GC-MS Analysis and HPTLC Fingerprinting Profile of Hydroalcoholic Extract of *Polygonum barbatum* Linn. Leaves

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Lakshmi Narayanan, *et al.*: GC-MS and HPTLC Analysis of *P. barbatum*

An important medicinal plant, *Polygonum barbatum* was undertaken for gas chromatography-mass spectrometry analysis and high performance thin layer chromatography finger print profile to investigate the chemical constituent present in the hydroalcoholic extract of *Polygonum barbatum* leaves. Gas chromatography-mass spectrometry analysis shown that twenty two compounds were identified and named from the extract. Among these constituents, terpenoids are the major constituents present in this extract. Other major constituents were present in the extracts were carbohydrate and fatty acids. High performance thin layer chromatography fingerprint analysis of hydroalcoholic extract of *Polygonum barbatum* was carried out by using ethyl acetate-hexane (7:3 v/v) as a mobile phase. From the high performance thin layer

chromatography analysis, peak numbers 4, 5 and 6 shown highest % of peak area of 29.79, 21.42 and 10.67, respectively at 366 nm.

Key words: *Polygonum barbatum* linn, hydroalcoholic extract, GC-MS analysis, HPTLC fingerprint

Polygonum barbatum belongs to the family Polygonacaea is one of the most important herbal used in the traditional medicine. The genus *Polygonum* is comprised of nearly 300 species, which are widely distributed around the world. The genus primarily grows in northern temperate regions and Asian countries. They vary widely from prostrate herbaceous annual plants under 5 cm high to erect herbaceous perennial plants growing up to 3-4 m tall to perennial woody vines growing up to 20-30 m high in trees. Several are aquatic, growing as floating plants in ponds. The smooth-edged leaves range from 1-30 cm long and vary in shape between species from narrow lanceolate to oval, broad triangular, heart-shaped, or arrowhead forms. The stems are often reddish or red-speckled. The small flowers are, pink, white, or greenish, forming in summer in dense clusters from the leaf joints or stem apices[1].

Various secondary metabolites like tannins[1], proanthocyanidins[2], flavonoids[3,4], anthraquinones[5], stilbenoids[6], resveratrol[7], lignans[8], phenylpropanoids[9], triterpenoids[10], coumarins and neoflavonoids[11], sesquiterpenoids[12], anthraquinones[13], phenylpropanoids[14], proteins, amino acids and carbohydrates[15] have been reported from species of genus *Polygonum*.

Among the various species of genus *Polygonum*, biological activities such as antiinflammatory activity of *P. glabrum*[16], anticancer activity of *P. hydropiper* L[17], antihypertensive effect of *P. perfoliatum*[18], myocardial protective action of *P. multiflorum*[19], antiviral activity of *P. punctutam*[20], antiallergic effect of *P. tinctorium*[21], analgesic, antiinflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from *P. viscosum*[22], antioxidant activity of *P. paleaceum*[23] and antiulcer activity of *P. barbatum*[24] were reported. Review of literature shown that there was limited number analytical studies carried out on the species of *P. barbatum*.

The present communication deals with the gas chromatography mass spectrometry (GC-MS) and high performance thin layer chromatography (HPTLC) fingerprinting analysis of hydroalcoholic extract of *P. barbatum* (HAPB) leaves. *P. barbatum* leaves were collected from an Ukkadam area in Coimbatore, Tamil Nadu, India. It was identified and authenticated (FRL/CRJ/RDR No. 04/2014-15) at the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore, Karnataka, India. The plant materials (leaves) were cleaned, shade dried and powdered. The powdered leaves of the plant (100 g) was transferred into stoppered flask and treated with ethanol (70 % v/v) until the powder was fully immersed. The flask was shaken every hour for the first 6 h and then it was kept aside and shaken after 24 h. This process was repeated for 2 d and then the extract was filtered. The extract was collected and evaporated to dryness by vacuum. The final residue thus obtained was then subjected to GC-MS and HPTLC analysis. Preliminary phytochemical screening of HAPB was carried out by the standard methods and it was reported[24].

The HPTLC fingerprint profile was determined for HAPB by the method of Harborne[25] and Wagner *et al*.[26]. About 1.0 g of the extract was dissolved in ethanol 70% and was taken for analysis. HAPB was spot (2 µl) as bands with the help of the auto sampler fitted with a 100 µl Hamilton syringe on a 10×10 cm silica gel precoated 60F-254 aluminium HPTLC plates of thickness 0.2 mm was used. Different combination of solvent systems like toluene:ethyl acetate, benzene:ethyl acetate, ethyl acetate:ethanol, chloroform:ethanol, chloroform:methanol, chloroform:water, ethyl acetate:benzene, ethyl acetate:hexane with various proportion were tried to obtain an excellent separation and sharp peaks for analysis. The satisfactory resolution of the separation of compounds presented in HAPB was obtained in ethyl acetate: hexane (7:3 v/v) solvent system. After

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Accepted 26 April 2017
Revised 10 January 2017
Received 28 July 2016
Indian J Pharm Sci 2017;79(3):477-482

the application of sample, the plate was developed in twin trough glass chamber 10×10 to a distance of 8 cm. Twin trough glass chamber was previously saturated with the solvent system by 30 min. The developed plate was air dried and scanned at 366 nm using Camag scanner 3 with WINCATS software. The plate was photographed at 366 nm using Camag Reprostar 3. The retention factor (Rf) value of each compound was separated on plate and % peak area of each band was recorded.

The GC-MS analysis of HAPB was performed using a GC-MS equipment GC Clarus 500 Perkin Elmer. Experimental conditions of GC-MS system were as follows: Elite-5MS (5% diphenyl/95% dimethyl poly siloxane), dimention: 30×0.25 mm×0.25 µm df was used and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1 injector temperature 250°). The oven temperature was programmed from 110° (isothermal for 2 min) with an increase of 10°/min up to 200°, then 5°/min up to 280° and ending with a 9 min isothermal at 280°. Mass spectra were taken at 70 eV, scan interval of 0.5 s and fragments from 45 to 450 Da. Source and inlet line temperature was 200° and the mass spectra detected at 36 min.

The GC-MS spectrum of HAPB was interpreted by using the database of National Institute Standard and Technology (NIST). The unknown individual constituents were identified based on direct comparison of the retention time (RT) and their mass spectra of known compounds stored in the NIST spectral database library. The RT, name, molecular formula, molecular weight and structure of the unknown compound were ascertained.

HPTLC fingerprint profile of HAPB was carried out by using ethyl acetate: hexane (7:3 v/v) solvent system to confirm the presence of various phytoconstituents in the extract. HPTLC chromatogram showed a total of 11 peaks at different Rf values and peak area at 366 nm was obtained (fig. 1, Table 1).

The results pertaining to GC-MS analysis lead to the identification of a number of compounds from gas chromatography fractions of the HAPB. They were identified through mass spectrum attached with GC. The GC-MS analysis of the HAPB was reported in Table 2.

The results revealed that the presence of 22 different phytoconstituents *viz*., D-mannose (18.32%), alloaromadendrene oxide-(1) (8.75%), 1H-3a,7-methanoazulene, octahydro-1,4,9,9-

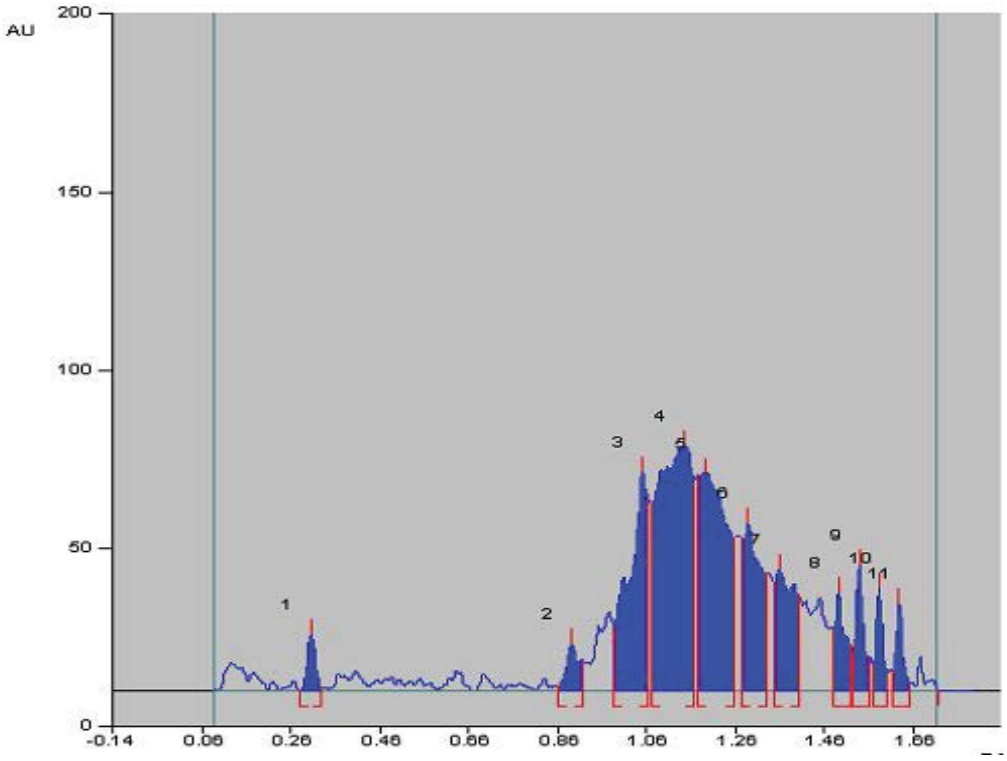

Fig. 1: HPTLC chromatogram of the hydroalcoholic extract of *P. barbatum* at 366 nm
Rf values of peak 1=0.30, peak 2=0.89, peak 3=1.05, peak 4=1.14, peak 5=1.19, peak 6=1.29, peak 7=1.36, peak 8=1.49, peak 9=1.54, peak 10=1.58, peak 11=1.63

TABLE 1: HPTLC FINGERPRINT PROFILE OF HYDROALCOHOLIC EXTRACT OF *POLYGONUM BARBATUM* LINN

Wavelength	Peaks	R_f values	%Peak area
366 nm	1	0.30	1.60
366 nm	2	0.89	2.13
366 nm	3	1.05	14.62
366 nm	4	1.14	29.79
366 nm	5	1.19	21.42
366 nm	6	1.29	10.67
366 nm	7	1.36	8.20
366 nm	8	1.49	3.55
366 nm	9	1.54	3.43
366 nm	10	1.58	2.46
366 nm	11	1.63	2.13

TABLE 2: PHYTOCONSTITUENTS IN THE HYDROALCOHOLIC EXTRACT OF *POLYGONUM BARBATUM* BY USING GC-MS ANALYSIS

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Nature of the compounds
7.07	7-Octene-1,2-diol	$C_8H_{16}O_2$	144	1.01	Mono terpene diol
7.98	3,7-dimethyl-6-Octen-1-ol	$C_{10}H_{20}O$	156	1.42	Alcoholic compound
8.12	1,4a,5,6,7,8,9,9a- octahydro-4a-methyl-, trans-2H-benzocyclohepten-2-one	$C_{12}H_{16}O$	178	2.78	Ketone
8.84	4,5,5a,6,6a,6b- hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)- 2H-cyclopropa[g]benzofuran	$C_{15}H_{22}O$	218	1.55	Terpenoid
9.58	1-methyl-4-(2- methyloxiranyl)-7-oxabicyclo[4.1.0] heptane	$C_{10}H_{16}O_2$	168	1.50	Terpenoid
10.23	3,3,5-trimethyl-cyclohexene	C_9H_{16}	124	3.80	Terpenoid
10.69	D-Mannose	$C_6H_{12}O_6$	180	18.32	Carbohydrate
10.91	Methyl ester of 2'-hexyl-[1,1'-Bicyclopropyl]-2-octanoic acid	$C_{21}H_{38}O_2$	322	6.55	Fatty acid
11.09	Longipinene epoxide	$C_{15}H_{24}O$	220	6.21	Terpenoid
11.17	Limonene-1,2-epoxide(fr.1)	$C_{10}H_{16}O$	152	6.56	Terpenoid
12.30	Aristolene epoxide	$C_{15}H_{24}O$	220	5.72	Terpenoid
12.74	4,6-diisopropylidene-8,8-dimethyl-bicyclo[5.1.0]octan-2-one	$C_{16}H_{24}O$	232	2.86	Ketone
13.20	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	220	5.50	Terpenoid
13.63	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.23	Fatty acid
13.92	Methyl ester of2,5-octadecadiynoic acid	$C_{19}H_{30}O_2$	290	4.10	Fatty acid ester
14.52	trans-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220	2.86	Terpenoid
15.10	cis-Z-à-bisabolene epoxide	$C_{15}H_{24}O$	220	4.43	Terpenoid
15.52	1b,5,5,6a-Tetramethyl-octahydro-1-oxa- cyclopropa[a] inden-6-one	$C_{13}H_{20}O_2$	208	3.70	Terpenes
16.19	octahydro-1,4,9,9- tetramethyl-1H-3a,7-methanoazulene	$C_{15}H_{26}$	206	7.71	Sesquiterpenes
17.96	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	8.75	Sesquiterpenoid
22.91	3,7,11-trimethyl-, (Z,E)-2,6,10-dodecatrien-1-ol	$C_{15}H_{26}O$	222	0.61	Fatty alcohol
26.90	Phenylmethyl ester 9-Octadecenoic acid	$C_{25}H_{40}O_2$	372	0.83	Fatty acid ester

tetramethyl-(7.71%), limonene-1,2-epoxide(fr.1) (6.56%), [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (6.55%), longipinene epoxide (6.21%), aristolene epoxide (5.72%), aromadendrene oxide-(2) (5.50%), cis-Z-à-bisabolene epoxide (4.43%), 2,5-octadecadiynoic acid, methyl ester (4.10%), cyclohexene, 3,3,5-trimethyl-(3.80%), 1b,5,5,6a-tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one (3.70%), 9,12-octadecadienoic acid (Z,Z)-(3.23%), 4,6-diisopropylidene-8,8-dimethyl-(2.86%), trans-Z-à-bisabolene epoxide (2.86%), 2H-benzocyclohepten-2-one, 1,4a,5,6,7,8,9,9a- octahydro-4a-methyl-,

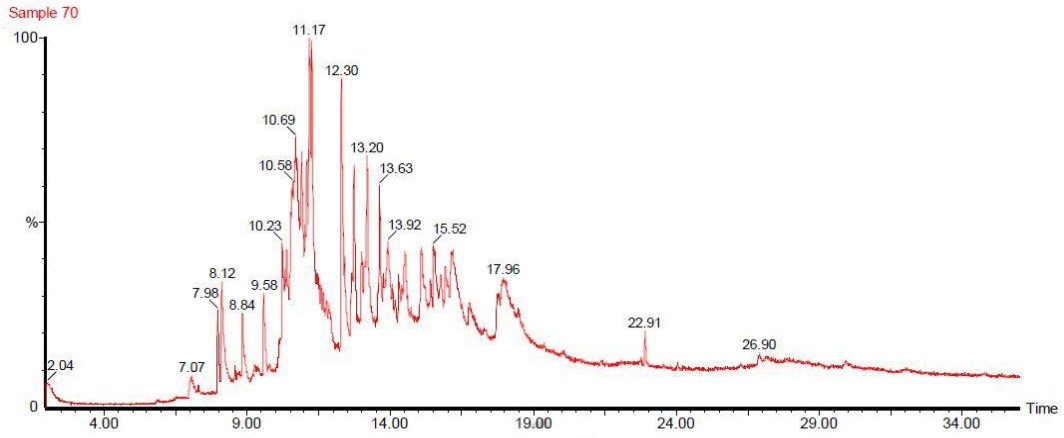

Fig. 2: GC-MS chromatogram of the hydroalcoholic extract of *P. barbatum*

trans-(2.78%), 2H-cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-(1.55%), 7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(2- methyloxiranyl)-(1.50%), 6-octen-1-ol, 3,7-dimethyl-(1.42%), bicyclo[5.1.0]octan-2-one, 7-octene-1,2-diol (1.01%), 9-octadecenoic acid (Z)-, phenylmethyl ester (0.83%), 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-(0.61%). The GC-MS spectrum of HAPB confirmed the presence of 22 compounds with the RT 7.07, 7.98, 8.12, 8.84, 9.58, 10.23, 10.69, 10.91, 11.09, 11.17, 12.30, 12.74, 13.20, 13.63, 13.92, 14.52, 15.10, 15.52, 16.19, 17.96, 22.91, 26.90, respectively (fig. 2).

The results revealed that D-mannose, alloaromadendrene oxide-(1), tetramethyl- octahydro-1,4,9,9-1H-3a,7-methanoazulene, limonene-1,2-epoxide, methyl ester of 2'-hexyl- [1,1'-bicyclopropyl]-2-octanoic acid, longipinene epoxide and aromadendrene oxide were found as a major components in HAPB. We found that the majority of compounds were terpenes and it was used for pesticide[27,28], antidiabetic, antidiarrheal effects[29], antiinflammatory[30,31], antioxidant, antileukemic, antitumor, anticancer, antiulcer, hepatoprotective[32-34].

Nowadays, the interest in the study of natural products is growing rapidly, especially as a part of drug discovery programs. It was concluded that the present mobile phase combination is suitable for eluting the phytoconstutuents from the extract. GC-MS analysis of HAPB concluded that the presence of various terpenes, terpenoids and fatty acids with varied degree. These various bioactive compounds present in the extract conform the application of *P. barbatum* for various diseases by traditional practitioners.

Thus, this type of GC-MS analysis is the preliminary step towards understanding the nature of active constituents present in the medicinal plants and this will be useful for further detailed study of plant. It could be concluded that, HAPB contains various bioactive components. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

Conflict of interest:

We declare that we have no conflict of interest.

Acknowledgement:

We would like to acknowledge Mr. S. Kumaravel, Quality Manager, Food Testing Laboratory, Indian Institute of Crop Processing Technology (Ministry of Food Processing Industries, Government of India), Thanjavur, Tamil Nadu, India for his assistance to carry out GC-MS analysis of the sample.

Financial support:

Nil.

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