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Aqueous extraction of *Pogostemon benghalensis (Burm. F.) Kuntze* aerial part and its efficacy of pharmacological activities

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Abstract
The present study Investigation of Aqueous extract of *Pogostemon benghalensis* (Burm. f.) Kuntze preliminary phytochemical screening, quantitative analysis, antibacterial and antioxidant. In previous years medicinal plants are used for therapeutic purpose. This study aim evaluate the aqueous extract various biological activities. Antibacterial activity of aqueous extract was performed by disc diffusion method against three strains of gram positive bacteria staphylococcus, Enterococcus faecalis, Enterococcus phyogens, and two strains of salmonella typhi, Enterobacter aerogenes. Aqueous extract exhibited the highest antibacterial activity against Enterobacter aerogenes (15mm), the lowest antibacterial activity against to salmonella typhi (5mm). The preliminary phytochemical screening shows Reducing sugar, Sugar, Phenolic compound, catechins, Flavonoid and amino acid. The present study suggest that aqueous extract can be used as Potentional of therapeutic drug.

Keywords: antibacterial, *Salmonella typhi, Enterobacter aerogenes Flavonoids*

Introduction

The Lamiaceae is a very large plant family occurring all over the world in a wide variety of habitats from alpine regions through grassland, woodland and forests to arid and mountain side. Each family usually contains a number of genera and each genus a number of species. These plants originated in Southern Europe around the Mediterranean [1]. *Flora of Britis India).* The plants are occur in most tropical, sub-tropical and temperate regions of the world Africa, Asia, the Pacific islands, and Australia. [2, 3, 4] Lamiaceae is a perennial herb belongs to the part of the mint family of plants and has long been cultivated in India, Thailand and parts of South East Asia as a spice and as a condiment for heart ailments and stomach cramps. The medicinal plants are greatest economic source of the world. Nature has bestowed on us a very rich botanical resources and a large number of various types of plants grow in several parts of world [5] Plants are richest resource of drugs of modern medicines, food supplements, nutraceuticals, pharmaceutical intermediates, flok medicines and chemical entities for synthetic drugs [6]. Herbal medicine is still the basis of about 75 to 80 % of the whole population and the plant extracts and their active constituents are used in the major part of traditional therapy [7]. Indigenous medicinal systems like Ayurveda, Unani, Siddha are used around 1500 plants systematically [5] It also mainly contributed to the pharmacological activities [8] and the therapeutic properties of the essential oils were directly correlated with their qualitative and quantitative compositions which were obviously different and derived from various cultivation regions of Patchouli [9] The main components of the essential oils extracted from *P. cablin* collected from Wanning City were patchouli alcohol (37.74%, relative content) and pogostone (0.85%) [10] While essential oil was mainly composed of patchouli alcohol (30.87%) and pogostone (18.90%) in the population from Guangzhou city [11]

The family of Lamiaceae is an aromatic plants are possessing odoriferous and volatile substances that occur as essential oil, gum exudates, balsam and oleoresin in one or more parts. The chemical nature of these aromatic substances may be due to a variety of complex chemical compounds. Many of these aromatics are powerful germicides and have antibacterial properties, but the bulk of the produces finds use in perfumery and food flavouring industries. Among the two, the perfumery and allied industries consume much large amount of the natural fragrant material. A large quantity of the aromatic material of plant origin is utilized in cosmetics, toiletries and allied industries. They serve as the basic raw material for perfumery, flavour and cosmetic industries. They are used in a number of products like soap detergents, cosmetic lotions, agarbattis, disinfectants, deodorants, mosquito repellants, flavouring of food, soft drink, pharmaceuticals, tobacco and variety of perfumery products. The application of essential oil in plant protection as antifeedant, repellants, biopesticides, natural herbicides and growth boosters Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, Dyeing repellents, fragrances, Cosmetics. Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. digestive stimulation action, [12, 13, 14, 15].

Edible antioxidants commonly found in plants are ascorbic acid, tocopherols, carotenoids, and several phenolic compounds [16] such as phenolic acids, flavonoids, and tannins [17] Phenolic acids, for example caffeic, ferulic, and vanillic acids have been known as natural antioxidants widely distributed in the plant kingdom. Besides, naturally occurring polyphenols whose oxidation inhibiting activities have been known for a long time are tannins. Additionally, some studies have revealed that low amounts of tannins

(0.15–0.2%) in the diet can be beneficial to human health and will create a more astringent feel to the taste, while at higher concentration; they inhibit the digestive enzymes and reduce the bioavailability of iron and vitamin B12 [18] Most of the Lamiaceae sources of antioxidants belong to the subfamily Nepetoideae, including basil, lemon balm, marjoram, mint, oregano, rosemary, sage, etc. They contain rosmarinic acid and are frequently abundant in fragrant volatile terpenes [19] Moreover, the extracts of rosemary were the first marketed natural antioxidants. In the study of [20] thyme, sage, rosemary, and marjoram showed the greatest antioxidant capacity among the investigated herbs [21]. Have shown that besides thyme, rosemary, and sage, peppermint, lemon balm, and basil also contain a considerable amount of phenolic compounds with strong total antioxidant and DPPH radical scavenging activities. The species are among the most frequently investigated species of the family Lamiaceae and their antioxidant activity has been demonstrated in numerous studies [22, 23, 24, 25, 26].

Materials and Methods

Collection and Processing of Plant Materials

The healthy leaves of *pogostemon benghalensis (Benth) kuntze* were collected from the Kolli hills of Eastern Ghats, Tamil Nadu India. The leaves were shade dried for one week and powdered for the phytochemical studies.

Solvent extraction

The *pogostemon benghalensis* Sample material was well washed with water and then successively with tap water and distilled water to remove the other wastes. Finaly the leaves samble materials (50g dry weight) were graind to fine powder and extracted with following solvent The powdered leaves were soaked in different solvents like petroleum eather, chloroform, methanol and water each in 50 soaked in different solvents like petroleum eather, chloroform, methanol and water each in 500 mL for 72 hours at room temperature. After that the soxhlet extracts completed. This extract was transfer to the Petridis extract were kept for dry for some time to eliminated the solvent after stored at 4 ^{0}C after used for phytochemical screening.

Phytochemical Screening

The extracts were subjected for the analysis of various phytochemical. Present the dried sample of *pogostemon benghalensis* the procedure for screening of phytochemical through preliminary screening was followed by using standard manual phytochemical tests were carried out for the presence and absence for the alkaloids, flavonoids, phenols, amino acids, saponins, sterols, tannins, terpenoids, Anthroquinines, sugars, reducing sugar and catechins the chemicals and reagents used in the above tests were freshly prepared in our laboratory. The presence and absence of phytochemicals in the differents extracts of *pogostemon benghalensis* were recorded in note book as and respectively the detailed methodology for each test is given below.

Preliminary phytochemical screening

Preliminary phytochemical analysis of different solvents extracts of plant samples were carried out according to [27].

Steroids

To the 2 ml of test solution, minimum quantity of chloroform, 3 or 4 drops of acetic anhydride and one drop of Conc.H_2SO_4 was added. Change of purple colour to blue or green indicates the presence of steroids.

Triterpenoids

To the 2 ml of test solution, piece of 2 drops of Thionyl chloride was added. Development of violet or purple colour indicates the presence of triterpenoids.

Reducing sugar

To the 2 ml of test solution, 2 ml of fehlings reagent and 1 ml of H_2O were added. Formation of red orange colour indicates the presence of reducing sugar.

Sugars

To the 2 ml of test solution, very small quantity of an Anthrone and 2 drop of Con. H_2SO_4 was added and heated gently. Appearance of green or purple colour indicates the presence of sugars.

Alkaloids

2 ml of test solution with 2 N HCl forms an aqueous layer. The aqueous layer was decanted. To the remaining aliquot, one or few drops of Meyer's reagent were added. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenolic compounds

To the 2 ml of test solution in alcohol, one drop of neutral ferric chloride (5%) solution was added. Development of intense blue colour indicates the presence of phenolic compounds.

Catechins

To the 2 ml of test solution in alcohol, Ehrlich reagent and few drops of concentrated HCl was added. Pink colour formation indicates the formation of catechins.

Flavonoids

To the 2 ml of test solution in alcohol, a bit of magnesium and one or two drops Conc. HCl was added. Appearance of red or orange red colour formation indicates the presence of flavonoids.

Saponins

2 ml of test solution was taken along with H_2O and shaken well. Formation of foamy lather indicates the presence of saponins.

Tannins

To the 2 ml of test solution, H_2O + lead acetate were added. Development of white precipitate indicates the presence of tannins.

Anthraquinones

To the 2 ml of test solutions, few drops of Magnesium acetate solution were added. Appearance of pink colour indicates the presence of anthraquinones.

Aminoacids

To the 2 ml of test solution, 1% ninhydrin in alcohol was added. Development of blue or violet colour indicates the presence of amino acids.

Determination of total phenol content

The amount of totel phenolics was determined following the modified folinciocalteu method [28, 29] Gallic acid was used as the standard and the phenolic content were expressed in mg/g gallic acid equivalents (GAE). Concentration of 10, 20, 30, 40, mg/ml galic acid were prepared in methanol. The concentrated extracts from various solvents dissolved in 5 ml of methanol and 0.05 ml of the solution was taken the study. 0.05 ml of each samble were treated with 0.125 ml of folin ciocalteu (FC) Reagent (FC: Distilled water-1:1) in test tubes and were incubated at room temperature for 6 minutes. Then 1.25 ml of 7% sodium carbonate was added to each tube and incubated for another 90 minutes at room temperature. The absorbance was read spectrophotometrically at 650 nm. Determination for all the sambles were done in triplicate.

Determination of total flavonoid content

The rutin acid was used as the standard.The concentration of 20, 40, 60, 80 mg/ml rutin acid were prepared in methanol. The estimation of totel flavonoids was conducted using standard procedure 0.1 ml of each extract dissolved in methanol was made upto 5ml with disstiled water. To this 0.15 ml of 5% sodiem corbonate was added and kept at room temperature for 5 minutes 0.3ml of 10% aluminium chloride followed by1.0 ml of sodium hydroxide was added mixed thoroughly and the readings were taken at 510 nm spectrophotometrically. All the readings were in triplicate.

Determination of total tannin content

Tannin content was determined in all the six extract using FC Reagent. Tannic acid was used as the standard and the result were expressed as mg/g equivalents of tannic acid. Concentrations of 25, 50, 75 and 100 mg/ml of tannic acid were prepared in methanol. The concentrated extracts from various solvents were dissolved in 5ml of methanol and 0.05 ml of the solution was taken for the study. 0.05 ml of each samble was mixed with 5 ml of 2% sodium corbonate and was incubated in boiling water bath for 10 minutes. Then 0.5 ml of FC Reagent was added and absorbance were taken spectrophotometrically at 760 nm. Determinations for all the samples were done in triplicate.

Antibacterial Activity

Zone of inhibition determination by agar well diffusion assay

Antibacterial activities of the crude drug extracts were screened for their zone of inhibition by agar disc-diffusion method. Crude extracts were prepared concentration of 100 mg/ml with dimethyl sulphoxide (DMSO,) as solvent. The Nutrient Agar Medium (NAM) medium (Hi Media) prepared and sterilized at 121^0C 15 lp/sq for 20 minutes in the autoclave. Twenty millilitres of this sterilized agar medium (NAM) were poured in to each 9 cm sterile petridishes under aseptic conditions and allowed to settle for the preparation of the inoculation 24 h culture.

The antibacterial analysis were done on human pathogenic bacteria like *Staphylococcus aureus, Salmonella typhi, Enterococcus faecalis, Entrococcus phyogenes, Enterobacter aerogenes* by standard disc diffusion method. Nutrien agar (NA) broth/ agar media was used to cultivate bacteria. Fresh overnight cultures of inoculum (100µl) of each culture were spread on to NA agar plates. Sterile paper discs of 5mm diameter containing DMSO 20µl (Nagative control), antibiotic disc were used as positive control separate NA mediam plates and 20µl, 40µl, 60µl, and 80µl of Aqueous extract along with four discs were placed in each plates (Bauer *et al.,* 1966).

Diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

The assay for the scavenging of free radical DPPH was done as reported earlier (Yamazaki *et al.,* 1994). Briefly, in a 96-well microplate, 25µL 50µ 75µ 100µ of various solvents plant extract, 100 µL and 100 µL DPPH solution 0.6 mM in ethanol were added. The reaction mixture was shaken well. DPPH decolorisation was recored (520nm) on a Bio Tek synergy HT multi-mode microplate reader after 30 min of incubation in the dark. Free radical scavenging capacity was calculated by the following equation: % inhibition of DPPH radical = (control – test / control).

$$\text{DPPH scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Result and Discussion

In the present study, preliminary phytochemical screeing was analyzed in aerial part aqueous extract of *Pogostemon benghalensis*. The result showed that, steroids and tannins were the presence of Reducing Sugar, Sugar, Phenol, Catechins, Flavanoid, Aminoacid Presented in (Table.1).[30] reported that, alkaloids, Phenolics, essentional oils and terpenes,sterols, flavonoids, lignin, tannin are secondary metabolites produced as the by Product of primary metabolism. [31] Reported the presence of alkaloids, glycosides, flavonoids and phytosterols in *Pogostemon auricularis.*[32] Investigated and reported the presence of steroids, saponnins, glycosides, proteins and aminoacids in *Pogostemon quadrifolius.*

The antimicrobial investigation aqueous extract of *P. benghalensis* were tested for its antibacterial activity against few pathogenic bacterial strains like *Staphylococcus aureus, Salmonella typhi,* Enteriococcus faecalis, Enteriococcus phyogenes, Enterobacter aerogenes results presented in table 2. The present study of antibacterial investigated both gram+ and gram-bacteria. The highest zone of inhibition was observed in Enterobacter aerogenes (15mm). And the lowest zone of inhibition was observed in salmonella typhi (5mm). All these results indicates aqueous extract can be used as antibiotic drug. (Table 3). The current study was in contradiction with [34] that methanol extract showed maximum inhibition against wild strains Staphylococcus, pseudomonas and E. coli with a zone of inhibition of 5.4, 4 and 5mm in *Pogostemon quadrifolius.* The present study qualitative investigation aqueous extract contain total phenol = 0.14mg/g, total flavonoid =0.15mg/g and total tannin = 0.11mg/g the extraction values of phenols, flavonoids and tannins are greatly depending on the solvent polarity (Table 2). The relatively stable organic radical, DPPH has been widely used in the determination of anti-oxidant activity y of single compounds, as well as different plant extracts [35]. Antioxidant measured by DPPH showed the variation in the range of solvent. Total antioxidant activity, measured by DPPH method ranged from 0.10 to0.34mg/ml. methanolic fraction of *Pogostemon benghalensis anti-oxidant activity measured by this method gave highest anti-oxidant concentration (57.27µg/ml).*

Table 1: Preliminary phytochemical analysis of aerial part of *Pogostemon benghalensis*

S.No	Phytochemical Constituents	Aqueous Extract (*Pogostemon Benghalensis*)
1.	Steriod	-
2.	Tritepenoid	-
3.	Reducing sugar	+
4.	Sugar	+
5.	Alkaloid	-
6.	Phenol	+
7.	Catechins	+
8.	Flavanoid	+
9.	Saponins	-
10.	Tannin	-
11.	Anthroquinones	-
12.	Amino acid	+

Table 2: Quantitative estimation of Total phenol, Flavonoid, Tannin present in aerial parts of *Pogostemon benghalensis*

S. No	Solvent	Total Phenol	Total Flavonoid	Total Tannin
1.	Aqueous extract	0.14+0.45	0.15+0.24	0.11+0.19

Table 3: Antibacterial activity of *Pogostemon benghalensis Aerial part* aqueous extract

S.No	Name of the Bacterial species	Zone of Inhibition (mm)				
		Standard disc	20µl	40µl	60µl	80µl
1.	*Staphylococcus aureus*	22	6	7	10	12
2.	*Salmonella typhi*	20	5	8	11	14
3.	*Enterococcus faecalis*	23	6	9	11	13
4.	*Enterococcus phyogenes*	26	6	7	10	14
5.	*Enterobacter aerogenes*	25	7	10	13	15

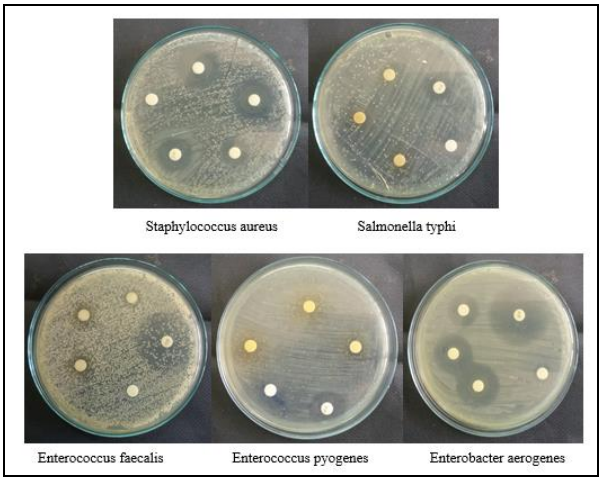

Fig 1: Antibacterial Activity *of Pogostemon benghalensis Aerial part* Aqueous extract

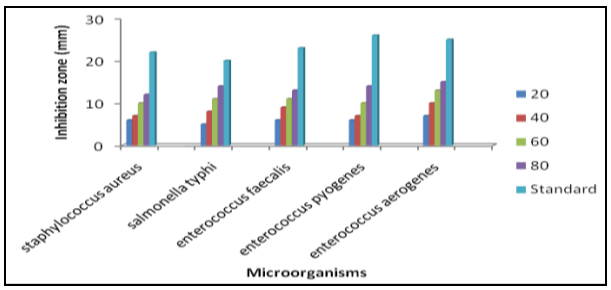

Fig 2: Antibacterial Activity *of Pogostemon benghalensis Aerial part* Aqueous extract

Conclusion

In conclusion the present investigation aqueous extract of pogostemon benghalensis preliminary phytochemical analysis, antioxidant, antimicrobial activity. Which indicates aqueous extract of pogostemon benghalensis can be used as effective therapeutic drug against bacterial infection.

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Conflict of Interests

The authors have nothing to declare**.**

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