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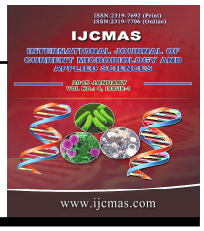

Original Research Article

Phytochemical Analysis and Antibacterial Properties of Some Selected Indian Medicinal Plants

Abhijit Balasaheb Shinde[1*] and Yogini Ramkrishna Mulay[2]

[1]Department of Microbiology, D. A. B. Naik College, Chikhali, Tal- Shirala, Dist- Sangli, Maharashtra, India- 415408
[2]Department of Microbiology, T. C. College, Baramati, Dist- Pune, Maharashtra, India- 413102

**Corresponding author*

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A B S T R A C T

For a long period of time, plants have been a valuable source of medicines. In the present study, antibacterial activity of leaf extracts of *Azadiracta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi) was evaluated against four human pathogens *Escherichia coli*, *Salmonella typhi*, *Shigella* sp. and *Staphylococcus aureus* using agar well diffusion method. Four different organic solvents acetone, chloroform, diethyl ether and methanol were used for extraction. Growth of all test pathogens was inhibited by methanol extracts of the plants, while chloroform extracts of the plants were found to be ineffective against all test pathogens. Maximum zone of inhibition (17mm) was obtained with methanol extract of *Azadiracta indica* (Neem) against *Salmonella typhi*. Amongst the four plants used, highest antibacterial activity was shown by *Azadiracta indica* (Neem). *Salmonella typhi* was found to be most sensitive while *E. coli* was found to be least sensitive to different plant extracts. Phytochemical analysis of the alcoholic extracts revealed the presence of alkaloids, glycosides, flavonoids, steroids, tannins and reducing sugars. The occurrence of these biologically active chemicals in the selected plants may justify their wide usage in traditional medicine.

Introduction

Since ancient times, different plants have been used as a source of medicines. A variety of drugs could be obtained from medicinal plants. About 80 % individuals from developing countries rely on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care (Ellof, 1998).

Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine (Nair *et al.*, 2005). Mostly, these compounds are secondary metabolites such as alkaloids, flavonoids, steroids, resins, fatty acids, tannins and phenol compounds, etc. Compounds extracted from different parts of

the plants can be used in treatment of diarrhea, dysentery, cough, cold, fever, bronchitis, cholera, etc. (Joshi *et al.,* 2011).Plant derived products can be exploited with a large number of sustainable advantages like more effectiveness, less side effects, reduced cost, and easy availability (Moorthy *et al.,* 2007).

Now a day, antibiotic resistance in medically important bacteria is the major problem faced by the world. The indiscriminate use of commercial antimicrobial drugs has resulted in multiple drug resistance.

Antibiotics may also cause adverse effects on the host including allergies, hypersensitivity and immune-suppression. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously minimizing many of the side effects that are often associated with synthetic antimicrobials (Cunha, 2001). Though antimicrobial properties of medicinal plants have been investigated by a number of researchers worldwide, very little information is available on such activities of medicinal plants and only a small number of plants have been systematically investigated for their antimicrobial activities.

In present study, four important commonly used Indian medicinal plants *Azadiracta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi) were selected and antibacterial activity of their leaf extracts was evaluated against some bacterial pathogens.

Materials and Methods

Isolation of pathogens

Four test pathogens *Escherichia coli, Salmonella typhi, Shigella* sp. and *Staphylococcus aureus* used in this study were isolated from water samples by standard methods. Identity of the isolates was confirmed by morphological characteristics and conventional biochemical tests (Harley and Prescott, 2002). Pure cultures were preserved at 4°C on nutrient agar and MacConkeys agar slants.

Collection of plant materials

The medicinal plants used for the experiment were *Azadiracta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi). The plant materials were collected from the agricultural fields in Baramati region.

Preparation of extracts

Collected plants were washed thoroughly and chopped into small pieces by using mortar and pestle, shade dried and grinded into powdered form. 5 gm of powdered plant material was dissolved in 50 ml of four different organic solvents Acetone, Chloroform, Diethyl ether or Methanol respectively. The mixture was centrifuged at 2000 rpm for 10 min. Then supernatant was placed in evaporating dish for evaporation. Afterwards, DMSO was added in evaporated extract for solublization (Nair *et al.,* 2005; Rahman *et al.,* 2011).

Determination of antibacterial activity

Antimicrobial activity of different plant extracts was determined by agar well diffusion method. 0.1 ml of freshly grown

culture of test organisms (10^6cfu/ml) was aseptically introduced and spread on surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter were made in agar plate with the help of sterile cork-borer. Fifty micro-liters of different plant extracts and same volume of extraction solvent for negative control were filled in the wells with the help of micro pipette.

Standard reference antibiotics like streptomycin and tetracycline were used as positive controls for the test organisms. Plates were left for some time at 4°C till the extract diffuses in the medium with the lid closed and incubated at 37°C for 24 hr. The plates were observed for zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition against the tested bacterial pathogens. Each assay in this experiment was replicated three times (Jain, 2009; Joshi *et al.*, 2011).

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, glycosides,terpenoids, steroids, tannin and reducing sugars by the following procedure.

Test for Alkaloid

The alcoholic extract of plant was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (Evans, 2002).

Test for Glycoside

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiqui and Ali, 1997).

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red or orange colour was observed for flavonoids (Siddiqui and Ali, 1997).

Test for Steroid and Terpenoid

10 mg of extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of concentrated sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer shows the presence of terpenoids (Siddiqui and Ali, 1997).

Test for Tannin

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour shows presence of gallic tannins while green black colour shows presence of catecholic tannins (Iyengar, 1995).

Test for Reducing Sugar

To 0.5 ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars (Iyengar, 1995).

Result and Discussion

In this study, one Gram positive bacterial pathogen *Staphylococcus aureus* and three Gram negative bacterial pathogens *E. coli*, *Salmonella typhi*, and *Shigella* sp. were selected and antibacterial activity of leaf extracts of *Azadiracta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi) was evaluated against them.

Acetone and methanol extracts of *Azadiracta indica* (Neem) showed highest inhibitory activity against all four test organisms. Maximum zone of inhibition (17mm) was obtained with methanol extract of *Azadiracta indica* (Neem) against *Salmonella typhi.* Methanol and diethyl ether extracts of *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi) showed moderate antibacterial activity. Acetone and methanol extracts of *Cymbopogon citratus* (Lemon grass) leaves also showed considerable antibacterial activity. Chloroform extracts of all four plants did not show any inhibitory activity against test organisms. Similarly, diethyl ether extracts of *Azadiracta indica* (Neem) and *Cymbopogon citratus* (Lemon grass) did not show any antibacterial activity against all test organisms. The detailed observations are given in table 1.

Salmonella typhi was found to be most sensitive while *E. coli* was found to be least sensitive to different plant extracts. All plants were useful in inhibiting growth of pathogens used for study if proper solvent is used for extraction. Methanol extract of *Azadiracta indica* (Neem) showed maximum antibacterial activity against *Salmonella typhi* comparable to that of standard antibiotics. Antibacterial activity of methanol extracts of different medicinal plants against bacterial pathogens is shown graphically in figure 1.

The antimicrobial properties of many medicinal plants have been previously studied (Hoffman, 1987; Rahman, 2004; Nair *et al.,* 2005; Joshi *et al.,* 2011). In the present study, antibiotic potential of leaf extracts of four important medicinal plants has been determined against four pathogens named *E. coli, Salmonella typhi*, *Shigella* sp. and *Staphylococcus aureus.* For the comparison positive and negative controls were used. Negative controls did not show inhibitory action on any of the test organisms, while positive controls significantly inhibited growth of all four test organisms. The findings match with that of other workers (Rahman *et al.* 2011; Bhattacharjee *et al.,* 2006). It is often reported that Gram positive bacteria are more sensitive than Gram negative bacteria to plant based organic extracts (Reynolds, 1996; Benzic *et al.,* 2003; Rahman *et al.,* 2009). But in our study, both gram positive and gram negative bacteria were found to be sensitive to plant extracts. In present study, *Salmonella typhi* was found to be most sensitive while *E. coli* was found to be least sensitive to plant organic extract than other organisms. The findings agree with that of other workers (Joshi *et al.,* 2011).

The antibacterial properties of medicinal plants may be due to presence of different chemical agents which were classified as bioactive antimicrobial compounds (Arulmozhi *et al.,* 2007). Phytochemical constituents such as alkaloids, glycosides, flavonoids, tannins, steroids, terpenoids and several other compounds are secondary metabolites of plants that serve as a defense mechanism against many microorganisms, insects and other herbivores. The present study also revealed the presence of medicinally active compounds like alkaloids, glycosides, flavonoids, steroid, terpenoid and tannins in most of the selected plants which could be responsible for the observed antibacterial property.

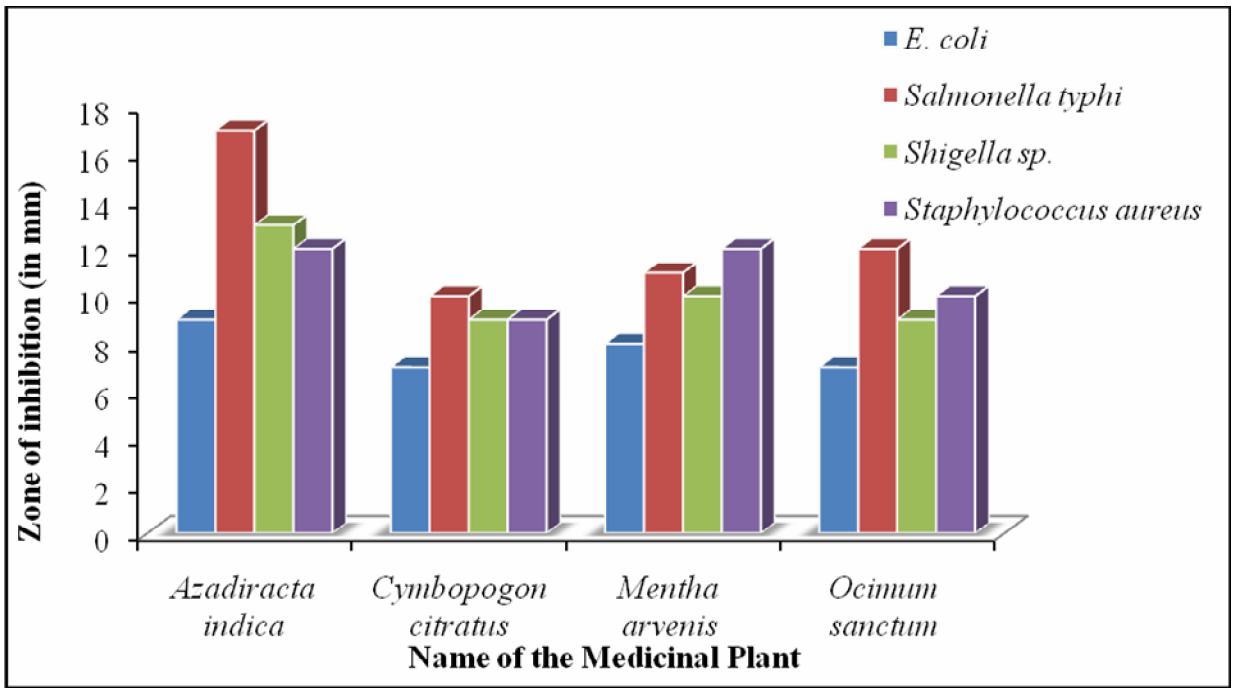
Table.1 Antibacterial property of organic extracts of different plant leaves against selected human pathogens

Name of the Plant	Solvent used for extraction	Diameter of zone of inhibition in mm			
		E. coli	***S. typhi***	***Shigella* sp.**	***Staph. aureus***
***Azadiracta indica* (Neem)**	Acetone	08	14	12	13
	Diethyl ether	-	-	-	-
	Chloroform	-	-	-	-
	Methanol	09	17	13	12
***Cymbopogon citratus* (Lemon Grass)**	Acetone	07	10	09	08
	Diethyl ether	-	-	-	-
	Chloroform	-	-	-	-
	Methanol	07	10	09	09
***Mentha arvenis* (Pudina)**	Acetone	-	12	09	10
	Chloroform	-	-	-	-
	Diethyl ether	-	10	10	11
	Methanol	08	11	10	12
***Ocimum sanctum* (Tulsi)**	Acetone	-	-	-	-
	Chloroform	-	-	-	-
	Diethyl ether	07	09	09	09
	Methanol	07	12	09	10
Positive Control	Tetracycline	13	14	14	15
	Streptomycin	20	15	16	15

Table.2 Phytochemical analysis of alcoholic extracts of medicinal plants

Medicinal Plants	Alkaloid	Glycoside	Flavonoid	Steroid	Terpenoid	Tannin	Reducing Sugar
A. indica	+	+	-	+	+	+	+
C. citrates	-	+	+	-	-	+	+
M. arvenis	-	-	+	+	-	+	-
O. sanctum	+	+	-	+	-	+	+

Figure.1 Antibacterial activity of methanol extracts of different medicinal plants against human pathogens



The phytochemical constituents of the selected medicinal plants are summarized in table 2. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al.,* 1979). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (Mantle *et al.,* 2000). Flavonoids complex with extra cellular and soluble proteins and with bacterial cell walls (Marjorie, 1999). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Raquel *et al.,* 2007). Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada *et al.,* 2006). Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al.,* 1996).

The demonstration of antimicrobial activity against both gram positive and gram negative bacteria by the plant may be indicative of the presence of broad spectrum of antibiotic compounds (Lans, *et al.,* 2001). The optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant (Bhandarkar *et al.,* 2003).Though the results of this study agree with results of other workers, diameter of zone of inhibition formed varies from other study results. Probably the sources of microorganisms used may be the reason for the difference. Moreover, the effectiveness of plant extract against a particular pathogen is affected by various intrinsic and extrinsic factors.

Most of the medicinal plants have great antimicrobial potential. All of the plants used in present study showed antibacterial activity against all four test pathogens. Maximum antibacterial activity was shown by organic extracts of *Azadiracta indica* (Neem). Methanol extract of *Azadiracta indica* (Neem) was found to be equally potent against *Salmonella typhi* compared to standard antibiotics such as streptomycin, tetracycline. The results confirm the validity of the use of such medicinal plants in traditional medicines and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug. But further studies must be carried out to enhance the activity of plant extracts. It is also necessary to check safety and toxicity of plant extracts before their pharmaceutical uses. The study scientifically proves the importance of plant products in development of a potent antibacterial agent.

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