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RESEARCH ARTICLE

GC-MS ANALYSIS OF PHYTOCOMPONENTS OF *PIPER SCHMIDTII*, HOOK. F. (PIPERACEAE)

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ABSTRACT

The present investigation was carried out to determine the possible bioactive components of *Piper schmidtii*. The chemical compositions of the methanolic extract of aerial parts of P. schmidtii were investigated using GC-MS, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Results showed that essential oil of the sample has a complex mixture of thirty compounds, many of which are found in trace amounts. Hence, the identified phytocomponents can be used as a pharmacognostical tool.

INTRODUCTION

Plants are used medicinally in different countries, because they are the source of many potent and powerful drugs (Sathyaprabha *et al*., 2010). Utilizing simple substances (water, carbon dioxide, nitrogen and inorganic salts) plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates), which are further transferred into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) to protect itself. India is called the botanical garden of the world for its rich natural resources (Agarwal and Raju, 2006). Many of the indigenous medicinal plants are used as spices and food (Okwu, 1999). Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae. The spiciness of black pepper is due to the chemical piperine. Analytical techniques are a powerful tool for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995). The hyphenated technique developed from the coupling of GC and MS, was the first of its type to become functional for research and development purposes (Badyal *et al*., 2015). GC-MS analysis can identify pure compounds present at less than one nanogram (Liebler *et al*., 1996). This technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acid, lipids and alkaloids (Mythili *et al*., 2013). Since there are no reports on the *P. schmidtii*, the plant was chosen as the subject of this study. Hence the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique.

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MATERIALS AND METHODS

Plant Identification, Collection and Sample Extraction

The plant sample was collected from Jawadhu Hills, Eastern Ghats, India. The herbarium of the plant was cross verified with 'The Flora of Presidency of Madras' (Gamble, 1921) and 'Materials for a Flora of Tamilnadu Carnatic' (Mathew, 1981). It was identified as a flowering vine *Piper schmidtii,* Hook. f. belongs to family Piperaceae. Aerial part (Stem, Leaf and Seed) of *P. schmidtii* was thoroughly washed, shade dried and pulverized to powder in a mechanical grinder. Then the powder was sieved and used for the extraction of active constituents of the plant materials. The extraction of essential oil or volatile oil from the plant was performed by using soxhlet apparatus with various solvents. The crude extract was collected and dried after which yield was weighed and then performed. The final residue thus obtained (from methanol extract) was then subjected to GC-MS analysis.

Gas Chromatography - Mass Spectroscopy analysis

GC-MS analysis was performed at 'The South India Textile Research Association' (SITRA), Coimbatore. The name of the instrument is 'Thermo GC-Trace Ultra- version 5.0, Thermo MS DSQ II'. Autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument equipped with a fused DB 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN. The components were separated using Helium as carrier gas at a constant flow of 1 ml/min. An aliquot (1 µl) of oil was injected into the column with the injector heater. The oven temperature was programmed from 70°C with an increase of 6°C/min and ending to 260°C.The source temperature were maintained as 220°C. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operated in scan mode from 20 to 600 atomic mass units (amu).

Table 1. Components identified in methanol extract of Aerial part of *P. schmidtii*.

Peak no.	RT	Compound Name	Molecular Formula	Mol. Weight	Peak area %
1	3.55	Methyl 4-(Phenylmethoxy)-5-phenoxypentanoate	C19H22O4	314	3.35
2	3.94	11-[(3-Hexyl-5-propyl)-2-furyl]undecanal	C24H42O2	362	2.47
3	5.98	(2S)-(-)-2-(3,4-Dimethoxyphenyl)-N-methylbutylamine	C13H21NO2	223	3.97
4	10.93	Dimethyl 3,7-Diacetoxy-6-[1,1-di(carboxymethoxy)-4-methylpenta-2,3-dien-1-yl]cycloheptene	C19H24O6	348	2.48
5	12.54	Diethyl N-{p-[(3-Amino-2-mercaptopropyl)amino]benzoyl}glutamate	C19H29N3O5S	411	3.44
6	12.80	(1R,2S,5R)-5-Methyl-2-[1-methyl-1-(2-naphthylyl)ethyl]cyclohexyl(1R,4S)-2-Oxa-3-azabicyclo[2.2.2]oct-5-ene-3-carboxylate	C27H33NO3	419	2.30
7	14.82	1-(N,N-Diacetylamino)-2-(isopropylcarbonyl)benzene	C14H17NO3	247	2.45
8	17.24	Ethyl (E)-5-[(1S,6R)-6-(p-Tolylthio)bicyclo[4.1.0]heptan-1-yl]-2-pentenoate	C21H28O2S	344	2.15
9	18.16	Ethyl (e)-3-(4-methoxy-2-methylphenyl)-2-propenoate	C13H16O3	220	1.85
10	20.69	3-[(Methoxycarbonyl)methyl]-2-methylbut-2-enolide	C10H14O4	198	1.84
11	22.62	1-Methylbutyl 2-methylene-3-acetoxypentanoate	C13H22O4	242	2.43
12	23.84	2-Allyl-4-methyl-2-azaspiro[4.5]decan-1,6-dione	C13H19NO2	221	2.04
13	28.73	Thiourea, N-(2-methoxyphenyl)-N'-(2-propenyl)-	C11H14N2OS	222	2.77
14	31.22	Dimethyl 2-phenyl-c-5-(3'-pyridyl)pyrrolidine-r-2,c-4-dicarboxylate	C19H20N2O4	340	4.95
15	31.65	5-(Tolylsulfinyl)-1,3-diiminoisoindoline	C15H13N3OS	283	2.25
16	32.42	4-(p-Chlorophenyl)-3-methyl-5,6-dihydrobenzo[h]thiochroman-2,3-dicarboxylic anhydride	C22H17ClO3S	396	6.23
17	32.85	syn-(1RS,2SR)-2-Methyl-1-(4-(phenylsulfanyl)-3,4,5,6-tetrahydro-2H-pyran-4-yl)propane-1,3-diol	C15H22O3S	282	1.81
18	33.85	10,10-dimethylanthrone	C16H14O	222	2.96
19	34.15	4-(4-allyl-5-pyridin-3-yl-4H-[1,2,4]triazol-3-ylsulfanylmethyl)-6-Piperidin-1-yl-[1,3,5]triazin-2-ylamine	C19H23N9S	409	2.37
20	34.66	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	C12H38O5Si6	430	4.68
21	35.11	Ethyl 2-methyl-4-(4-fluorophenyl)-6-trifluoromethylpyridine-3-carboxylate	C16H13F4NO2	327	8.05
22	35.84	2-(3',5'-Ditrifluoromethylphenyl)-1,1,3,3-tetramethylguanidine	C13H15F6N3	327	5.55
23	36.82	(PM,3SR)-3-Ethoxy-3-methoxy-9,11,13,15-tetramethyl-4-oxatricyclo[8.5.0.0(2,6)]pentadeca-1,6,8,10,12,14-hexaen-5-one	C21H24O4	340	2.82
24	37.21	Chloro(2',2'-dimethylcyclopropyl)methyl Tolyl sulfone	C13H17ClO2S	272	3.89
25	38.02	3,6-diacetoxy-9-hydroxy-7-methoxy-1-methyl-5,8-dioxo-5,8-dihydroanthracene-2-carboxylate	C22H18O10	442	3.10
26	38.29	Iron, dicarbonyl(ü5-2,4-cyclopentadien-1-yl)[2-(2-piperidinyl)ethyl]-	C14H19FeNO2	289	2.84
27	38.59	3-Hydroxyphenylacetic acid ethyl ester tms	C13H20O3Si	252	2.05
28	38.78	4-(Dimethylamino)azoestrone 3-methyl ether	C21H29N3O2	355	2.77
29	39.12	Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-à,L-glcero-pent-2-enopyranoside	C21H26O6	374	7.47
30	39.82	4,7-Dibromo-nido-5,6-dicarbaborane	C2H10B8Br2	280	2.66
	-------		----------	------	99.99
-----		Total Percentage of Peak Area			
-----	-------	Standard error	----------	------	0.01
-----	-------	Net Total Percentage of Peak area	----------	------	100

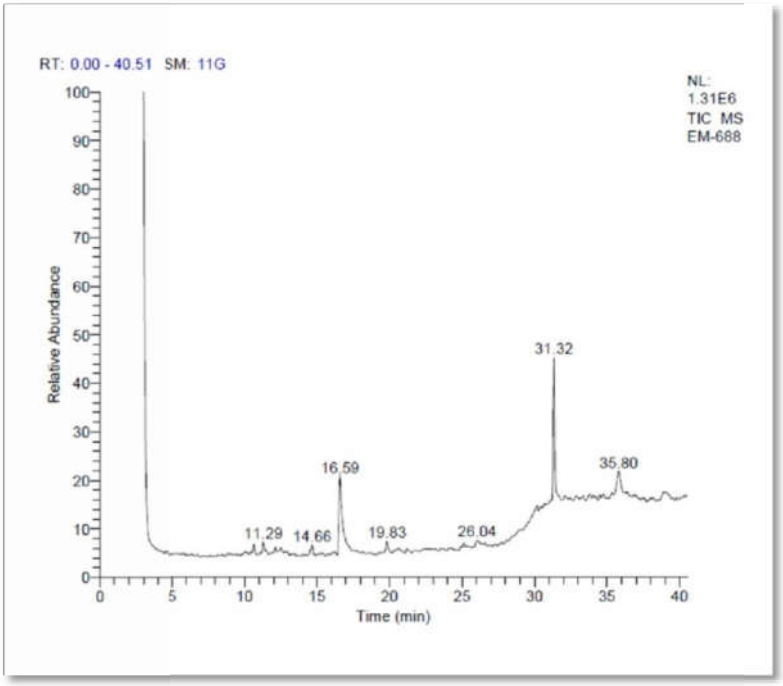
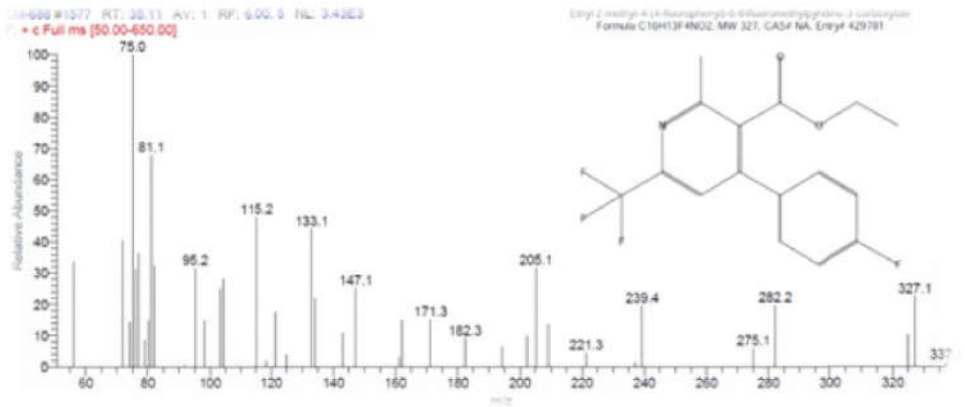
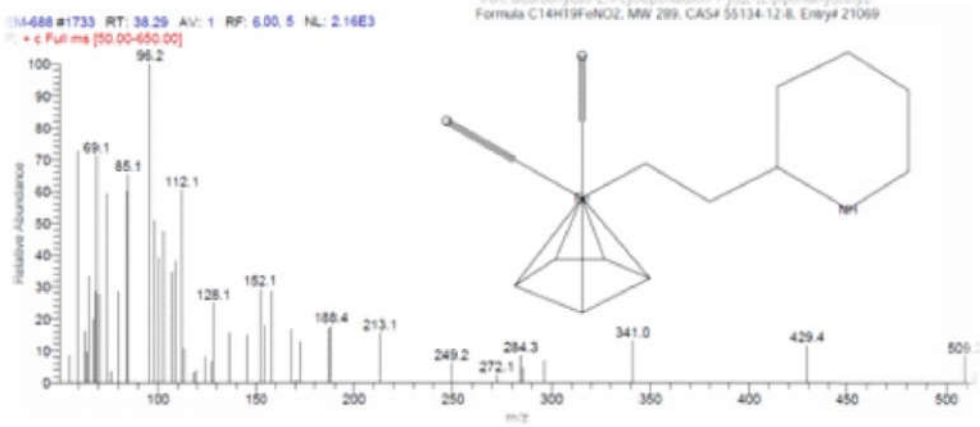


Figure 1. GC-MS Chromatogram of methanolic extract of aerial part of P. schmidtii



(b)



(b)

a) Ethyl 2-methyl-4-(4-fluorophenyl)-6-trifluoromethylpyridine-3-carboxylate

b) Iron, dicarbonyl(ü5-2,4-cyclopentadien-1-yl)[2-(2-piperidinyl)ethyl]-

Figure 2 (A and B). GC-MS Mass Spectrum of some compounds identified in the methanolic extract of aerial part of *P. schmidtii* with structure of compounds namely

Total GC running time was 37.51 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Identification of Components

Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and (NIST) 'National Institute Standard and Technology' having around 60,000 patterns. Identifications were based on the molecular structure, molecular weight and calculated fragmentations. The spectrum of the unknown component was compared with that of the spectrum of the known components stored in the NIST library. The listed components of the test materials were ascertained.

RESULTS

The compounds present in the methanol extract of aerial part of *P. schmidtii* were identified by GC-MS analysis (Figure 1). The active principles with their Peak number, Retention time (RT), Compound name, molecular formula, molecular weight (MW) and peak area percentage or concentration (%) were listed in Table 1. It reveals thirty compounds from the sample. Mass spectra are fingerprint of the compound which can be identified from the NIST data library.

Among them the predominant constituents are Ethyl 2-methyl-4-(4-fluorophenyl)-6-trifluoromethylpyridine-3-carboxylate, with a concentration of 8.05% (Figure 2A), followed by Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-à,L-glcero-pent-2-enopyranoside, with a concentration of 7.47%. In certain peaks the components are considered by research index value (RSI). The 19th peak's RSI value is very closer to the search index value (SI) (Figure 2B).

DISCUSSION

The structure of schimiditin be revised to lignan, which has assigned to Kadsurin B based on X-ray data. Since it is proved that lignan and schimiditin are identical, the name schimiditin should be removed from literature (Tyagi *et al*., 1995). The nature of compound Piperine was obtained as two different peaks (Peak no. 26 and 19). At Peak No. 26 (RT 38.29), Iron, dicarbonyl (ü5-2,4-cyclopentadien-1-yl) [2-(2-piperidinyl) ethyl]-was obtained with Peak area 2.84%. At Peak No. 19 (RT 34.15), 4-(4-allyl-5-pyridin-3-yl-4H-[1, 2, 4] triazol-3-ylsulfanylmethyl)-6-Piperidin-1-yl-[1,3,5] triazin-2-ylamine, was obtained with Peak area2.37%. Piperine, a substance present in black pepper has been found to increase the absorption of selenium, B-complex vitamins, beta-carotene, circumin as well as other nutrients from food. Piperine also inhibits pro-inflammatory cytokines that are produced by

tumor cells. During that process, it interferes with the signaling mechanisms between cancer cells, thereby reducing tumor progression (Kesari *et al*., 2006). Fluctuation of the oil composition can impart change in the organoleptic properties of the plant (Aziz *et al*., 2012).

Conclusion

The presence of bioactive components in *P. schmidtii,* paves the way for the development of several treatment regimens. So, it is recommended as a plant of pharmaceutical importance. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity. Thus, GC-MS analysis is the first step towards understanding the nature of active principles in this plant and such study acts as a base for further studies.

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