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Phytochemical investigation of selected medicinal plants and their antimicrobial activities against human pathogens

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ABSTRACT

Phytochemicals present in the plants are having medicinal values. These phytochemicals possess potentiality to cure number of diseases.The present study phytochemical screening of some important medicinal plants was carried out. Qualitative and qualitative phytochemical analysis of plants confirms the presence of various biologically active secondary metabolites like alkaloids, terpenoids, saponins, steroids, anthocyanin and tannins. The results imply that the phytochemical with medicinal properties used for curing various ailments and possess potential antioxidant, antibacterial and the isolation of novel bioactive compounds. Antibacterial activity of selected plants against *Pseudomonas aeroginosa* and *Escherichia coli* was carried out.

Key words: Medicinal plants,Phytochemicals, Antibacterial.

INTRODUCTION

Humans, since ancient times, have been exploiting the nature, particularly plants in search of new drugs. This has resulting in the use of large number of medicinal plants with curative properties to treat various diseases. Approximately 65% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 90% of the prescriptions are plant based in the traditional systems of Ayurveda, Homeopathy and Siddha. The study of plants continues for the discovery of novel secondary metabolites. Uses of natural drug are more efficient than chemical drugs and no side effects. There is increasing need to search for new compounds with antimicrobial activity to microbial infection. The available commercial [14] antimicrobial drugs are unsatisfactory due to the problem of microbial resistance in various strategies of their life cycle [15] Plant products have part of phytomedicines since time immemorial. These can be derived from any part of the plant like leaves, bark, flowers, seeds, roots etc. contains active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex biochemical substances. In the present work, qualitative and quantitative phytochemical analysis with antimicrobial activity of selected medicinal plants.

MATERIALS AND METHODS

Collection of plant materials

The plant material were collected from Chandanapuri ghat, (Located 19° 29' 41" N, 74° 11' 47" E) Maharashtra during October 2019. A voucher specimen of all plants has been deposited in the herbarium of Department of Botany. These plants were identified with the help of the available literature.

Plant extract preparation

The leaves were washed thoroughly 2-3 times with running tap water, then air dried under shade and the plant material was grinded in mixer. The powder was kept in small plastic bags with paper labeling. Crude plant extract was prepared by Soxhlet extraction method. About 5gm of powdered plant material was uniformly packed into a thimble and extracted with 125ml of different solvents separately. Solvents used were methanol, petroleum ether, ethanol, and acetone. The process of extraction continues till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot air oven at 30-40ºC till all the solvent got evaporated. Dried extract was kept in small glass bottle for their future use in phytochemical analysis.

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods. [1,2,3]

Test for Protein

Biuret test

Crude extract mix with 2ml biuret reagent, a bluish violet colour appeared the presence of protein.

Ninhydrin test

Crude extract boiled with 2ml of 0.2% Ninhydrin violet colour appeared the presence of amino acids and proteins.

Test for Carbohydrate

Molisch's test

Crude extract was mixed with 2ml of Molisch's reagent and 2ml of concentrated sulphuric acid was poured carefully along the side of the test tube, appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Benedicts test

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

Test for Flavonoids

Shinoda test

Crude extract mixed with fragments of magnesium ribbon and concentrated hydrochloric acid. Pink colour appeared after few minutes which indicated the presence of flavonoids.

Lead acetate test

The extract treated with a few drops of lead acetate solution. Formation of yellow ppt indicates the presence of flavonoids. Orange to crimson colour shows the presence of flavonoids.

Test for Alkaloids

Wagner's test

Extract was mixed with dilute HCL and placed in steam bath for 5 min.and then filter 1-2 ml filtrate, 2 mL of Wagner's reagent was added. Reddish brown coloured precipitate indicates the presence of alkaloid.

Dragendroff's test

The filtrate was mixed with Dragendorff's reagent and the formation of orange precipitate indicates the presence of alkaloids.

Test for Phenolics

Ferric chloride test

Extract was dissolved in distilled water.2 mL of 5% ferric chloride solution was added. Formation of violet colour or blue green indicates the presence of phenolic compounds.

Lead acetate test

Extract was dissolved in distilled water.1 to 2 drops of lead acetate solution was added. Formation of white ppt indicates presence of phenolic compounds.

Test for Glycosides

Libermann's test

Crude extract mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated sulphuric acid was added. A colour changes from violet to blue to green indicated the presence of steroidal nucleus, i.e. presence of glycoside.

Keller-kilani test

Extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of ferric chloride. The mixture was then poured into another test tube containing 2ml of concentrated sulphuric acid.A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for Saponins

Extract was mixed with 5 to 10 ml of distilled water in a test tube and shaken vigorously. The formation of stable foam indicate for the presence of saponins

Test for Tannin

Extract was mixed with 2 to 3 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of tannins.

Test for steroids

Extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test mixing extract with 2ml of chloroform. Then 2ml of each of concentrated sulphuric acid and acetic acid were poured into the mixture. A greenish coloration indicated the presence of steroids.

Test for Microorganisms

Clinical isolates of bacteria were used for the antibacterial screening. The isolates were Escherichia coli and Pseudomonas aeroginosa .These strain obtained from the laboratory unit of the Department of Microbiology, P.M.T. Pravaranagar and authenticated using standard biochemical tests as described by Cheesbrough (2002). The isolates were maintained on a freshly prepared nutrient agar slant and kept refrigerated at 5^0C until required for use.

Preparation of sensitivity disc

The sensitivity discs prepared using sterile Whatman's grade one filter paper [4]. A paper puncher used to obtain disc 5.0mm in diameter. These were then placed in sterile screw-capped bottles and sterilized in an autoclave at 121oC and 15psi for half an hour. The discs were allowed to cool until use. Stock solution of the plant extract was prepared: 100 micro gram of each fraction was weighed and dissolved in 10 ml of dimethyl sulphoxide.

Antibacterial Assay

Agar diffusion method [5]. The freshly prepared nutrient agar plates were dried in a drier for about 15 minutes to remove surface moisture. The plates were aseptically inoculated uniformly with the test organism by spread plate method. With the aid of a sterile forceps, paper discs that have been impregnated with the plant extracts at different concentrations were arranged radially and pressed onto the inoculated surface with each disc sufficiently spaced out. Positive control discs containing standard antibiotic (Ampicillin) and negative control discs containing DMSO the extract were used. The plates were incubated at 37OC for 24 hours in incubator. The result was observed by measuring of zone of inhibition in a diameter and recorded in millimeter [6].

RESULTS AND DISCUSSION

The phytochemical characteristics of ten medicinal plants tested were summarized in the table-1. The results revealed the presence of medicinally active compounds in the ten plants studied. From the table, flavonoid and alkaloid present in all the plants. Tannin present in six plants. Glycosides present in five plants. Phenolic and steroids present in only four plants Saponin were absent in Terminalia arjuna and Terpenoid were absent in Adhatoda vasica.

The results of plant extracts against antibacterial activities are presented in table-2. The methanol extracts of ten medicinal plants tested against two Gram-negative bacteria using agar disc diffusion method. The plants exhibited antibacterial activity to a certain degree. Plumbago zeylanica showed highest zone of inhibition in all bacteria followed by Terminalia arjuna,Withania somanifera, Tridax procumbens,Woodfordia fruticosa and Azadiracta indica.Butea monospermous extracts was inactive against Pseudomonas aeroginosa Gram-negative strains tested. Out of ten-nine medicinal plant species showed significant antimicrobial activity in both the strains.

The results of the present study support the folkloric usage of the studied medicinal plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new novel drugs for the therapy. The most active extracts subjected to isolation of the therapeutic antimicrobial and undergo further pharmacological evaluation. The antibacterial substances in the higher plants are well established. The successful evaluation of plant substances from plant material is largely dependent on the type of solvent used in the extraction procedure. Commonly performed water extracts, water as the solvent but, plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. The compounds being extracted in addition to their intrinsic bioactivity, by their

ability to dissolve or diffuse in the different media used in the development of medicine, a natural blueprint for the development of drug. Esteemed workers have identified plant compounds that are effective antibiotics. Traditional systems around the world which utilize herbal remedies are an important source for the discovery of new antibiotics. Some traditional remedies have produced compounds that are effective against antibiotic resistant strains of microorganism. The antibiotics property of the herbal compounds that indicates the need for further research in to traditional healing system [18]. It is also important pharmacological studied leading to synthesis of more potent drug with reduced toxicity.

The present study was initiated because of increasing resistance of antibiotics including bacteria. Plant extracts (compounds) are most important in the field of antiseptic and antimicrobial agents. As a result, the microbial activity of ten medicinal plants was screened against common pathogens. The methanol plant extracts of Plumbago zeylanica , Terminalia arjuna,Withania somanifera and Woodfordia fruticosa showed the most remarkable activity. These plants can be further subjected to isolation of the therapeutic antimicrobial and carry out further pharmacological evaluation.

Table 1: Phytochemical Screening of Methanol Extract of Plants.

Sr.No.	Plant Species	Fla	Alk	Phe	Gly	Sap	Tan	Ste	Ter
1.	*Boerhavia erecta L.*	+	+	-	-	+	-	+	+
2.	*Adhatoda vasica L.*	+	+	+	-	+	+	-	-
3.	*Terminalia arjuna Roxb.*	+	+	+	+	-	+	-	+
4.	*Tridax procumbens L.*	+	+	-	+	+	+	+	+
5.	*Azadirachta indica A.*	+	+	-	-	+	-	-	+
6.	*Plumbago zeylanica L.*	+	+	+	+	+	+	-	+
7.	*Terminalia bellerica* L.	+	+	-	-	+	+	+	+
8.	*Butea monosperma* F.	+	+	+	-	+	-	+	+
9.	*Woodfordia fruticosa L.*	+	+	-	+	+	-	-	+
10.	*Withania somnifera* L.	+	+	-	+	+	+	-	+

1. Fla-Flavonoid,Alk-Alkaloid,Phe-Phenolic,Gly-Glycoside,Sap-Saponin, Tan-Tannin,Ste-Steroid,Ter-Terpenoid.
2. + indicates presence and – indicates absence of activity.

Table 2: Showing antibiotic activities of methanol plant extracts.

Sr.No.	Plant Species	Zone of inhibition(in mm diameter)	
		Ampicillin (Gram-negative)	
		Escherichia coli	***Pseudomonas aeroginosa***
1.	*Boerhavia erecta L.*	11	09
2.	*Adhatoda vasica L.*	14	10
3.	*Terminalia arjuna Roxb.*	13	15
4.	*Tridax procumbens L.*	13	14
5.	*Azadirachta indica A.*	12	14
6.	*Plumbago zeylanica L.*	15	18
7.	*Terminalia bellerica* L.	11	11
8.	*Butea monosperma* Faub	07	-
9.	*Woodfordia fruticosa L.*	13	12
10.	*Withania somnifera* L.	13	15

CONCLUSION

Now a days increasing interest in the natural remedies, with a basic approach towards nature, due to people's awareness towards the potency and side effect of the synthetic drugs. Hence, it is necessary to analyze the herbal and phytochemicals for achieving sustainable and environmental friendly novel pharmaceutical products. To ensure the safety and efficacy of herbal medicines used, standardization and the development of the processing aspects of phyto-medicines are very important. The present study has provided supportive scientific evidence, the chemical elements present in the selected medicinal plant parts pharmacologically important. Currently available herbal products are not adequate to meet the demand. Therefore, there is a need to expand the research in much more variety of plants in future.

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