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A study of antimicrobial activity of few medicinal herbs

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ABSTRACT

The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine. The development of new antimicrobial agents against resistant pathogens is increasing interest. Therefore, the methanolic extracts from different parts of four medicinal plants used locally in folk medicine were evaluated for antimicrobial activity. It was found that most plant extracts studied had antibacterial and antifungal activities. The methanolic extract of leaf of the plant Azadiracta indica, Acacia nilotica and Witania somnifera showed significant antibacterial activity against Bacillus subtilis, Escherchia coli, stphaylocuccus aureus and pseudomonas fluorescence. Azadiracta indica and A.tinolica showed significant antifungal activity against A. flavus, Ziziphus mauritiana. The rhizome extract of curcuma longa showed significant activity against all tested bacteria and showd higher anti fungal activity against Fusarium verticillioides .

Keywords: Antibacterial screening, Medicinal plants, Antifungal, Antibacterial

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INTRODUCTION

Infectious disease are the world's leading cause of premature deaths, killing almost 50 000 people every day. Morbidity and mortality due to diarrhea continues to be a major problem in many developing countries, specially amongst children. Infections due to variety of bacterial etiologic agents such as pathogenic Escherichia coli, Salmonela spp., Staphylococcus aureus are most common. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1][2][3].

With the continuous use of antibiotics microorganism have become resistant. I addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions[4].This has created immense clinical problems in the treatment of infectious diseases[5].

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important recourse to combat serious diseases in the world. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field.

The traditional medicinal methods, specially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries.

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity [6][7][8][9].

In recent times, the search for potent antibacterial agents has been shifted to plants. Most plants are medicinally useful in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists.

There are about 120 plant-based drugs prescribed worldwide and they come from just 95 plant species. Approximately 250,000 species of flowering plants and only 5000 have had their pharmaceutical potential assessed. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day-medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics [10]. Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable [11].

There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, and as a result plants are still recognized as the bedrock for modern medicine to treat infectious diseases [12].

Neem (*Azadirachta indica* A. Juss)

Neem is one of the most important native medicinal plants of India, as it has a wide spectrum of biological activity and is the most useful traditional medicinal plant in India.

Each part of neem tree has some medicinal property. Neem leave, bark extracts and neem oil are commonly used for therapeutic. Neem oil suppresses several species of pathogenic bacteria such as *S. aureus* and *S. typhosa*, all strains of *M. tuberculosis* [13].

The growth of *S. paratyphi* and *V. cholerae* was inhibited [10]. Considerable progress has been achieved regarding the biological activity and medicinal applications of neem compounds, which have chemical and structural diversity.. This versatile tree is now considered a valuable source of unique natural products for the development of medicines, including non-antibiotic drugs, against bacterial infections and various other human disorders; thus, the tree is still regarded as the “village pharmacy” in India.

Curcuma longa (Turmeric)

Curcuma longa (*C. longa*), a perennial herb, is a member of the *Zingiberaceae* family and has a Long tradition of use in the Chinese and Ayurvedic systems of medicine. Curcuminoids, a group of phenolic compounds isolated from the roots of *C. longa*, exhibited a variety of beneficial effects on health and has the ability to prevent certain diseases [14].

In East Asia, the rhizomes from *C. longa*, are considered to have natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic, and analgesic activities because they contains a number of moniterpenoids,sesquiterpenoids, and curcuminoids[15][16].

It is also reported to have insecticidal activity [17].In addition, wound healing and detoxifying properties of curcumin have also received considerable attention [18].

Study by Limtrakul 9 et al. (2004) using RT-PCR showed that all three curcuminoids isolated from *C. longa* inhibited multidrug resistance -1 (MDR-1) gene expression. Fraction II of the oil extract from the turmeric oleoresin containing ar-Turmerone, turmerone, and curlone showed antibacterial activity by the pour plate method against *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa[19][20]*

Curcumin is the most important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin, C2H2OO6, is 184 ºC. It is soluble in ethanol and acetone, but insoluble in water.

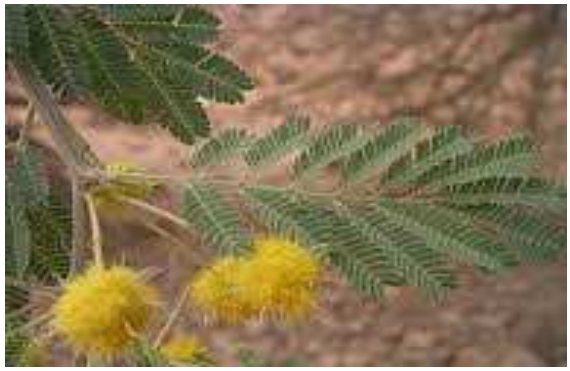
Curcumin 95%, a potentantioxidant is believed to be the most bioactive and soothing portion of the herb turmericand posses the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol lowering antibacterial and anti-fungal effects. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system [21].

C. longa oil was tested against cultures of *Staphylococcus albus*, *S. aureus* and *Bacillus typhosus*, inhibiting the growth of *S. albus* and *S. aureus* in concentrations up to 1 to 5,000[22].

Keeping in view the important role of turmeric in inhibition of different cultures of bacteria and its role as antioxidant and antibacterial, the present study was conducted to compare the antibacterial activity extracts of *C. longa* varieties and potency of turmeric varieties on some bacteria.

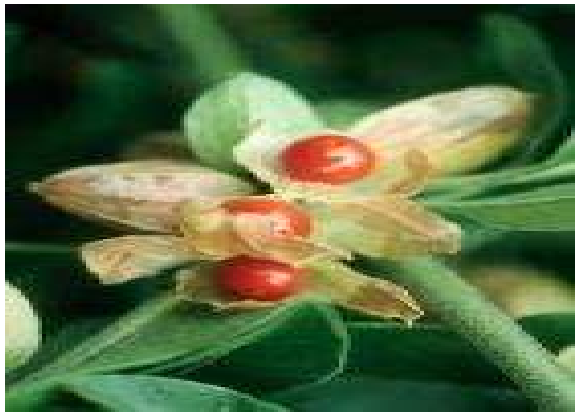
Acacia nilotica

Acacia nilotica L. is a common, medium sized tree, locally known as ‘Babul’ or ‘Kikar’ belongings to the family Mimosaceae. *Acacia* is the most significant genus of family Leguminosae[23].



The plant is considered to be antispasmodic and antidysenteric.Pods and tender leaves are reported to treat diarrhoea.The plant has been shown to exhibit antibacterial anti-inflammatory [24].antiplatelet aggregatory activity[25].spasmogenic, vasoconstrictor actions[26].cytotoxic activity[27]and antioxidant activity[28]. The stem bark of *Acacia nilotica* is well-known for its diuretic properties[29].

Withania Somnifera:



Withania somnifera (family Solanaceae) is a medicinally important herb used in number of Indian herbal formulations. In India, it is locally known as 'Ashwagandha' and is considered as *Indian Ginseng*. Roots of the plant are major source of active chemical substances and are traditionally used to cure ulcers, fever, cough, dyspnoea, consumption, dropsy, impotence, rheumatism, toxicosis and leucoderma[30].

These activities are mainly attributed towards the presence of different withanolides mainly withaferine A and withanolide A.[31]

The plant had been reported to grow in wild and is also cultivated in selective areas of India. Their pharmacological properties are diverse ranging from anti-inflammatory,anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoetic and cardio-protective effects[32].

the traditional medical system of India. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy[33][34].

The herbal root extract has been traditionally used as a tonic and as a sedative Anti-inflammatory agent; it us used to treat rheumatic pain and arthritis in Ayurveda, the berries and leaves of W. somnifera are locally applied to tumors.tubercular glands, carbuncles, and ulcers.

MATERIALS AND METHODS

2.1 Collection of Plant Material:
Fresh leaves of selected plants viz., Azadiracta indica, Acacia nilotica, Tinospora cordifolia, Withania somnifera and rhizome of Curcuma longa free from disease were collected from Mysore, Karnataka. The plants parts were washed two times thoroughly with distilled water.

2.2 Solvent Extraction:
Thoroughly washed dried leaves of five plants of azadiracta indica , Acacia nilotica and Withania somnifera and rhizome extract of curcuma longa , of plant material were dried in shade for four days. Plant parts were placed in an oven at 38 °C and then powdered with mortar and pestle. 100 grams of each respective plant parts and 300 ml of methanol were place in mortar and it was grind thoroughly with the pestle. The methanol extract kept at room temperature for 24 hours. The extracts were filter using whattman filter paper (No. 1) and dried at temperature below 45°C for methanol removal to obtain the dense extract and then they were kept in sterile bottles under refrigerated conditions until use.

2.3 Preparation of Inoculum:
The gram positive (Bacillus subtilis and Staphylococcus aureus) and gram negative bacteria (Escherichia coli, Pseudomonas fluorescens and Xanthomonas axonopodis pv. malvacearum) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 6 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A nm). The fungal inoculums (F. verticillioides, D. turcica and A. flavus) were prepared from 6 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 9 to 11 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A595 nm) to obtain a final concentration of approximately 105 spores/ml.

2.4 Anti-bacterial testing:
Antibacterial activity was measured using agar dilution technique. Briefly, the methanol extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton Agar (MHA, Sigma) in petridishes (100 mm×15 mm) to obtain final concentrations: 100, 50, 25 and 12.5 µg/ml. The solvent did not exceed 1% concentration and did not affect the growth of the organisms. All bacterial strains were grown in Mueller Hinton Broth (MHB, Sigma) for 4 h at 37°C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 108 cfu/ml were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24 h at 37 °C. After incubation, all dishes were observed for microbial inhibition by the disc diffusion method[35]

Streptomycin sulphate (10 µg mlG) used as positive control and methanol solvent (100 µg mlG) used as negative control. The antibacterial assay plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in mm.

2.5 Antifungal Activity:
The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg mlG concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control and Nystatin (10 µg discG) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm[36].

2.7 Preparation of test samples
For the antimicrobial tests, ethanolic extracts were diluted in dimethylsulfoxide (DMSO): methanol (1/1: v/v) solvent to a concentration of 20 mg/ml.

2.8 Antimicrobial bioassay
For bioassays, suspension of approximately 1.5×10^8 bacterial cells/ml in sterile normal saline were prepared and about 1.5 ml of it was uniformly seeded on Mueller-Hinton-Agar medium with 3–4 mm thickness in 12 cm × 1.2 cm

glass petridishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. Wells were filled with 0.1 ml of 20 mg/ml concentration of each sample (2 mg/well) and incubated at 37 °C for 48 hours. Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm. Each experiment was repeated three times and the mean of the diameter of the inhibition zones was calculated. Pure solvent was used as negative control.

RESULTS

The antimicrobial activity of plant parts from four medicinal plant species has been evaluated *in vitro* against pathogens including four bacterial species (*Escherichia coli*, *Bacillus subtilis* and *Pseudomonas. fluorescence, Staphylococcus aureus)* and three fungus species (*Aspergillus flavus, Dreschlera turcica, and Fusarium verticillioides*) . In general, most plant extracts of the different plant parts exhibited broad spectrum of antimicrobial activity (Table 1).

Table 1 illustrated that leaf extracts of *Azadiracta indica and A.niloticas*howed antimicrobial activity against all test microorganisms. On the other hand, methanolic extracts of *curcuma longa* exhibited antibacterial activity against all test microorganisms. It was observed that alcoholic extracts of Tinospora cardifolia exhibited the highest significant antibacterial activity against *Staphylococcus aureus* with mean inhibition zone equal to 17.0 ±1.5 and 17±1.22 mm, respectively.

Table 1: Antibacterial activity of some medicinal plant methanol extracts (100 µg mlG1) and antibiotic (10 µg mlG1) against bacterial species tested by disc diffusion assay Zone of inhibition (mm)

Bacterial sp.	*Azadiracta indica*	*Acacia nilotica*	*Tinospora cordifolia*	*curcuma longa*
Bacillus subtilis	23±0.08	22±3.20	16±0.91	22±1.2
Escherichia coli	22±2.33	17±0.98	15±0.56	19±0.98
Pseudomonas. Fluorescence	21±0.48	*15±1.33*	16±0.13	*19±0.33*
Staphylococcus aureus	22±1.04	16±2.88	17±1.51	20±0.88

Table 2: Antifungal activity of some medicinal plant methanol extracts (100 µg mlG1) and fungicide (10 µg mlG1) against fungal species tested by disc diffusion Zone of inhibition (mm)

Fungal sp.	*Azadiracta indica*	*Acacia nilotica*	*Tinospora cordifolia*	*Curcuma longa*
Aspergillus flavus	16±0.24	12±0.33	10±0.78	14±0.22
Dreschlera turcica	17±0.10	10±0.33	15±0.67	15±0.63
Fusarium verticillioides	17±0.23	9±0.00	13±0.22	16±0.00

DISCUSSION

The current study was initiated because of the increasing resistance to antibiotics including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. As a result, the antimicrobial activity of different medicinal plant parts extracts of four plants was screened against the most common pathogens. In general, methanol leaf extracts of the selected plants appeared to be effective source of active antimicrobial agents. However, extracts of Azadiracta indica and A.nilotica recorded to posses higher antimicrobial activity among the other tested medicinal herbs.

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