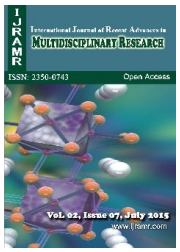

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Research Article

SCREENING OF ACTIVE PHYTOCOMPOUNDS BY GC-MS ANALYSIS AND IN VITRO ANTIBACTERIAL ACTIVITY OF ENDEMIC PLANT *POGOSTEMON MOLLIS* BENTH

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ABSTRACT

Medicinal plants have has a vital role in human culture and civilization. Herbal medicine as a choice for health care and the screening of medicinal plants for bioactive compounds is important. The study aimed to determine the antibacterial activity and photochemical constituents through Gas Chromatography- Mass Spectroscopy Analysis (GC-MS) and qualitative phytochemical screening of *Pogostemon mollis* Benth. Belongs to family Lamiaceae and also to determine the purity of crud drug by ash values. According to GC-MS analysis, totally 47 compounds were isolated by their retention indices (RI), retention time (RT) and mass spectra. The primary phytochemical analysis of the plant extracts showed the presence of various secondary metabolites like phenol, flavonoids, terpenoids, Pyosteroids, cardic glycosides, quinine and coumarians among this the methanolic plant extract showed the significant results. The ash values showed high purity of drug. A wide range of compounds were identified and they have many medicinal importances so that it can be recommended as a plant of phytopharmaceutical importance.

INTRODUCTION

Around the world millennia people have healed in sick by herbal derived remedies and handed down through generations. Traditional medicine is the sum total of knowledge, skills, practices. Based on the theories, beliefs and experiences indigenous of different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness **(**WHO**).** Potentially valuable treasures in medicinal plants remain unexplored. We have to consider the scope of these medicinal plants by use more amounts of time and resources into developing medicines (Kirtikar and Basu, 1918). Medicinal plants are source of important therapeutic aids for alleviating human ailments. The present plant phytochemical compounds are valuable source of food, medicine and its products of plant metabolism are mainly used by the plants for their defence. The chemical components in plants have various biological roles in their therapeutic value (Nisa *et al*., 2011). The plants are sources of pharmaceuticals for human ailments, either as totally pure compounds or as synthetic analogy. Medicinal plants are the source for the development of new molecules.

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Currently Pharmaceutical research is extensively focusing on natural compounds for developing active molecules of plant origin (Dev S. 2010). Approximately 25% of drugs prescribed in the United States are plant derived natural products and 74% of the 119 most important drugs contain ingredients from the traditional medicinal plants (Cochrane *et al*., 2008).

The members of Lamiaceae family include aromatic plants that are being used in traditional medicine for various disorders. The therapeutic application of these plants is attributed to the presence of secondary metabolites or phytochemicals such as alkaloids, saponins, flavonoids, glycosides and phenols. They are known to have various biological activities such as antimicrobial, antifungal, antioxidant, etc (Vaishali Rai *et al*., 2013). Particularly *Pogostemon* genera are used by tribals mostly for its roots and leaves. The Fresh root or poultice of the leaves is applied on the snake bite of Phursa snake and other snake bites (Sen *et al*., 2008), Haemorrhage especially in uterine haemorrhage (BhuiyanMd *et al*., 2011) and leaf extract used as an insect repellent (The Wealth of India, 1998). Plant extract is used for the treatment of food poisoning, vomiting and stomach troubles (Sen *et al*., 2008). Respiratory tract infection (BhuiyanMd *et al*., 2011) and pollen and nectar were the source of panagol honey in Maharashtra (The Wealth of India, 1998).

Some plants have been used for antiseptic activity and in the treatment of enteritis, eczema and mycotic enteritis (Dymock *et al.*, 1810). Therefore, the present investigation aims to evaluating the phytochemical composition by qualitative method, GC-MS analysis and antibacterial activity in various extracts of the Lamiaceae family member *Pogostemon mollis* Benth.

MATERIALS AND METHODS

Collections of plant material

Aerial and root parts of *Pogostemon mollis* Benth. were collected from Vellyangiri hills, Coimbatore district, Tamilnadu, India. They were identified by Dr. V. Balasubramaniam taxonomist and voucher specimen has been deposited in Kongunadu Arts and Science College, Coimbatore.

Preparation of plant extract

The fresh aerial and root parts of *P. mollis* were washed with tap water and shade dried for a week and powdered coarsely. Then they were finely powdered mechanically using Pulverizer and passed through 40 mesh sieve and stored in airtight containers. About 250g of powdered aerial and root were extracted in soxhlet apparatus with petroleum ether, chloroform, aqueous, methanol and also the whole plant methanolic extract were taken for GC-MS (Gas Chromatography Mass Spectroscopy). The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent were removed under Vaccum drier and the solid mass obtained was stored at 4°C until further use.

Phytochemical Study

Qualitative analysis

The qualitative tests were done to find out the presence of the active phytochemical constituents in the defatted extracts (Harborne**,** 1984; Wagner *et al.,* 1984).

Gas Chromatography analysis

Gas Chromatography (GC) analysis was carried out using Varian 5975 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fit with VF 5 MS capillary column (30 m × 0.25 mm). The injector temperature was set at 240ºC, and the oven temperature was initially be at 70ºC then programmed to 300ºC at the rate of 10ºC / minute and finally held at 300ºC for 10min. Helium was used as carrier gas with the flow rate of 1.51ml/min. The percentage of composition of extract was calculated by GC peak areas. The compounds were identified based on comparison of their retention indices (RI), retention time (RT) and mass spectra.

GC-MS- identification of compounds

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name of the components of the test materials was ascertained.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the WILEY8 and NIST08 library version (2012) and turbomas 5.2 software.

Determination of Ash value

- **Determination of total ash**

Total ash was determined by weighing 2 gm of the air dried crude drug in the tarred platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed.

$$\% \text{ of total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \times 100$$

- **Determination of acid insoluble ash**

The ash obtained from the previous process was boiled with 25ml of 2M HCl for 5 min. The insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug.

$$\% \text{ of acid insoluble ash value} = \frac{\text{Wt. Of acid insoluble ash}}{\text{Wt. Of crude drug takken}} \times 100$$

- **Determination of water soluble ash**

The ash was boiled with 25ml of water for 5 min. The insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

$$\% \text{ of water soluble ash value} = \frac{\text{Wt. of water soluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Antibacterial activity

Antibacterial activity of the various extracts of *Pogostemon mollis* was determined by disc diffusion method (Bauer *et al.,* 1966).Nutrient agar medium was prepared and transferred into sterile petriplates. 25ml of the standardized bacterial inoculums was spread on agar medium using sterile cotton swab. The discs impregnated with extracts were placed on the inoculated agar medium. Ampicillin (10µg/disc) was used as standard to determine the sensitivity of each microbial species. All the petriplates were incubated at 37°C for 24 hours. After the incubation period, diameter of zone of inhibition was measured.

Composition of nutrient agar medium for bacterial culture

S. No.	Composition	Quantity (g)
1	Peptone	5.0
2	Beef extract	3.0
3	Sodium chloride	5.0
4	Agar	15.0
5	Distilled water	1000 ml
	pH	7.0

RESULTS AND DISCUSSION

Phytochemicals are classified as primary or secondary constituents based on their role in plant metabolism (Wadood et al., 2013). The present study progress the qualitative phytochemical screening with peteroleum ether, chloroform, aqueous and methanol extracts of *Pogostemon mollis* and the result showed the presence of secondary metabolites which are clearly depicted Table 1. Which indicated the presence of phenol, flavonoids, terpenoids, Pyosteroids, cardic glycosides, quinine and coumarians. In our study, phenolic compounds are the major phytoconstituents. Whereas Subhadra Devi, (2014) documented the phenolic compounds are largest and the most widely distributed category of phytochemicals in the plant kingdom. The flavonoids, phenolic acids and polyphenols are the most important and complex group of chemical constituents in plants.

The most of the phytoconstituents were present in methanolic extract. Gas Chromatography-Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Hites, 1997). In GC-MS analysis, 47 bioactive phytochemical compounds were identified in the methanolic extract of *P. mollis*. The identification of phytochemical compounds is based on the peak area and index kovats. According to that the percentage composition of the various components are listed in Table 2. There are many reports on the *Pogostemon* species phytoconstituents though GC-MS likewise *P. cablin* and *P. travancoricus* (Sundaresan *et al*., 2009). Similar study was reported by Shobo *et al*., (2015) in the lamiaceae plant methanolic leaves extracts of ocimum canum.

Table 1. Preliminary qualitative phytochemical screening with various extracts of *Pogostemon mollis* Benth

S.No	Test	Parts	Petrolum ether	Chloroform	Methanol	Aqueous
1	Alkaloids					
	A) Mayer`s test	Aerial	-	-	-	-
		Root	-	-	-	-
	B) Wagner`s test	Aerial	-	-	-	-
		Root	-	-	-	-
	C) Dragedroff`s test	Aerial	-	-	-	-
		Root	-	-+	-+	+-
2	Carbohydrate					
	A) Fehiling test	Aerial	-	-	++	++
		Root	-	-	++	++
	B) Barfoed`s test	Aerial	-	-	++	+-
		Root	-	-+	++	-
	C) Benidic test	Aerial	-	-	+-	+-
		Root	-	-	++	++
3	Glycoside					
	Leqals test	Aerial	-	-	-	-
		Root	-	-	-	-
4	Protiens					
	A)Millon`s test	Aerial	-	-	++	-+
		Root	-	-	-	-
	B)Biuret test	Aerial	-	-	++	-+
		Root	-	-+	++	++
5	Amino acid					
	Ninhydrine test	Aerial	-	-	-	-
		Root	-	-	-	-
6	Pytosterols	Aerial	++	++	-	-
		Root	-	-	-	-
7	Tannins	Aerial	-	-	-	++
		Root	-	-+	++	-
8	Phenols	Aerial	-+	-+	++	++
		Root	-	-	++	++
9	Flavaniods	Aerial	-	-	++	-+
		Root	-	-	++	++
10	Coumarins	Aerial	+-	-	++	++
		Root	-	-+	++	+-
11	Saponin	Aerial	-	+-	++	+-
		Root	-	-+	++	-+
12	Quinine	Aerial	-	++	++	++
		Root	++	++	++	-
13	Cardiac glycosides	Aerial	-	++	++	++
		Root	++	++	++	-
14	Terpenoid	Aerial	-	++	++	++
		Root	++	++	++	-
15	Steroids and phytosteroids	Aerial	-	++	-	++
		Root	++	++	-	-
16	Fixed oils	Aerial	-+	-	-	-+
		Root	+-	+-	+-	+-
17	Gum and mucilages	Aerial	-	+-	-	-
		Root	-	-	-	-

Table 2. Gas Chromatography - Mass Spectroscopy analysis in methanolic extract of *Pogostemon mollis* Benth

Peak#	R.time	Area%	Formula	Mol. Weight	Mark	Name
1	5.613	1.92	C6H6O2	110	MI	2-FURANMETHANOL
2	6.995	1.33	C5H6O2	98	MI	1,2-CYCLOOCTANEDIONE
3	7.567	0.85	C6H6O2	110		2-FURANCARBOXALDEHYDE, 5-M
4	7.834	0.59	C6H8O4	144		2,4-Dihydroxy-2,5-dimethyl-3(2H)-fura
5	8.815	0.12	C4H6O3	102	MI	2-Hydroxy-gamma-butyrolactone
6	9.067	0.58	C8H16O	128	MI	2-OCTANONE
7	9.555	1.09	C10H18O2	170		2-FURANMETHANOL, 5-ETHENYL
8	9.808	1.83	C5H8N2O2	128		Hydrouracil, 1-methyl-
9	10.325	1.23	C6H6O3	126		4H-PYRAN-4-ONE, 3-HYDROXY-2-M
10	10.542	2.68	C5H8O4	132	V	Butanedioic acid, monomethyl ester
11	10.850	0.29	C16H34O5Si2	362		1,1,3,3-TETRAMETHYL-1,3-BIS[3-(2
12	11.039	9.27	C6H8O4	144	V	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydr
13	12.059	2.34	C8H8O	120		Benzofuran, 2,3-dihydro-
14	12.475	22.21	C6H6O3	126	V	2-FURANCARBOXALDEHYDE, 5-(H
15	12.529	8.82	C6H6O3	126	V	5-(HYDROXYMETHYL)-2-FURALDE
16	13.342	2.63	C9H10O2	150		2-METHOXY-4-VINYLPHENOL
17	13.917	0.18	C8H10O3	154		Phenol, 2,6-dimethoxy-
18	14.525	0.33	C10H11ClO3	214	MI	4-CHLOR-2-METHYLPHENOXYESS
19	15.323	0.73	C10H18O3	186	MI	Hexanoic acid, 2-acetyl-, ethyl ester
20	15.811	1.44	C8H10O2	138	MI	Benzeneethanol, 4-hydroxy-
21	17.551	-0.16	C9H16O	140	MI	2-CYCLOHEXEN-1-OL, 1,4,4-TRIME
22	19.076	1.08	C13H21NO2	223		DOET P1009
23	21.489	1.02	C13H18O	190		Megastigmatrienone
24	22.033	0.67	C20H40O3l	328	MI	2-HYDROXYHEXADECYL BUTANO
25	24.100	5.61	C8H8O4	168		Benzeneacetic acid, 3,4-dihydroxy-
26	24.208	1.29	C8H8O4	168	V	Benzeneacetic acid, 3,4-dihydroxy-
27	24.350	1.10	C10H12O4	196	V	2-BUTYNYL-5-HYDROXY-3-OXO-4
28	26.017	-0.20	C15H32O	228	MI	n-Pentadecanol
29	26.826	0.41	C10H22O6	238	MI	Pentaethylene glycol
30	28.515	1.80	C17H34O2	270		HEXADECANOIC ACID, METHYL E
31	28.837	0.42	C18H28O3	292		METHYL ESTER OF 3-(3,5-DI-TERT-
32	29.222	6.78	C17H34O2	270		n-Hexadecanoic acid
33	31.003	1.05	C19H34O2	294		9,12-Octadecadienoic acid (Z,Z)-, methy
34	31.094	2.36	C19H32O2	292	V	9,12,15-OCTADECATRIENOIC ACID
35	31.259	0.36	C20H40O	296	V	2-HEXADECEN-1-OL, 3,7,11,15-TETR
36	31.411	0.80	C19H38O2	298		Octadecanoic acid, methyl ester
37	31.583	1.67	C18H32O2	280	V	9,12-OCTADECADIENOIC ACID (Z,Z
38	31.700	4.91	C18H30O2	278	V	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
39	31.933	1.85	C18H36O2	284	V	Octadecanoic acid
40	32.192	1.26	C19H34O2	294		9,12-Octadecadienoic acid (Z,Z)-, meth
41	32.679	0.74	C19H34O2	294		9,12-Octadecadienoic acid (Z,Z)-
42	33.433	0.36	C19H36O3	312		9-OCTADECENSAEURE, 12-HYDRO
43	35.152	0.30	C20H28O2	300	MI	1-PHENANTHRENECARBOXYLIC A
44	35.603	1.36	C20H30O	286		1-PHENANTHRENEMETHANOL, 1,2
45	36.372	0.26	C19H38O4	330	MI	Hexadecanoic acid, 2-hydroxy-1-(hydro
46	36.678	0.37	C29H50O	414	MI	STIGMAST-5-EN-3-OL, (3.BETA.,24S
47	38.222	2.10	C28H46O	398		ERGOSTA-5,24(28)-DIEN-3-OL, (3.BE

Table 3. Antibacterial activity of various extract of *Pogostemon mollis* Benth

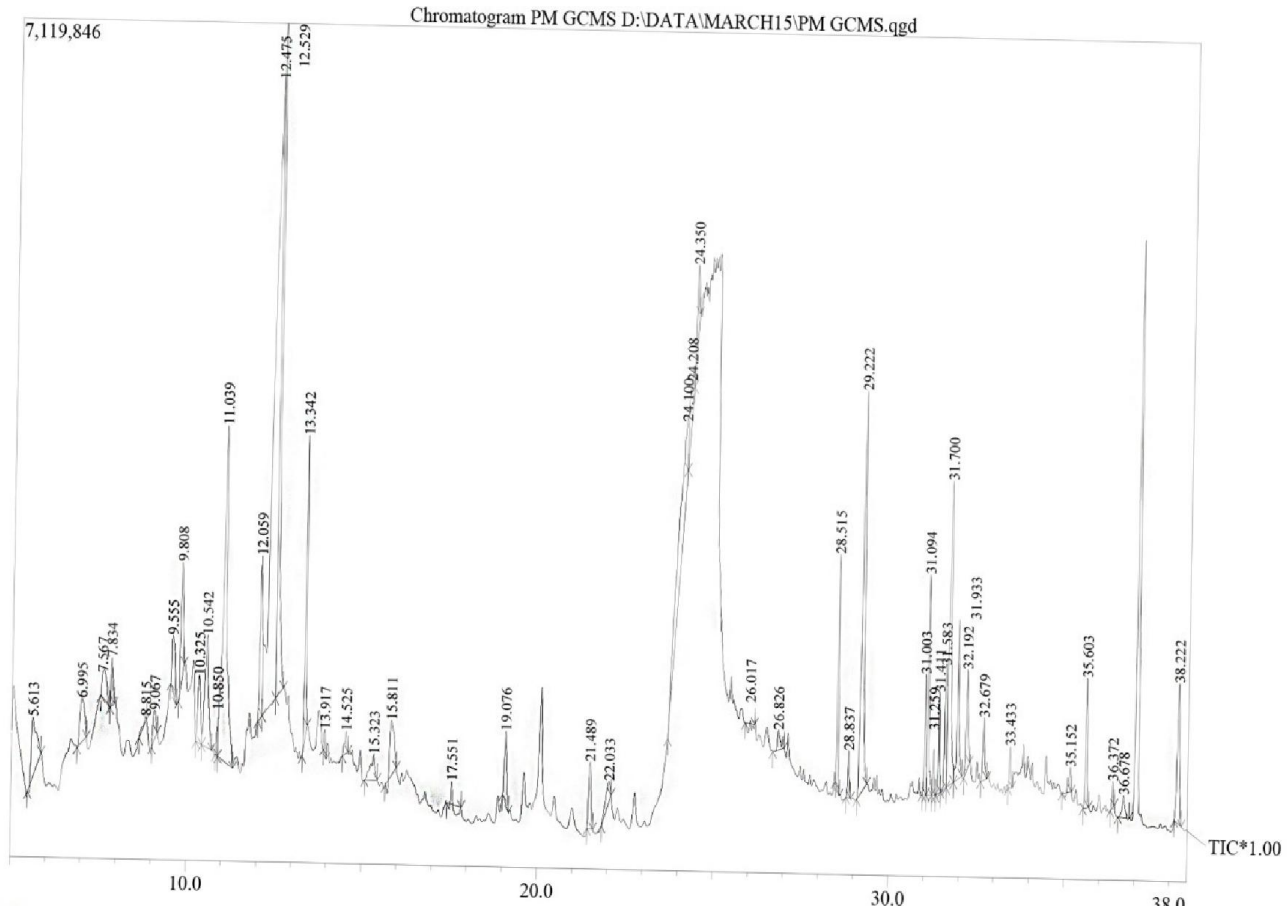
Bacterial stein	Control	Plant extracts			
		Petroleum ether	Chloroform	Methanol	Aqueous
E. coli	21±0.43	11±0.23	10±0.22	15±0.18	13±0.24
Proteus mirabilis	26±0.26	10±0.31	12±0.27	10±0.25	14±0.42
Streptococcus pyogenes	22±0.17	13±0.42	15±0.19	18±0.33	16±0.38
Bacillus thuriginesis	23±0.52	8±0.14	8±0.26	7±0.32	9±0.21
Eutrococcus faccalis	20±0.29	7±0.11	9±0.36	15±0.27	12±0.22

Ash value is useful in determining authenticity, purity of sample and also these values are important in qualitative standards. The total ash containing physiological ash derived from plant tissue and non- physiological ash derived from environment contaminations. The plant quality not only determined by total ash value but also by water soluble and acid insoluble ash (Rao and Xiang, 2009). In this investigation the result were indicated the high purity, quality in plant extract (Table: 3) and determination of these parameters are important in order to maintain the purity of the herbal medicine (Gami and Parabia 2010; Kunle *et al*., 2012).

The analysis of various extracts effects on the bacterial strains of *Pogostemon mollis* plant. The plant extracts showed the highest microbial efficiency while the wild thyme the lowest efficiency.

Table 4. Physiological ash values of *Pogostemon mollis* Benth

Total ash (%)	Water soluble ash(%)	Acid insoluble ash(%)
17	15	7



By using disk diffusion method according to the standard conditions the diameter of the inhibition zone is proportional to the logarithm of the various extracts of the substance studied. The results obtained with all tested bacteria showed that the inhibition zone diameter was proportional to the logarithm of the tested extracts. The significant differences were found in antibacterial activities in all bacterial samples (Tables 2 and 3). The methanolic extract were showed positive result in *Streptococcus pyogenes* (18±0.33mm) followed by *Proteus mirabilis* (14±0.42mm) and in others appeared that the difference in antibacterial activities due to the nature of contents.

In the last few years, a number of studies have been conducted in different countries to prove such efficiency. The plant extracts and essential oils of the herbal belonging to Lamiaceae family, which known antimicrobial properties of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. Several studies indicate that possess biological activity against several bacteria (Delamare *et* al., 2007; Faleiro*et al*., 2003; Sarac and Ugur, 2007).

Conclusion

The results of this study showed that the plant extract contain significant phytochemical compounds and significant antimicrobial activity. The antimicrobial activity seemed to be dependent mostly on herbal source and also on bacterial genera. Further investigations regarding the in vitro and in vivo toxicity should be conducted in order to develop such products.

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