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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL PROPERTY OF LEAVES OF *POGOSTEMON BENGHALENSIS* (BURM. F.) KUNTZE

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Abstract– Phytochemical analysis and antimicrobial property of *Pogostemon benghalensis* leaf was studied. The cold extracts of leaf were prepared in acetone and methanol solvents. The phytochemical analysis of bioactive acetone and methanol extract was carried out. The phytochemical analysis of different solvent extract of this plant showed the presence of different classes of metabolites such as alkaloids, flavonoids, tannins, steroids, saponins glycosides, carbohydrates and proteins. Agar cup technique was used to assess the antimicrobial activity of acetone and methanol leaf extracts against five human pathogenic bacteria strains (*Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Streptococcus pyogenes, Bacillus subtilis*). The acetone leaf extract showed antimicrobial activity against all the organisms expect for *B. subtilis* as compared to methanol leaf extract. The minimum inhibitory concentration was 12.5 mg/mL in acetone extract. The present study gives an insight for further phytochemical and pharmacological investigation of this plant.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Plants consist of a number of biologically active ingredients therefore they are used for the treatment of a large number of infectious diseases. These biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds (Balakumar *et al.*, 2011; Paulraj *et al.*, 2011). Plant derived natural products have received considerable attention due to their diverse pharmacological properties including antibacterial, antifungal and antioxidant activities. Thus, it becomes necessary to analyze the vast untapped potential of the medicinal plants in combating the antibiotic resistant organisms. The aromatic plant of *Pogostemon benghalensis* is a very common member of Lamiaceae family and it occurs in open riverine forest,but is also cultivated in almost all parts of India, Bangladesh, Sri Lanka, Nepal, Thailand and China (Bhuiyan *et al.*, 2011). *P. benghalensis* is commonly known as Phangli in Marathi language. Almost all the parts of the plant namely root, leaves, shoots, stem and bark are used as medicine, manure for paddy crops and other miscellaneous purpose such as scenting woolens, wardrobes, insect and leeches repellent (Shigwan *et al.*, 2013). The tribals use poultice of leaves and root juice as remedy for snake bites. *P. benghalensis* is reported to posse antibacterial, antifungal, antitubercular and anti-rheumatic activities. Hence in the present investigation, *P. benghalensis* has been tested for presence of therapeutically active metabolites and efficacy of the leaves to inhibit some bacterial strains.

MATERIALS AND METHODS

Plant Collection

P. benghalensis leaves were collected from the Tamhini Ghat, Mulshi district of Pune, Maharashtra, India in the month of February. The collected plant was identified taxonomically by Blatter Herbarium, St. Xavier's College, Mumbai. The specimen matched with Blatter Herbarium specimen 22949 of

H. Santapau.

Cold Extraction

Leaves of the plants were washed with water and shade dried. After complete shade drying, the leaves were ground in mixer and sieved to obtain fine powder. Cold extraction of leaves was prepared by using maceration process. 5g of leaf powder wassoaked in 50 mL of each solvent viz. acetone and methanol. The extracts were kept on shaker for 24 hrs at RT. After 24 hrs, the extracts were filtered using Whatman filter paper No.1. The extracts were stored in clean glass bottles at -4 ^{o}C in a refrigerator until use.

Phytochemical screening

Chemical tests were carried out on the acetone and methanol extract using standard methods (Harborne, 1972).

Tests for Alkaloids

Dragendorff's Test (Potassium Bis. Iodide)

To 2-3 mL of filtrate, few drops of the Dragendorff's reagent were added. Formation of orange brown precipitate indicated the presence of alkaloids.

Mayer's Test ($HgCl_2$)

To 2-3 mL of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Hager's test (Picric acid)

To 2-3 mL of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Wagner's test ($I_{2/}$KI)

To 2-3 mL of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

Tests for flavonoids

Lead acetate test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline reagent test

The extract was treated with few drops of dilute sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Ferric chloride test

To the filtrate few drops of ferric chloride solution was added. Intense green color confirmed flavonoids.

Alkaline reagent test

The extract was treated with few drops of dilute sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Ferric chloride test

To the filtrate few drops of ferric chloride solution was added. Intense green color confirmed flavonoids.

Tests for tannin and phenolic compounds

Ferric chloride test

Approximately 1 mL of extract was dissolved in distilled water. To this solution 2 mL of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Lead acetate test

Approximately 1 mL of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

Tests for triterpenoids and steroids

Salkowski's test

The extract was treated with chloroform and filtered. A few drops of concentrated sulphuric acid were added to this filtrate, shaken and allowed to stand. If the lower layers turn red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

Libermann- burchard's test

The filtrate was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation

of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Tests for Glycosides

Borntrager's test

To 3 mL of extract, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and mixed well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

Legal's test

One mL of extract was dissolved in pyridine. 1 mL of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Alkaline test

Filtrate when made alkaline, blue or green fluorescence confirms coumarin glycosides.

Tests for Saponins

Foam test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of Saponins.

Lead acetate test

To 1 mL of extract solution 1% Lead acetate solution was added. White precipitate confirms saponins.

Tests for Carbohydrates

Barfoed's test

One mL of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Fehling's test

To 1 mL of extract, 1 mL of Fehling's A and 1 mL of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for Proteins

Ninhydrin Test

3 mL of extract was heated with 3 drops of ninhydrin solution in water bath for 10 minutes. Formation of blue color indicates presence of amino acid.

Tests for Quinones

A small amount of extract was treated with concentrated HCl. Formation of yellow colored precipitate confirms quinones.

Test Micro-organisms and Media

The organisms used in this study were *Staphylococcus aureus* (ATCC 29213), *Streptococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pyogenes* (ATCC 19615) and *Bacillus subtilis* (ATCC 6633). The strains were procured from NCIM, Pune. The strains were maintained on nutrient agar slants at 4 °C.

Bioassay

The antimicrobial activity of different extracts was evaluated using agar cup technique. Four wells were punched into agar plate using a sterile cork borer of 6mm diameter per plate. 50 µL of the extracts (100mg/mL) were filled into wells. The plates were left on bench for about 2 hrs to allow the extract diffuse properly into nutrient agar, i.e. pre diffusion. The plates were incubated for 18-24 hours at 37°C. Test plates were maintained in duplicates and each positive result was tested again to ensure its efficacy. Zone size was measured in millimeters using simple scale.

Minimum inhibitory concentration (MIC)

The MIC was performed for the potent extract at different concentrations: 100, 50, 25, 12.5, 6.25,3.125mg/mL (Jayashree *et al*., 2015). Standard drug Kanamycin was used as positive control at concentration of 10 mg/mL in Dimethyl sulfoxide (DMSO). The plates were left on bench for about 2 hrs to allow the extract diffuse properly into

nutrient agar, i.e. pre diffusion. The plates were incubated for 18-24 hours at 37 °C. Test plates were maintained in duplicates and each positive result was tested again to ensure its efficacy. Zone size was measured in millimeters using simple scale.

RESULTS

Phyto-constituents of *P. benghalensis* leaves: The present study was carried out on the leaves of *P. benghalensis* revealed the presence of metabolites that may have medicinal value (Fig.1).

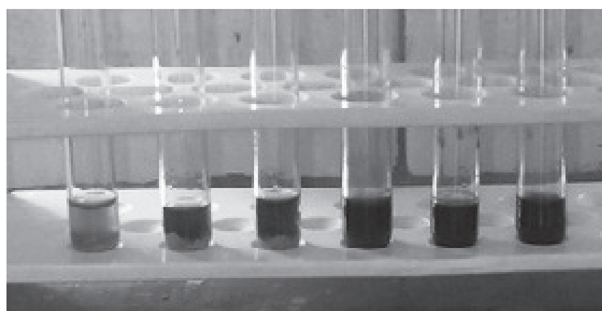

Fig. 1. Phytochemical test showing positive test for alkaloids, Flavonoids, tannins, steroids, proteins and carbohydrate

Alkaloids, flavonoids, tannins and phenolic compounds, steroids, saponins, carbohydrates were present in both the solventextracts (Table 1). Quinones were absent in acetone extract.

Antibacterial Property

Most of the test bacteria were significantly inhibited by the 100 mg/mL acetone extract except for *B.subtilis*. The highest zone of inhibition (mm) was observed against *P.aeruginosa* (22mm) followed by *S.pyogenes* (21 mm), *S.aureus* (20mm) and *S.faecalis*

Table 2. Antibacterial activity of cold acetone leaves extract (100 mg/mL) by Agar cup method (zones in mm)

Microorganism	Acetone Extract	Methanol Extract
Bacillus subtilis	-	-
Pseudomonas aeruginosa	22	-
Staphylococcus aureus	20	12
Streptococcus faecalis	20	-
Streptococcus pyogenes	21	-

Table 1. Phyto-chemical screening of *Pogostemon benghalensis* leaves extracts.

Sr. No.	Name of Phytochemical tests	Acetone	Methanol
1.	**Alkaloids**		
	Dragendorff's test	+	+
	Hager's test	+	+
2.	**Flavonoids**		
	Lead acetate test	+	+
	Alkaline reagent test	+	+
	Ferric chloride test	+	+
3.	**Tannins and phenolic compounds**		
	Ferric chloride test	+	+
	Lead acetate test	+	+
4.	**Steroids**		
	Salkowski's test	+	+
	Libermann- Burchard's test	+	+
5.	**Glycosides**		
	Coumarin glycosides(Alkaline test)	-	-
	Cardiac glycosides(Legal's test)	-	-
	Anthraquinone (Borntragers)	-	+
6.	**Saponin**		
	Foam test	+	+
	Lead acetate test	+	+
7.	**Carbohydrates**		
	Barfoed' test (Monosaccharides)	-	-
	Fehling's test(Reducing sugar)	+	+
	Benedict's test (Reducing sugar)	+	+
8.	**Proteins**		
	Ninhydrin test	-	+
9.	Quinones	-	+

Table 3. MIC of Acetone extracts of leaves of *Pogostemon benghalensis* against five bacteria strains

Organisms	50	25	12.5	6.25	3.125	1.5625	K (10 mg/mL)
Pseudomonas aeruginosa	21	20	18	-	-	-	33
Staphylococcus aureus	12	10	-	-	-	-	33
Streptococcus faecalis	18	-	-	-	-	-	33
Streptococcus pyogenes	-	-	-	-	-	-	34

-: No Inhibition K: Kanamycin

(20mm). 100 mg/mL of methanolic extract inhibited only *S. aureus* (12 mm). Methanolic extract didn't show any activity against other test organism used (Table 2).

MIC

Overall acetone extract results were better therefore selected for MIC. The best MIC of 12.5 mg/mL was observed against *P. aeruginosa* followed by *S. aureus*at 25 mg/mL and *S. faecalis*at 50 mg/mL (Table 3).

DISCUSSION

The phytochemical screening of leaves of the plant *P. Benghalens* is showed the presence of various bioactive metabolites constituents. Primary metabolites like proteins and carbohydrates were present in both the extracts. It was observed that these metabolites of the plant can be easily extracted in both solvents used. Further, the phytochemical analysis of both leaf extracts showed presence of secondary metabolites viz. alkaloids, steroids, tannins, saponins, and reducing sugar. These components are well known to have curative activity against several human problems such as diuretic, Chloretic, spasmodic, chronic eczema, diarrhoea, and dysentery. Each of these secondary metabolites possesses specific properties and physiological activities. There are many reports indicating the preliminary phytochemicals in various medicinal plants (Shirsat *et al*., 2012). Our results are in analogy with the above reports. The availability of the major phytocompounds like alkaloids, phenolics, flavonoids, tannins and steroids in various extracts of *P. benghalensis* can be correlated with the medicinal potential of the plant. Hence this could suggest the folk use of this plant.

The highest zone of inhibition (mm) was exerted against *P. aeruginosa* (22 mm) followed by *S. pyogenes* (21 mm), *S.aureus* and *S. faecalis* (20 mm). The 100mg/mL methanol extract only exhibited mild activity against *S. aureus* (12 mm). These results showed that acetone extract is more effective than methanolic extract. The MIC was found to be 12.5 mg/mL against *P. aeruginosa* while for *S. aureus* it was 25 mg/mL. It indicates that *P. benghalensis* contains potent broad spectrum of antimicrobial principles. These results are in agreement with Ashwini *et al.* (2013). The present investigation provides a starting material for further pharmacological investigation. It can be concluded that, this plant could be further used for making specific drugs provided it could be exploited in detail, phytochemically and pharmacologically.

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