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PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIBACTERIAL PROPERTIES OF SELECTED MEDICINAL PLANTS

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ABSTRACT

Crude extracts from leaves, stem and root viz., five different medicinal plants namely, *Abrus precatorius* L., *Decalepis hamiltonii* Wight & Arn., *Mucuna atropurpurea* (Roxb.) DC., *Santalum album* L. and *Senna tora* (L.) Roxb, were examined using agar well diffusion method against some bacterial microbes namely, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus.* Plant leaves were extracted using different solvents such as Aqueous, Ethanol, Methanol, Acetone, Ethyl acetate, Chloroform and Petroleum ether. Phytochemical screening of these plants was performed for constituents: Carbohydrates, Amino acids, Proteins, Vitamin, Tannins, Phenolic compounds, Flavanoids, Alkaloids, Saponins, Steroids and Glycosides. Among these different extracts, methanol extract showed more antibacterial activity and moderate recorded with aqueous, ethyl acetate and chloroform extracts. *Decalepis hamiltonii* showed a maximum antibacterial activity against all the tested bacterial strains than the other plants. All the bacterial strains were more susceptible to methonolic extracts than the other organic extracts. In future these plants can be subjected to isolate of the major antimicrobials constituents after pharmacological evaluation.

KEYWORDS: Medicinal plants, Phytochemical, Antimicrobial activity, Kathiri hills.

INTRODUCTION

Plants provide basic raw materials for the indigenous pharmaceutical industries such as medicines, cosmetics and perfumeries etc.[1] The medicinal plants are referred to plants that are used for their therapeutic or medicinal values. The whole plant or its different parts may be valued for its therapeutic, medicinal, aromatic or savory qualities.[2] These plants produce and contain a variety of chemical substances that act upon the human body. Industrial sources reveal that Ayurveda and Unani are the two systems of herbal medicines, together have pegged an amount of foreign exchange of 220 crores in 2000-2001 which is nearly 20 percent of the business.[3] The use of the leaves, flowers, stem, seed, berries and roots of the plants are known to prevent, relieve and treat various ailments. They also play vital role as antimicrobial agents.[4] From a scientific perspective, many herbal treatments are considered experimental. Recently, there has been resurgence in the consumption and demand for medicinal plants.[5] Today, science has isolated the medicinal properties of a large number of plants and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories to exploit the pharmaceuticals productions. Resistant to antimicrobial agents such as antibiotics is emerging worldwide of variety of organisms and multiple drug resistant organisms pose serious threats to treat infectious diseases. Hence plant derived antimicrobial have received considerable attention in recent years. As per an estimate of World Health Organization, about 80% of the population in developing countries relay on traditional medicines for their primary health care. Moreover, 20% of the prescribed drugs presently are formulated from the herbal plants.[6] Plants cells have highly sophisticated chemical factors where a large variety of chemical compounds are manufactured with great precision from simple raw materials at normal temperature and pressure. Plants thus produce a variety of phytochemicals. It is estimated that there are 2, 50,000 to 5, 00,000 species of plants on earth. A relatively small percentage (1-10%) of these is used as foods by all animals including human beings. It is possible that even more are used for medicinal purpose.[7] The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds.[8] The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants. In plants, these compounds are mostly secondary metabolites such as Carbohydrates,

Amino acids, Proteins, Vitamin C, Tannins, Phenolic compounds, Flavanoids, Alkaloids, Saponins, Steroids and Glycosides which are capable of producing definite physiological actions in our body. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants.[9] Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. In the present study, we selected five different medicinal plants, such as *Abrus precatorius*, *Decalepis hamiltonii*, *Mucuna atropurpurea*, *Santalum album* and *Senna tora* for screening phytochemical constituents and antimicrobial activities against *Escherichia coli*, *Klebsiella* pneumonia, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh Leaves, Stems and Roots of *Abrus precatorius* L., *Decalepis hamiltonii* Wight &Arn., *Mucuna atropurpurea* DC., *Santalum album* L. and *Senna tora* (L.) Roxb, were collected from Kathiri hills, Erode District, Tamil Nadu, India, during 2014-2015. The plants were identified taxonomically and authenticated at the Herbarium, Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), University of Madras, Chennai, Tamil Nadu, India.

Bacterial culture and Growth Conditions

Microorganisms chosen were isolated from the clinical specimens that came for culture and sensitivity testing done in the laboratory of Department of Biotechnology, Sri Sankara Arts and Science College, Kanchipuram, Tamil Nadu, India. The plant extracts were used against the following test organisms; *Escherichia coli* (MTCC-890), *Klebsiella pneumonia* (MTCC-7162), *Pseudomonas aeruginosa* (MTCC-2295), *Bacillus subtilis* (MTCC-1305) *and Staphylococcus aureus.* Five bacterial species were employed as test organisms which include *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus* (MTCC-1305). The bacterial culture was maintained in Mueller-Hinton Agar (MHA). Inoculates were prepared by adding an overnight culture of the organism in MH broth to obtain an OD 600 in 0.1. The cells were allowed to grow until they obtain the McFarland standard 0.5 (approximately 108 CFU/ml).

Preparation of plant extract

The powdered plant leaves were extracted in soxhlet apparatus with Ethanol, Methanol, Ethyl acetate, Acetone, Chloroform and Petroleum ether. It was extracted with solvent for 3 hours by soxhelation. The obtained extracts were evaporating at room temperature to set a crude dried extract. The yield was determined and stored in air tight container until used to prevent the loss of biological activity.[10]

Qualitative analysis phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing for the presence of chemical constituents.[11, 12]

Tests for Carbohydrates (Benedict's test)

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Tests for Amino Acids (Ninhydrin test)

For the analysis of amino acid, 3 ml test solution and 3 drops 5% Ninhydrin solution were heated in water bath for 10 min. and observed for purple or bluish colour, the appearance of colour indicated the presence of amino acids.

Tests for Proteins (Biuret test)

3 ml of each test solution was added to 4% NaOH and few drops of 1% CuSO4 solution into separate tubes. The tubes were observed for violet or pink colour formation.

Tests for Vitamin C

1ml of 2% w/v solution was diluted with 5ml of water. 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of diluted sodium hydroxide solution were added. Then 0.6 ml of hydrochloric acid was added drop wise and stirred, the yellow colour turns blue which indicated positive results.

Tests for Tannins

With 2-3 ml test solution, 5% FeCl3 solution was added and observed for deep blue-black colour reactions.

Tests for Alkaloids (Wagner's test)

2-3 ml filtrate was taken into separate tubes. To that few drops of Wagner's reagent was added and observed the reddish brown precipitate.

Detection of flavanoids Lead acetate Test

The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavanoids.

Tests for Steroids (Salkowski Reaction)

To 2 ml of sample, 2 ml chloroform and 2 ml Concentrated H2SO4 were added and observed chloroform layer for red color and acid layer for fluorescence.

Test for Phenolic compounds (Ferric chloride test)

The extract was diluted to 5 ml with distilled water. To that a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presences of phenolic compounds.

Test for saponins

The fruits samples were diluted with distilled water and made into 20 ml. The suspension was shaken well in

graduated cylinder for 15 minutes; 2cm layer of foam indicates the presence of saponins.

Test for Glycoside

To 2 ml of plant extract, 1 ml of glacial acetic acid and 5 % ferric chloride was added then a few drops of concentrated sulphuric acid were added Presence of greenish blue colour indicates glycosides.

Antimicrobial Assay

Disc diffusion method was adopted for evaluation of antimicrobial activity of five different Medicinal plants. Muller Hinton agar was prepared and autoclaved at 15 1bs pressure for 15 minutes at 121°C. The cooled media was poured on to sterile Petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The disc impregnated with respective plants extract at individually were placed on the four corners of each Petri dishes, control disc was also placed. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

The preliminary phytochemical tests were performed; all extracts of selected medicinal plants was carried out different extracts were performed to know the secondary metabolites present among the selected medicinal plants such as alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenolic compounds, vitamin C, proteins, aminoacids and carbohydrates. The present study clearly shown that all the extracts was observed in alkaloids, flavanoids, phenolic, saponins and carbohydrates whearas amnio acids, protein, steroids and glycosides were absent but moderately present vitamins and tannins comparatively than to the other phyto compounds (Table 1).

The present study documents the antibacterial activity of five plant species of seven extracts was assayed *in vitro* by agar disc diffusion and agar well diffusion methods against 5 bacterial species. In this study, Two Gram positive (Gr+) bacterial pathogen such as *Bacillus subtilis; Staphyloccocus aureus* and three Gram negative (Gr-) bacterial pathogens *E. coli, Klebsilla pneumonia; Pseudomonas aeruginosa* were selected and antibacterial activity of extracts of selected medicinal plants *Abrus precatorius* L., *Decalepis hamiltonii* Wight & Arn., *Mucuna atropurpurea* (Roxb.) DC., *Santalum album* L. and *Senna tora* (L.) Roxb.

Ethanol and methanol extracts of leaves of *Senna tora* showed highest inhibitory activity against all the four tested organisms (Table 2). Maximum zone of inhibition was obtained with methanol extract of leaves of *Senna tora* against *Bacillus subtilis.* Ethanol and acetone extracts of leaves of *Abrus precatorius* and Stem of *Santalum album* showed moderate antibacterial activity. Petroleum ether extracts of leaves of *Mucuna atropurpurea* also showed highest antibacterial activity. Chloroform extracts of all the three plants did not show any inhibitory activity against test organism's only one show inhibitory activity which is the leaves of *Senna tora*. Similarly, aqueous extracts of medicinal plants did not show any antibacterial activity against all test organisms. The detailed observations are given in table 2.

The antimicrobial properties of many medicinal plants have been previously studied.[13]; [14]; [15] In the present study, antibacterial potential of leaf extracts of five important medicinal plants have been determined against four pathogens named *Escherichia coli, Klebsilla pneumonia, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus.* For 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 the four test organisms. The findings match with that of other workers.[16]; [17] It is often reported that Gram positive bacteria are more sensitive than Gram negative bacteria to plant based organic extracts.[18]; [19]; [20] But in our study, both gram positive and gram negative bacteria were found to be sensitive to plant extracts. In the present study, *Bacillus subtilis* was found to be the most sensitive while *Staphylococcus aureus* was found to be least the sensitive to plant organic extract than the other organisms. The findings agree with that of other workers.[21]

The antibacterial properties of medicinal plants may be due to presence of different chemical agents which were classified as bioactive antimicrobial compounds.[22] Phytochemical constituents such as alkaloids, glycosides, flavanoids, tannins, steroids, terpenoids and several other compounds are secondary metabolites of plants that serve as defense mechanism against many microorganisms, insects and other herbivores. The present study also revealed the presence of medicinally active compounds like alkaloids, glycosides, flavanoids, steroid and tannins in most of the selected plants which could be responsible for the observed antibacterial property.

The various phytochemical tests were performed to know the secondary metabolites present in the leaves of *Abrus precatorius;* leaves and roots of *Decalepis hemiltonii;* in the leaves of *Mucana atropurpurea;* leaves and stem of *Santalum album* such as alkaloids, flavanoids, glycosides, saponins, steroids, tannins, phenolic compounds, Vitamin C, proteins, aminoacids and carbohydrates. The results clearly evident that the highest activity was observed in alkaloids and flavanoids compare to the other compounds (Table 2). The Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores.[23] Alkaloids are formed as metabolic by products and have been reported to be responsible for the antibacterial activity.[24] Flavanoids are complex with extra cellular and soluble proteins and with bacterial cell walls.[25] 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.[26] Tannins bind to proline rich proteins and interfere with the protein synthesis.[27] Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell.[28] 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.[29] 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.[30] Though the results of this study agree with results of other studies, 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 the four test pathogens. Maximum antibacterial activity was shown by leaves of *Senna tora*, Ethanol and Methanol extract of leaves of *Senna tora*, was found to be equally potent against *Bacillus subtilis* 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 drugs. It is also necessary to check safety and toxicity of plant extracts before their pharmaceutical applicability. The study scientifically proves the importance of plant products in development of potent antibacterial agents.

Table 1 Qualitative phytochemical analysis of selected medicinal plants

Plants	Extracts	CH	AA	P	V	T	PC	F	A	Sa	St	G
Leaves of *Abrus Precatorius*	Acetone	+	-	-	-	+	+	+	+	+	-	-
	Ethyl acetate	+	-	-	+	-	+	+	+	+	-	-
	Chloroform	+	-	-	-	+	+	+	+	+	-	-
	Petroleum ether	+	-	-	+	-	+	+	+	+	-	-
	Aqueous	+	-	-	+	-	+	+	+	+	-	-
	Ethanol	+	-	-	-	+	+	+	+	+	-	-
	Methanol	+	-	-	-	+	+	+	+	+	-	-
Root of *Decalepis hamiltonii*	Acetone	+	-	-	-	+	+	+	+	+	-	-
	Ethyl acetate	+	-	-	+	-	+	+	+	+	-	-
	Chloroform	+	-	-	-	+	+	+	+	+	-	-
	Petroleum ether	+	-	-	+	-	+	+	+	+	-	-
	Aqueous	+	-	-	-	+	+	+	+	+	-	-
	Ethanol	+	-	-	+	-	+	+	+	+	-	-
	Methanol	+	-	-	-	+	+	+	+	+	-	-
Leaves of *Mucuna atropurpurea*	Acetone	+	-	-	-	+	+	+	+	+	-	-
	Ethyl acetate	+	-	-	+	-	+	+	+	+	-	-
	Chloroform	+	-	-	-	+	+	+	+	+	-	-
	Petroleum ether	+	-	-	+	-	+	+	+	+	-	-
	Aqueous	+	-	-	+	-	+	+	+	+	-	-
	Ethanol	+	-	-	-	+	+	+	+	+	-	-
	Methanol	+	-	-	-	+	+	+	+	+	-	-
Stem of *Santalum album*	Acetone	+	-	-	-	+	+	+	+	+	-	-
	Ethyl acetate	+	-	-	-	+	+	+	+	+	-	-
	Chloroform	+	-	-	-	+	+	+	+	+	-	-
	Petroleum ether	+	-	-	+	-	+	+	+	+	-	-
	Aqueous	+	-	-	-	+	+	+	+	+	-	-
	Ethanol	+	-	-	+	-	+	+	+	+	-	-
	Methanol	+	-	-	+	-	+	+	+	+	-	-
Leaves of *Senna tora*	Acetone	+	-	+	+	-	+	+	+	+	-	+
	Ethyl acetate	-	-	+	-	-	+	+	+	+	-	+
	Chloroform	-	-	+	-	-	-	+	-	-	-	-
	Petroleum ether	+	-	+	-	-	-	+	-	+	-	+
	Aqueous	+	+	+	+	+	+	-	-	+	-	+

	Ethanol	+	-	+	-	+	+	+	+	+	-	+
	Methanol	+	-	+	+	+	+	+	-	-	+	+

Present (+); absent (-)

CH = Carbohydrates; **AA** = Amino acids; **P** = Proteins; **V** = Vitamin; **T** = Tannins; **PC** = Phenolic compounds; **F** =Flavanoids; **A** = Alkaloids; **Sa** = Saponins; **St** = Steroids: **G** = Glycosides.

Table 2 Antibacterial activity of various extracts against human pathogenic organisms

Plants	**Extracts**	**Zone of Inhibition in mm**				
		Escherichia coli	***Klebsilla pneumoniae***	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***Staphyloccocus aureus***
Leaves of *Abrus precatorius*	Aqueous	-	-	-	-	-
	Ethanol	9.8±0.10	10± 0.00	-	-	-
	Methanol		-	-	-	-
	Acetone	9.9 ±0.10	-	-	-	-
	Ethyl acetate	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Petroleum ether	-	16±0.10	-	-	-
Root of *Decalepis hamiltonii*	Aqueous	-	-	-	-	-
	Ethanol	12.9±0.10	-	14±0.10	12.8±0.10	-
	Methanol	-	-	-	18.9±0.20	-
	Acetone	-	-	-	-	-
	Ethyl acetate	-	-	-	16±0.10	-
	Chloroform	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
Leaves of *Mucuna atropurpurea*	Aqueous	-	-	-	-	-
	Ethanol	-	16±0.10	-	11.9±0.0	11.03±0.10
	Methanol	-	-	-	-	-
	Acetone	-	-	-	-	-
	Ethyl acetate	-	-	-	16.9±0.10	-
	Chloroform	-	-	-		
	Petroleum ether	-	19±0.10	-	13.8±0.10	17.03±0.10
Stem of *Santalum album*	Aqueous	-	-	-	-	-
	Ethanol	15.03±0.10	15.02±0.10	11.96±0.0	13.96±0.10	-
	Methanol	15.0±0.10	14.9±0.10	14.0±0.00	-	-
	Acetone		13.9±0.12	18.0±0.10	-	-
	Ethyl acetate	16.9±0.10	-	-	-	-
	Chloroform	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
Leaves of *Senna tora*	Aqueous	-	-	-	-	-
	Ethanol	15.0±0.10	12±0.10	11.0±0.10	18.1±0.10	-
	Methanol	16.0±0.10	14.0±0.10	13.0±0.10	-	-
	Acetone	-	-	-	-	-
	Ethyl acetate	11.1±0.10	-	-	-	-
	Chloroform	-	-	-	11.0±0.00	-
	Petroleum ether	-	-	11.0±0.10	-	-

No activity (-)

Each value is expressed as mean ± standard deviation (SD) of triplicate determination

CONCLUSION

The present study concluded that, the extract of five medicinal plants can be used as an antibacterial agent to threats against human pathogenic organisms. These plants have a good source of potentially useful phytochemical components, which serve as a traditional medicine and act as a drug. The present investigation provides the way for further attention of upcoming research to identify the active principles of these medicinal plants.

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