

GC-MS DETERMINATION OF BIOACTIVE CONSTITUENTS OF THE ETHANOLIC EXTRACT OF *PHOEBE WIGHTII* MEISN L. (STEM)

*Devika M and **Mary Kensa V.

*Research Scholar (Full time) Reg No: 18213152142037, Email Id: deviashi.mv@gmail.com, Abishehappatti, M.S. University, Tirunelveli.

**PG Research centre of Botany, S.T. Hindu College, Nagercoil. Email:Id: surejkensa@gmail.com.

Abstract

Plant is man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization. Plant continues to be a major source of medicine, as they have throughout human history. Plants offer novel bioactive compounds which added advantage of ethnobotanical observations, since many species are used in systems of natural and traditional medicine. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable. However, there has been not much information available on phytochemical components and biological activity in the stem extract of *Phoebe wightii*. The aim of the present study is to identify the phytochemicals of this plant and subjecting the methanol extract of the plant leaves to Gas chromatography – Mass Spectrum analysis. This study was designed to determine the phytochemicals in the stem ethanol extract of *P. wightii*. GC-MS analysis of the stem ethanol extract of *P. wightii* was performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC- MS). This analysis revealed that the ethanol extract of *P. wightii* (whole plant) contained Trimethylsilyl, tetradecanol, dodecanol, bromomethyl, phenylmethoxy, cyclododecane, 3,4- dimethoxyphenyl,N-ethylthiocarbamoyl,aminophenyl,methylimino,ethyl stearate etc. From the results, it is evident that the selected plant contains various bioactive compounds and is recommended as a plant of phytopharmaceutical importance. From the results, it could be concluded that *P.wightii* contains various bioactive compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance. Further in future, these components can be isolated and pharmacological activity may be studied to determine the traditional use.

Keywords: bioactive compounds, chemotherapeutants, non-phytotoxic, phytopharmaceutical and traditional use.

INTRODUCTION

From ancient times, medicinal plants have been used extensively for their tremendous healing properties and health benefits India has a treasure of medicinal plants due to the rich diversity in its agroclimatic condition (Maheswari, 2018). Awareness of medicinal plants usage is result of the many years of struggles against illness due to which man learned to pursue drugs in barks, seeds, fruit bodies, leaves and part o the plants (Biljanai, 2012). In India the medicinal systems using medicinal plants are Ayurveda, Siddha, and Homeopathy etc. to treat various ailments (Puspangandanet *al.*, 1984). Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (Kensa and Neelamegam, 2016).

Phoebe wightiis is a tree commonly found in wasteland of garden and plains. It is a monotypic to genus, native to Mexico. It belongs to the family Lauraceae. The plant is commonly known as blood berry or rouge plant. The investigation was carried out to determine the chemical components of *Phoebe wightii* stem using Perkin Elmer Gas chromatography-Mass spectrometry. Higher plants as source of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants these are non-phytotoxic, more systematic and easily biodegradable (Vyas., 1999; Kaushiket *al.*, 2002; ChamanLal and Verma., 2006). If we can come back to our nature, culture and tradition of use of medicinal plants it can bring up a bright and health new generation (Kirtikar and Basu, 1918).

In GC-MS method the unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald, 1997; Chauhanet *al.*, 2014). This plant is also known to possess antimicrobial activity, antioxidant activity, anti-inflammatory activity, therapeutic agent, and nematocidal activity. GC-MS analysis of aerial part of this plant revealed the presence of many bioactive components (Sudhaet *al.*, 2013). This plant is also known to possess antimicrobial activity, antioxidant activity, anti-inflammatory activity, therapeutic agent, and nematocidal activity.

Materials and Methods

Collection of plant sample

Stem of *P. wightii* was collected from Kotagiri, Coimbatore of Tamil Nadu, India and authenticated by Botanist Dr. R. Murugan, BSI, Southern circle, Kovai, India. A voucher specimen was deposited in the herbarium of the Botanical Survey of India Coimbatore; Herbarium code No. BSI/SRC/19/710-20/Tech.

Plant sample extraction

Stems were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighted and transferred to stoppered flask and treated with ethanol until the powder is fully immersed. Dark green residues were obtained after concentrating the extract under reduced pressure. The obtained extracts were stored in desiccators for further GC-MS.

GC-MS ANALYSIS

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 μm df, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV; Helium gas (99.9%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 μl was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0.

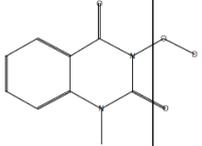
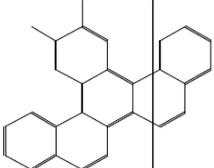
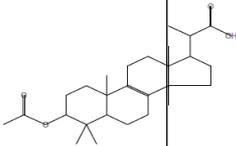
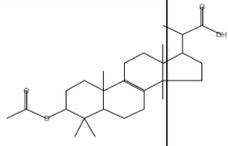
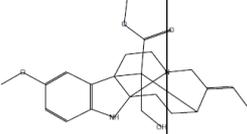
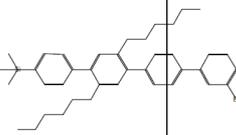
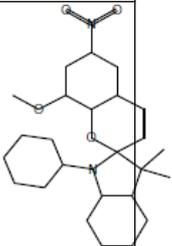
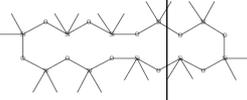
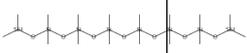
Identification of bioactive components

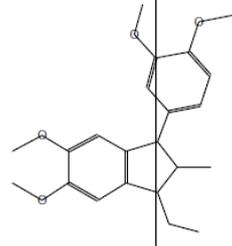
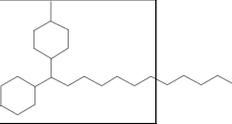
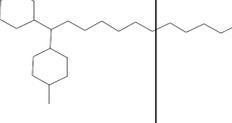
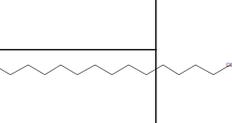
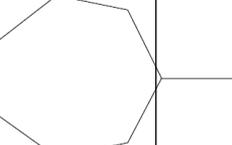
The relative percentage amount of each component was calculated by comparing its average peak area to the total peak areas. The detection employed the NIST (National Institute of Standards and Technology) Ver. 2.53 – year 2005 library. The compound prediction is based on Dr. Duke's phytochemical and Ethno botanical Database (Dukes, 1955) by Dr. Jim Duke of the Agricultural Research Service. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Results and Discussion

The GC-MS chromatogram of ethanolic extracts of stem of *Phoebe wightii* revealed the presence of various compounds with corresponding peaks at different retention time. GC-MS is one of the best techniques to identify the constituents in plants. The GC-MS analysis of *P. Wightii* stem revealed the presence of 14 compounds. Table 1 and figure 1 showed the various bioactive compounds were characterized and identified.

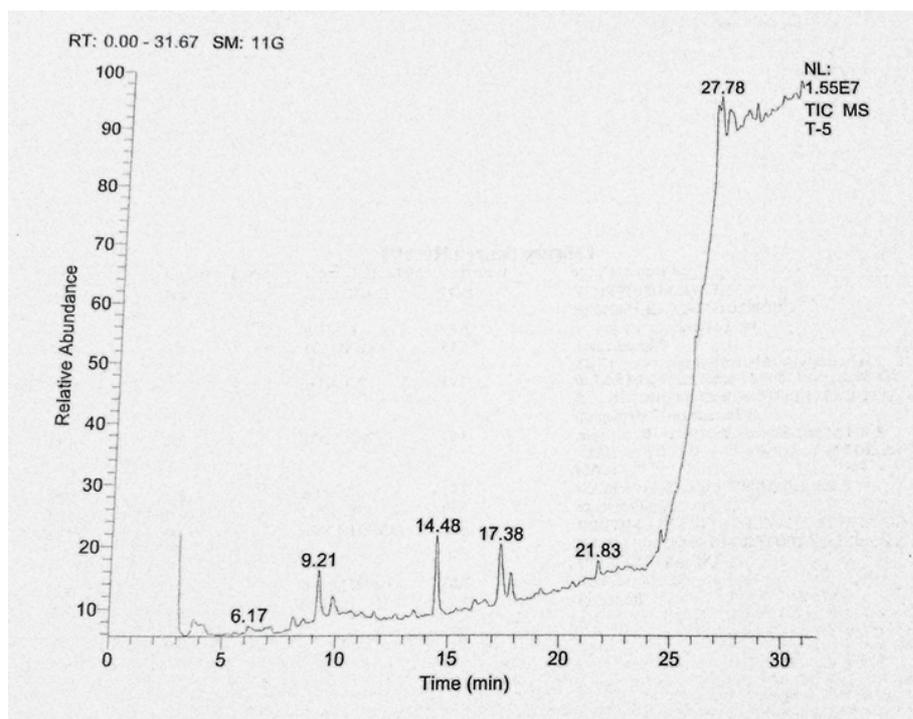
Table 1. GC-MS analysis of phytochemicals and their activities in the stem of ethanolic extracts of *PHOEBE WIGHTII*.

Sl. No.	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %	Structure
1	1-METHYL -3-DEUTEROXY QUINAZOLINE-2,4(1H,3H)DIONE	11.71	C ₉ H ₇ DN ₂ O ₃	192	5.24	
2	14,15-Dimethylbenzo[s]picene	6.39	C ₂₈ H ₂₀	356	5.24	
3	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl)-	5.15	C ₂₇ H ₄₂ O ₄	430	5.24	
4	2-(3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-Propioni	4.95	C ₂₇ H ₄₂ O ₄	430	5.24	
5	2,4(1H)-Cyclo-3,4-secoakammilan-16-carboxylic acid, 17-hydroxy-10-methoxy-, methyl ester, (16R)-(CAS)	3.99	C ₂₂ H ₂₈ N ₂ O ₄	384	5.24	
6	1'''-Trimethylsilyl-3-bromo[1-[4-(2-phenyl-1,4-dihexylphenyl)phenyl]]benzene	15.12	C ₃₉ H ₄₉ BrSi	624	1.02	
7	Spiro[2H-1-benzopyran-2,2'-[2H]indole], 1',3'-dihydro-8-methoxy-3',3'-dimethyl-6-nitro-1-phenyl-(CAS)	4.67	C ₂₅ H ₂₂ N ₂ O ₄	414	1.02	
8	Cyclodecasiloxane, eicosamethyl-	12.18	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	740	0.95	
9	Octasiloxane, 1,1,3,3,5,5,7,7,9	5.55	C ₁₆ H ₅₀ O ₇ Si ₈	578	0.95	

	,9,11,11,13,13,15,15-		i8			
10	trans,trans-1-(3,4-Dimethoxyphenyl)-2-methyl-3-ethyl-5,6-dimethoxyindan	4.90	C ₂₂ H ₂₈ O ₄	356	0.95	
11	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	8.99	C ₂₆ H ₅₀	362	1.48	
12	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-(CAS)	8.99	C ₂₆ H ₅₀	362	1.48	
13	1-Tetradecanol (CAS)	5.45	C ₁₄ H ₃₀ O	214	1.48	
14	Cycloheptane, methyl-	5.02	C ₈ H ₁₆	112	1.48	

Source: Dr. Duke's phytochemical and ethnobotanical databases.

Fig. 1. GC-MS chromatogram of the ethanol extract of *PHOEBE WIGHTII* stem.



The identification of the phytochemical compounds was confirmed based on the peak area, retention time. The results revealed the 1-METHYL -3-DEUTEROXY QUINAZOLINE-2,4(1H,3H)DIONE, 14,15-Dimethylbenzo[s]picene, Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl), 2-(3-acetoxy-4,4,10,13,14-pentamethyl 2,3,4,5,6,7,10,11,12,13,14,15,16,17 – tetradecahydro-1H- cyclopenta[a] phenanthren-17-yl)-Propioni,2,4(1H)-Cyclo-3,4-secoakumamilan-16-carboxylic acid, 17-hydroxy-10-methoxy-, methyl ester, (16R)-(CAS),1'''-Trimethylsilyl-3-bromo[1-[4-(2-phenyl-1,4-dihexyl phenyl)phenyl]]benzene, Spiro[2H-1-benzopyran-2,2'-[2H]indole], 1',3'-dihydro-8-methoxy-3',3'-dimethyl-6-nitro-1-pheny, 1-(CAS) Cyclodecasiloxane, eicosamethyl-Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-trans,trans-1-(3,4-Dimethoxyphenyl)-2-methyl-3-ethyl-5,6-dimethoxyindan,Cyclohexane, 1,1'-dodecylidenebis[4-methyl-Cyclohexane, 1,1'-dodecylidenebis[4-methyl- (CAS) 1-Tetradecanol (CAS) Cycloheptane, methyl-1-Dodecanol (CAS)revealed the presence of various compounds with corresponding peaks at different retention time. The GC-MS analysis of *Phoebe wightii* stem revealed the presence of 14 compounds (phytochemical constituents).

Plants synthesize an extensive array of secondary metabolites often highly compound structures. The chemical investigations of medicinal plants have largely been driven to find new drugs to treat human disease. The secondary metabolites have been of interest to humans as flavors, fragrance, dyes, pesticides and pharmaceuticals (Govindaraj and Rajangam, 2017).

Conclusion

In this present study about 14 bioactive compounds are identified from ethanol extract of *P. wightii* by GC-MS method. The presence of various phytoactive compounds in this plant is responsible for the pharmaceutical properties. Therefore, it is recommended as a plant of phytopharmaceutical importance. Present study may be useful in the identification of novel drugs from stem of *P. wightii*. It is concluded that the ethanol can be used for extracting active compounds from plants and incorporating into medicinal food products. In addition further research is necessary to identify the active compounds responsible for therapeutic activity and animal study to evaluate the dosage of the identified chemical compounds.

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