GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L.

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Submitted: 15-06-2011 Revised: 22-08-2011 Published: 22-12-11

ABSTRACT

Background: The presence of diverse secondary metabolites has been reported from species of the genus *Polygonum*. However, there has been not much information available on phytochemical components and biological activity in the whole plant ethanol extract of *Polygonum chinense* L. **Objective**: This study was designed to determine the phytocomponents in the whole plant ethanol extract of *P. chinense*. **Materials and Methods**: GC-MS analysis of the whole plant ethanol extract of *P. chinense* 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). **Results**: This investigation was carried out to determine the possible chemical components from *P. chinense* by GC-MS. This analysis revealed that the ethanol extract of *P. chinense* (whole plant) contained mainly a triterpene compound—squalene (47.01%), and a plasticizer compound—1,2-benzenedicarboxylic acid, mono[2-ethylhexyl]ester (40.30%). All identified compounds were, generally, reported as having antimicrobial activity. In addition, the squalene compound also having anti-cancer, anti-oxidant, anti-tumor, chemo-preventive, pesticidal and sun-screen properties, while the plasticizer compound —1,2-benzenedicarboxylic acid, mono[2-ethylhexyl] ester reported to have anti-oxidant and anti-inflammatory properties. No activity was reported in the alcoholic compound–4-hexene-1-ol, 5-methyl-2-(1-methylethanyl)-acetate-(R)–. **Conclusions**: From the results, it is evident that *P. chinense* contains various bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Key words: GC-MS analysis, phytocomponents, *Polygonum chinense*, whole plant ethanol extract

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.91028

Quick Response Code:

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines.[1] It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[2]

The genus *Polygonum* belongs to Polygonaceae comprises about 150 species. The presence of diverse secondary metabolites such as flavonoids,[3,4] anthraquinones,[5,6] phenylpropanoids,[7] and proanthocyanidines[8] have been reported from species of the genus *Polygonum*. Moreover, the biological activities of *Polygonum* species such as antihypertensive effect of *P. perfoliatum*,[9] myocardial protective action of *P. multiflorum*,[10] anti-allergic effect of *P. tinctorium*,[11] and anti-viral activity of *P. punctatum*[12] have been investigated, but there have not been much reports on the phytochemical components and biological activity of *P. chinense*. In this study, the bioactive components of *P. chinense* have been evaluated using GC-MS.

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MATERIALS AND METHODS

Plant material

P. chinense was collected from Mekkari, Chenkottai Taluk, Tirunelveli District, Tamil Nadu, India, and identified by Dr. Chelladurai, Research Officer, at Central Council for Research in Ayurveda and Siddha, Palayamkottai. Herbarium of the plant, *Polygonum chinense*, was prepared and preserved in the Department of Botany, S. T. Hindu

College, Nagercoil, Kanyakumari District, Tamil Nadu, India.

Preparation of extract

The whole plant material of *P. chinense* was collected from wild, shade dried, and pulverized to powder using a mechanical grinder. The required quantity of the whole plant powder of *P. chinense* was weighed, transferred to a flask, treated with ethanol until the powder was fully immersed, incubated overnight and filtered through a Whatmann No. 41 filter paper along with sodium sulfate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulfate was wetted with absolute alcohol. The filtrate is then concentrated to 1 ml by bubbling nitrogen gas into the solution. The extract contains both polar and nonpolar components of the plant material, and 2 µl of the sample of the solutions was employed in GC-MS for analysis of different compounds.

GC-MS analysis

GC-MS analysis of the ethanol extract of *P. chinense* 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) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °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 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin–Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of phytocomponents

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 spectrum of the unknown components was compared with the spectrum of 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

GC-MS chromatogram analysis of the ethanolic extract of *P. chinense* [Figure 1] showed five peaks which indicating the presence of five phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library, the five phytocompounds were characterized and identified [Table 1]. The various phytochemicals which contribute to the medicinal activities of the plant were shown in Table 2. The mass spectra of all the phytochemicals identified in the whole plant ethanolic extract of *P. chinense* were presented in Figure 2. Of the five compounds identified, the most prevailing compounds were squalene, a triterpine compound (47.01%) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, a plasticizer compound (40.30%). Among the compounds, four compounds were reported to have antimicrobial activity, in general, and no activity was reported in 4-hexen-1-ol, 5-methyl-2-[1-methylethenyl]-, acetate, (R)-, a third major compound (9.70%) of the sample [Table 2].

In addition to antimicrobial activity, the squalene was

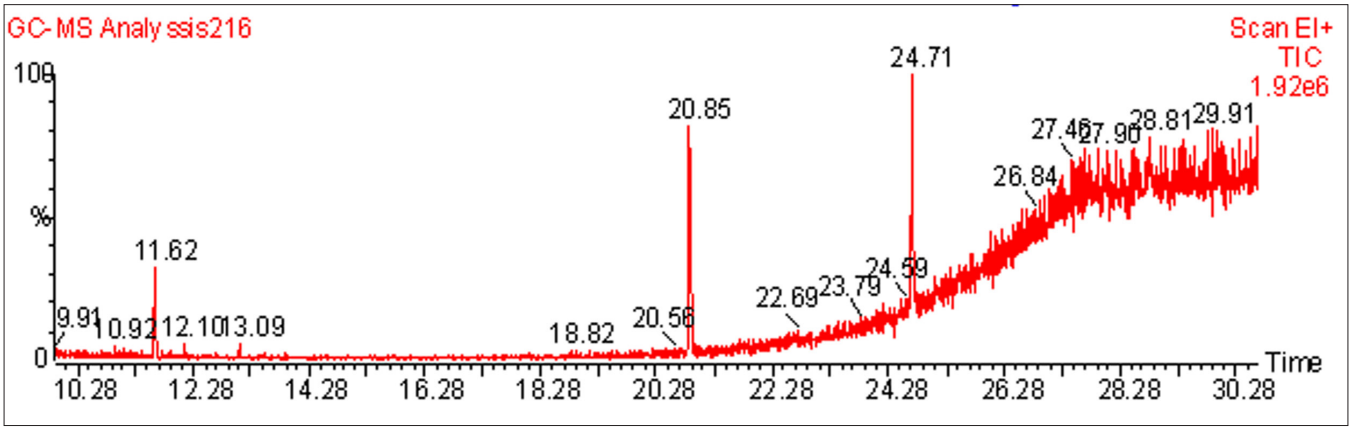

Figure 1: GC-MS chromatogram of *P. chinense* ethanolic extracts.

Table 1: Phytocomponents[a] identified in the ethanolic extract of *P. chinense* by GC-MS

No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1.	11.62	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate, (R)–	$C_{12}H_{20}O_2$	196	9.70
2.	12.10	8-Methyloctahydrocoumarin	$C_{10}H_{16}O_2$	168	1.49
3.	13.09	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	1.49
4.	20.85	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	40.30
5.	24.71	Squalene	$C_{30}H_{50}$	410	47.01

[a]Parameters tested are not covered under the scope of NABL accreditation.

Table 2: Bioactivity of phytocomponents identified in the ethanolic extracts of *P. chinense* by GC-MS

No.	RT	Name of the compound	Nature of compound	Activity[a]
1	11.62	4-hexen-1-ol, 5-methyl-2-[1-methylethenyl]-, acetate, (R)-	Alcoholic compound	No activity reported
2	12.10	8-Methyloctahydrocoumarin	Coumarin compound	Antimicrobial, antioxidant, anti-inflammatory
3	13.09	1,2-Benzenedicarboxylic acid, diheptylester	Plasticizer compound	Antimicrobial
4	20.85	1,2-Benzenedicarboxylic acid, mono[2-ethylhexyl] ester	Plasticizer compound	Antimicrobial
5	24.71	Squalene	Triterpene	Anticancer, antimicrobial, antioxidant, chemo preventive, pesticide, anti-tumor, sunscreen

[a]Source: Dr. Duke's: Phytochemical and Ethnobotanical Databases.

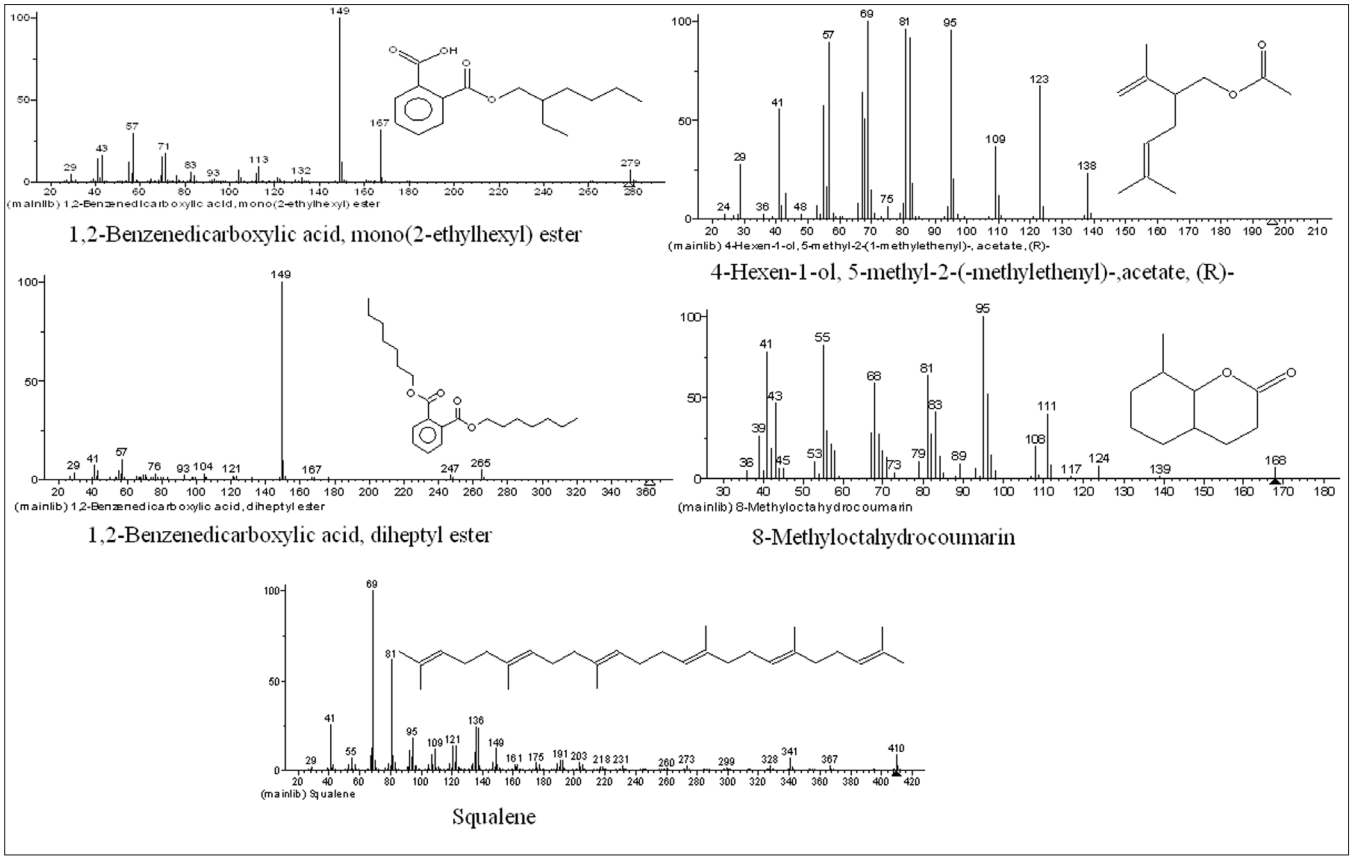

Figure 2: Mass spectrum and structure of phytocomponents identified by GC-MS in the ethanolic extracts of *P. chinense*.

also reported to have anticancer, anti-oxidant, chemo-preventive, pesticide, anti-tumor, and sunscreen properties, and the coumarin compound (8-methyloctahydrocoumarin) has antioxidant and anti-inflammatory properties [Table 2]. The squalene (triterpine) is a phenolic compound and that the terpines are found in latex and resines of some plants and physiological function of these compounds are generally believed to be a chemical in defense against certain pathogens causing human and animal diseases.[13] Their activity is a function of the lipophilic properties of

the constituent terpines, the properties of their functional groups, and their aqueous solubility.[14]

The presence of various bioactive compounds in the *P. chinense* justifies the use of whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. From the results, it could be concluded that *P. chinense* contains various bioactive compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance.

ACKNOWLEDGEMENT

The authors thank S. Kumaravel, Quality Manager, Food Testing Laboratory and the Director, Indian Institute of Crop Processing Technology (IICPT), Thanjavur, for providing all the facilities and support to carry out the work. The authors also express sincere thanks to the Management Authorities, The Principal, S. T. Hindu College, and Head of the Department of Botany and Research Centre, S. T. Hindu College, Nagercoil, Kanyakumari District, India, for providing necessary facilities and encouragement.

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Cite this article as: Ezhilan BP, Neelamegam R. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Phcog Res 2012;4:11-4.

Source of Support: Nil, **Conflict of Interest:** None declared.