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A REVIEW ARTICLE, HERBAL PLANTS USED AS ANTIMICROBIAL AGENT

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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 of different parts of medicinal plants used locally in folk medicine were evaluated for antimicrobial activity. It was found that most plant extract studies have antibacterial and antifungal activities. The methanolic extract of leaf of the plant *Azadirachta indica, Dendrocnide sinuata, Acacia nilotica, Capsicum chinense, Bambusa vulgaris* and *Syzygium aromaticum* showed

significant antibacterial activity against *Bacillus subtilis*, *Escherchia coli*, *Staphaylococcus aureus* and *Pseudomonas fluorescence*. *Azadirachta indica* and *Acacia nilotica* showed significant antifungal activity against *Aspergillus flavus*, *Ziziphus mauritiana*.

KEYWORDS: Antibacterial screening, Medicinal plants, Antifungal.

INTRODUCTION

Worldwide trend towards the utilization of natural plants remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.^[1] The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well dominated used of plants is their use as Antimicrobial agent. Antimicrobial agents are the agents which can kill or prevent the growth of unwanted

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microorganisms. For example antibiotics are used against bacteria and antifungal are used against fungi.^[2]

Antimicrobial agents are used against infectious disease caused by microorganisms. Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. [3] Infections due to variety of bacterial etiologic agents such as pathogenic Escherichia coli, Salmonela spp., and Staphylococcus aureus are most common. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world. [4] With the continuous use of antibiotics microorganism have become resistant. One addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions. [5] This has created immense clinical problems in the treatment of infectious diseases. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. There one approaches to screen local medicinal plants for possible antimicrobial properties.

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. 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. Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable. [6]

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.^[7]

Mechanism of Action of Antimicrobial Agents

Antimicrobial agents take advantage of the differences between animal cells and bacteria (prokaryotes), fungi, or protozoa. The goal is to have highly selective toxicity towards these microbes with minimal or no toxicity in humans.

The most common targets for antimicrobial drug actions fall into 5 basic categories

- A. Inhibition of Cell Wall Synthesis
- B. Inhibition of Protein Synthesis
- C. Inhibition of Nucleic Acid Synthesis
- D. Effects on cell membrane sterols (antifungal agents)
- E. Inhibition of unique metabolic steps

Table: Specific Mechanism of Action of Antimicrobial Agents.^[8]

Mechanism of Action	Drugs
Inhibition of Cell Wall Synthesis	Penicillins, Cephalosporins,
Inhibit cross-linking of peptidoglycan by	Aztreonam,
inactivating transpeptidases (PBPs)	Imipenem
Bind to terminal D-ala-D-ala & prevent	Vancomycin, Teicoplanin
incorporation into growing peptidoglycan	
Inhibition of transglycosylation	Oritavancin, Teicoplanin, lipophilic
	vancomycinanalogs, ramiplanin
Inhibit dephosphorylation of	Bacitracin
phospholipidcarrier in peptidoglycan structure	
Prevents incorporation of D-alanine into	Cycloserine
Peptidoglycan	
Inhibition of Protein Synthesis	Macrolides, Chloramphenicol,
Bind to 50S ribosomal subunit	Clindamycin
Bind to 30S ribosomal subunit	Aminoglycosides, Tetracycline
Inhibition of Nucleic acid synthesis	Quinolones
Inhibition of DNA gyrase & topoisomerase	
Inhibition of nucleic acid biosynthesis	Flucytosine, Griseofulvin
Inhibition of mRNA synthesis	Rifampin, Rifabutin, Rifapentine
Alteration of Cell Membrane Function	Imidazole antifungals
Inhibition of ergosterol biosynthesis	
Bind to membrane sterols	Polymyxins, Amphotericin B, Nystatin
Alteration of Cell Metabolism	Sulfonamides,
Inhibition of tetrahydrofolic acid	Trimethoprim, Trimetrexate
production(co-factor for nucleotide synthesis)	Pyrimethamine
Inhibition of mycolic acid biosynthesis	Isoniazid
Interference with ubiquinone biosynthesis &	Atovaquone
cell respiration	
Bind to macromolecules	Metronidazole, Nitrofurantoin

Herbal Treatment

Many thousands of plants worldwide are used in traditional medicine as treatments for bacterial infections. Some of these have also been subjected to in-vitro screening but the efficacy of such herbal medicines has seldom been rigorously tested in controlled clinical trials. Conventional drugs usually provide effective antibiotic therapy for bacterial infections

but there is an increasing problem of antibiotic resistance and a continuing need for new solutions. Although natural products are not necessarily safer then synthetic antibiotics, some patients prefer to use herbal medicines. Thus healthcare professionals should be aware of the available evidence for herbal antibiotics. This review was undertaken to assess critically those antibacterial herbal medicines that been have subjected to controlled clinical trials.

Herbs Used As Antimicrobial Agent

Medicinal plants, medical herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemical works on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effect of taking a plant medicine can be complex.

SOME HERBAL PLANTS EXERTING ANTIMICROBIAL ACTIVITY

Neem (Azadirachta indica A. Juss)

Azadirachta indica is also known as Neem, and it belongs to the family *Meliaceae*. It is one of the most important native medicinal plants of India and Indian subcontinent including Nepal, Pakistan, Bangladesh and Sri Lanka, 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 *Staphaylococcus aureus* and *Staphaylococcus typhosa*, all strains of *Mycobacterium tuberculosis*. ^[9]

Antimicrobial activity of *Azadirachta indica* leaf extract was studied by Parastoo Karimi Alavijeh et al. The antimicrobial activity of plant parts of this 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*). They used methanol extract of *Azadirachta indica* for evaluation of antimicrobial activity. They measured the antibacterial activity using agar dilution technique and the antifungal activity was by disc diffusion method. For the antimicrobial tests, they were diluted ethanolic extracts in dimethyl sulfoxide (DMSO): methanol (1/1: v/v) solvent to a concentration of 20 mg/ml.

The current study suggests that, in general methanol leaf extracts of the selected plants appeared to be effective source of active antimicrobial agents. However, extracts of Azadirachta indica recorded to possess higher antimicrobial activity.^[10]

Dendrocnide sinuata (Blume)

Dendrocnide sinuata (Blume) Chew has been widely used for varity of diseases by the local tribes of North East India. The plant belonging to the family *Urticaceae* is a perennial herb, dull-green in colour, armed with minute rigid hair or pickles, which transmit a venomous fluid when pressed. The steam is obtusely 4-angled, brunching, up to 1.5m high, arising from a branching root, with freshly shoots and many fibers. The leaves are opposite, petiolate, cordate, lanceolate, spreading, conspicuously acuminate, coarsely and acutely serrate, the point entire, armed with stings 7-10cm long and about 1.5cm wide. The flowers are small, green, clustered, axillary, interrupted spikes and longer than the petioles.

The antimicrobial activity of *Dendrocnide sinuata* (Blume) Chew leaves extract was studied by Bhaben Tanti et al. to investigate ethno medicinal uses particularly the antimicrobial and antioxidant activities of Dendrocnide sinuata leaves, traditionally used by different tribal communities in North East India. The ethno medicinal study was carried by conducting a survey to collect information on the medicinal use of *Dendrocnide sinuata* among few of the tribal communities. [11] For evaluation of antimicrobial activity, methanolic and water extract of Dendrocnide sinuata leaves was used. And by disc-diffusion assay method the anti-microbial activity of the extract under investigation was carried out. [12] The current study suggests that, the antimicrobial activity of the Dendrocnide sinuata leaf extract was exhibited mainly against the Gram-positive bacteria. Among the investigated extract the methanol extract exhibited the heights antimicrobial activity with inhibition zones more than 14mm was shown by the methanol extract of 75 and 100%. Results of phytochemical screening shows the presents of some active constituents like flavonoids, terpenoids, tannins etc. When methanol extract of the investigated plant was tested for DPPH radical scavenging activity, it was found that 75mg/ml and 100mg/ml of the extract lowered the DPPH radical levels above 64 and 74% respectively. Inhibition of DPPH radical above 50% is considered as significant for antioxidant properties of any compound. The total investigation obtained that Dendrocnide sinuata having the anti-oxidant and antimicrobial activity against the Gram-negative bacteria. [13]

Vachellia nilotica

Vachellia nilotica (Common name *Acacianilotica*) also known as Babul, belonging to the family *Fabaceae* is found in Africa, the Middle East and Indian subcontinent. *Acacia nilotica* is a tree 5-20 m high with a dense spheric crown, stems and branches usually dark to black coloured fissured bark, grey-pinkish slash, exuding a reddish low quality gum. The tree has thin, straight, light, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees. The leaves are bipinnate, with 3-6 pairs of pinnulae and 10-30 pairs of leaflets each. Flowers are 1.2-1.5 cm in diameter with bright golden yellow colour. Numbers of seeds are approximately 8000/kg.^[14]

The antimicrobial activity of the extract of Acacia nilotica leaf was studied by B. Mahesh and S. Satish. For evaluation of the antimicrobial activity, the bacterial cultures of *Bacillus* subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas fluorescens (P. fluorescens), Staphylococcus aureus (S. aureus) and Xanthomonas axonopodis pv. malvacearum (X. axonopodis pv. malvacearum) and fungal cultures of Aspergillus flavus(A. flavus), Dreschler aturcica(D. turcica) and Fusarium verticillioides (F. verticillioides) was used. The methanolic leaf extract of Acacia nilotica were tested by the disc diffusion method, and it showed significant activity against Escherichia coli, Staphylococcus aureus and Xanthomonas axonopodis pv. Malvacearum i.e around 15 mm. The highest antibacterial activity of 20 mm was found in Bacillus subtilis and least activity was recorded in Escherichia coli i.e 14 mm. Bark extract of Acacia nilotica exhibit highest activity against Bacillus subtilis and Staphylococcus aureus (15 mm) and lowest in Pseudomonas fluorescens. Antifungal activity of five leaf extract showed significant activity when compared with the bark/root extract. Acacia nilotica bark and leaf extract showed antifungal activity against Aspergillus flavus (12 mm). In the present study, the methanolic leaf, root/bark extracts of Acacia nilotica showed the activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, Xanthomonas axonopodispv. malvacearum, Aspergillusflavus, Dreschleraturcica and Fusarium verticillioides. Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. [15]

Capsicum chinense

Capsicum chinense, commonly known as the "bonnet pepper", belonging to the family Solanaceae, is a species of chili pepper native to the Americas. Increasing antimicrobial resistance and infections by the genus *Trichosporon* has led to interest in developing new

therapies for medicinal plants. The fruit of *Capsicum chinense*(pepper-pout) has capsaicinoids and capsaicin, is a compound extremely annoying to have analgesic, anti-inflammatory and antioxidant effects. Some substances of the genus *Capsicum* have pharmacological properties but still poorly studied, although recent studies show that the crude extract has antimicrobial activity.

The antimicrobial activity of *Capsicum chinense* was studied by Gayathri N et al. The present study investigated on the comparative evaluation of the extraction, quantification, phytochemical and antimicrobial activity of Capsaicin from acetone and acetonitrile extracts of Capsicum chinense Jacq. The polar aprotic solvent extracts showed high amount of Capsaicin with pungency level of 1,529,500 Scoville Heat Units. The Thin layer chromatography method is providing a fingerprint of plant extract. The Capsaicin extracted in the solvents on TLC chromatogram was viewed under UV 254 nm and UV 366 nm and documented. The extraction and estimation of chlorophyll and Carotenoids were also performed for the plant sample following standard procedure. Phytochemical analysis shows that acetone and acetonitrile extract of callus, leaf, shoot, fruit and seed which shows abundant presence of alkaloids, flavonoids, phenols, saphonins etc. The acetone and acetonitrile extract showed maximum zone of inhibition of Klebsiella pneumonia and Staphylococcus aureus against the gram positive and gram negative bacteria respectively through agar well diffusion method. The acetone and acetonitrile extract was found to be more effective at different concentration against all the tested bacteria and fungi. The results revealed that the Capsaicin and other secondary metabolites present in the acetone and acetonitrile extract of Capsicum chinense would contribute for the further extraction and purification of capsaicin as an antimicrobial agent. [16]

Bambusa vulgaris

The Leaf of *Bambusa vulgaris*, belonging to family *Poaceae* is used as anthhelmintic. Tabashir (bamboo manna) has stimulant, astringent, febrifuge, tonic, cooling, antispasmodic and aphrodisiac properties. Antimicrobial activity of the successive extract of the fresh leaves of *bamboo* was evaluated against both Gram positive and Gram negative bacterial strains by disc diffusion method. The results revealed that all extracts showed effective inhibitory action against *Staphylococcus aureas*. The aqueous and ethnolic extracts showed very effective as compared to standard penicillin.

The antimicrobial activity of bamboo leaves were studied by Vijay kumar Singh et al. Successive extraction of leaves of *bamboo* plant with petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and aqueous was carried out. In this study *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus subtilis* bacteria are used to check the antimicrobial activity. In vitro antibacterial activity was screened by using Nutrient agar with by disc diffusion method. These studies were performed in triplicate by using standard drugs (10mcg/disc Penicillin). Ethanolic and aqueous extracts were subjected for antimicrobial activity against the strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. The aqueous extract was also found to have very significant antimicrobial effect against *Escherichia* coli. Ethanolic extract showed moderate effect against *Escherichia coli* and *Staphylococcus aureus*, whereas the other extracts were found to have resistance. [17]

Syzygium aromaticum(Clove)

Cloves are the aromatic dried flower buds of a tree in the family *Myrtaceae*. Cloves are used in Ayurveda, Chinese medicine and Western herbalism. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. It is also used in dentistry where the essential oil of clove is used as anadyne for dental emergencies. In addition, the cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic.

Antimicrobial activity of *clove* was studied by Sabahat Saeed et al. to investigate the potency of aqueous infusion, decoction and essential oil of clove (*Syzygium aromaticum*) as natural antibacterial agents against 100 isolates belonging to 10 different species of Gram–ve bacilli viz., *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhi*, *Shigella dysentriae* and *Vibrio cholerae*. The screening was performed by standard disc diffusion method. The aqueous infusion and decoction of clove exhibited maximum activity against *Pseudomonas aeruginosa* with 10.43 mm mean diameter of zone of inhibition ± 1.76 standard deviation and 10.86 mm mean diameter ofzone of inhibition ± 1.46 standard deviation respectively. Essential oil of clove exhibited maximumactivity against *Vibrio cholera* with 23.75 mm mean diameter of zone of inhibition ± 3.03 standarddeviation. *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhi*, *Shigella*

dysentriae and Vibrio cholera were found resistant to aqueous infusion and decoction while essential oil showed strong antibacterial activity against all bacterial isolates tested. [18]

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