No zone and 8 mm). The ethyl acetate extract of *Ocimum sanctum* showed resistance against *Penicillium* sp. and *Aspergillus niger*. No zone of inhibition was observed against *Penicillium* sp., *Aspergillus niger* and negative DMSO control.

Saranraj *et al*. [28] evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Acalypha indica* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Acalypha indica* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (30 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (12 mm). The ethyl acetate extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (23 mm) against *Escherichia coli*.

Sivasakthi *et al*. [29] evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Datura metel* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (26 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (8 mm). The ethyl acetate extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (19 mm) against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*.

Ali Rehman *et al*. [30] 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 *Azadirachta indica* inhibit the growth of *Microsporum canis*. There was no inhibition zone of inhibition of ethanol and aqueous extract of leaves, seeds roots and stem of *Acalypha indica* against *Staphylococcus aureus* and *Escherichia coli*.

Ramasamy *et al*. [31] found the antibacterial activity of valuable compounds from various solvent extracts of *Azadirachta indica*, *Blumea lacera* and *Melia azadirachta* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* 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 marcescens* were relatively more sensitive.

The antimicrobial activity of methanol extract of *Azadirachta indica* was studied. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (28 mm, 32 mm and 35 mm) followed by *Staphylococcus aureus* (25 mm, 26 mm and 28 mm), *Pseudomonas aeruginosa* (19 mm, 22 mm and 25 mm) and *Streptococcus pyogenes* (17 mm, 20 mm and 23 mm). The fungi *Penicillium* sp. (15 mm, 17 mm and 20 mm) showed more inhibitory activity when compared to *Aspergillus niger* (12 mm, 14 mm and 18 mm).

The antimicrobial activity of chloroform extract of *Azadirachta indica* was tested. Maximum antibacterial activity was noticed in the bacteria *Bacillus subtilis* (23 mm, 27 mm and 33 mm) followed by *Staphylococcus aureus* (20 mm, 21 mm and 23 mm), *Pseudomonas aeruginosa* (14 mm, 17 mm and 20 mm) and *Streptococcus pyogenes* (12 mm, 15 mm and 18 mm). The fungi *Penicillium* sp. (10 mm, 12 mm and 15 mm) showed more inhibitory activity when compared to *Aspergillus niger* (7 mm, 9 mm and 11 m\Am).

The antimicrobial activity of ethyl acetate extract of *Azadirachta indica* was investigated. Maximum antibacterial activity was recorded in the bacteria

*Bacillus subtilis* (20 mm, 24 mm and 30 mm) followed by *Staphylococcus aureus* (17 mm, 18 mm and 20 mm), *Pseudomonas aeruginosa* (11 mm, 14 mm and 17 mm) and *Streptococcus pyogenes* (9 mm, 12 mm and 15 mm). The fungi *Penicillium* sp. (No zone, 9 mm and 12 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, No zone and 8 mm).

Siva Sakthi *et al*. [32] screened the pharmacological activity of the ethanol and ethyl acetate extract of *Datura metel* and *Acalypha indica* for its antifungal activity against pathogenic fungi. Six different fungal isolates *viz*., *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum* were tested for its antifungal activity. The collected leaf samples were powdered and the bioactive compounds were extracted by using ethanol and ethyl acetate in a Soxhelet extractor. The antifungal activity was determined by using Well diffusion method. Ethanol and ethyl acetate extracts with different concentrations (100 mg/ml, 200mg/ml and 300mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. The inhibitory effect of ethanol extract was relatively high when compared to ethyl acetate extract. The extract of *Datura metel* showed maximum zone of inhibition against fungal pathogens when compared to *Acalypha indica*.

Earlier research work on the medicinal plants *Phyllanthus amarus* grown in the same research location have shown that extracts from some plants posses antimicrobial properties [33]. However, the ability of the extracts to inhibit the growth of *Staphylococcus aureus, Pseudomonas* sp*., Klebsiella* sp. and *Escherichia coli* indicates that these organisms do not possess a mechanism inactivating the active ingredients in the extracts or other mechanisms which include exclusion of the substance from the cell and modification of the target site of the substance. 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 penicillin-C acid which could not inhibit its growth [34].

*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 [35] to severe life threatening infections, such as septicemia [36] and disseminated abscesses in all organs and *Escherichia coli* which causes Urinary Tract Infection (UTI), diarrhea, sepsis and meningitis.

The antimicrobial activity of methanol extract of *Phyllanthus amarus* was determined. Maximum antibacterial activity was noticed in the bacteria *Bacillus subtilis* (27 mm, 30 mm and 35 mm) followed by *Pseudomonas aeruginosa* (25 mm, 29 mm and 32 mm)*, Staphylococcus aureus* (22 mm, 28 mm and 31 mm) and *Streptococcus pyogenes* (17 mm, 20 mm and 24 mm). The fungi *Penicillium* sp. (15 mm, 18 mm and 21 mm) showed more inhibitory activity when compared to *Aspergillus niger* (12 mm, 15 mm and 19 mm). The finding of the present study was supported by Murugan and Saranraj [37], Saranraj and Stella [38], Saranraj *et al*. [39] and Saranraj and Sivasakthivelan [21].

The antimicrobial activity of chloroform extract of *Phyllanthus amarus* was evaluated. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (22 mm, 27 mm and 31 mm) followed by *Pseudomonas aeruginosa* (20 mm, 25 mm and 28 mm)*, Staphylococcus aureus* (17 mm, 23 mm and 26 mm) and *Streptococcus pyogenes* (12 mm, 15 mm and 19 mm). The fungi *Penicillium* sp. (10 mm, 13 mm and 16 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 14 mm).

The antimicrobial activity of ethyl acetate extract of *Phyllanthus amarus* was investigated. Maximum antibacterial activity was observed in the bacteria *Bacillus subtilis* (19 mm, 24 mm and 28 mm) followed by *Pseudomonas aeruginosa* (17 mm, 22 mm and 25 mm)*, Staphylococcus aureus* (14 mm, 20 mm and 23 mm) and *Streptococcus pyogenes* (9 mm, 12 mm and 16 mm). The fungi *Penicillium* sp. (No zone, 9 mm and 11 mm) showed more inhibitory activity when compared to *Aspergillus niger* (No zone, 10 mm and 13 mm). No zone of inhibition was observed in the negative DMSO control.

Saranraj and Sivasakthivelan [21] tested the antibacterial activity of *Phyllanthus amarus* was tested against Urinary tract infection causing bacterial isolates *viz*., *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter* sp., *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The *Phyllanthus amarus* was shade dried and the antimicrobial principles were extracted with methanol, acetone, chloroform, petroleum ether and hexane. The antibacterial activity of *Phyllanthus amarus* was determined by Agar Well Diffusion Method. It was found that methanol extract of *Phyllanthus amarus* showed more inhibitory activity against UTI causing bacterial pathogens when compared to other solvent extracts.

Sekar *et al*. [22] screened 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 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.

## CONCLUSION

The study of antibacterial activity of herbal plant extract of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* showed that the methanol extract showed promising antimicrobial activity against bacterial and fungal human pathogens followed by chloroform extract and ethyl acetate extract. Among the three plants, maximum inhibition activity was exhibited by *Phyllanthus amarus* followed by *Azadirachta indica* and *Ocimum sanctum*. 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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