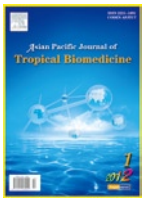
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae)

Janakiraman N[1], Johnson M[2*], Sahaya Sathish S[3]

[1]*Department of Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous), Tiruchirappalli-620 002, Tamil Nadu, India*
[2]*Centre for Biotechnology, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India*
[3]*Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli-620 002, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 9 January 2012
Received in revised form 7 February 2012
Accepted 18 March 2012
Available online 28 April 2012

Keywords:
Phytochemistry
Chemical profile
Medicinal plants

ABSTRACT

Objective: To characterize the chemical constituents of *Peristrophe bicalyculata* (*P. bicalyculata*) (Retz.) Nees. using GC-MS. **Methods:** Ten grams of powdered sample was extracted with 30 mL ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g) and the extract was concentrated to 1 mL by bubbling nitrogen into the solution. The Clarus 500 GC used in the analysis employed a column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1 μm df) and the components were separated using Helium (1 mL/min) as the carrier gas. The 2 μL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. **Results:** The GC-MS analysis provided different peaks determining the presence of seven different phytochemical compounds namely propane,1,1-diethoxy (68.89%), (6Z)-nonen-1-ol (24.00%), 4-methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene-1 (3.56%), cyclooctyl alcohol (1.78%), oxirane,butyl- (0.89%), (2H)pyrrole-2-carbonitrile,5-amino-3,4-dihydro- (0.44%) and ethaneperoxoic acid,1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl] pentyl ester (0.44%). **Conclusions:** The presence of various bioactive compounds confirms the application of *P. bicalyculata* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

1. Introduction

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects[1]. Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds[2]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate[3]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, *etc*[4]. The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[5].

There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[6-12]. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer[13] and Alzheimer's disease[14].

Peristrophe bicalyculata (*P. bicalyculata*) (Retz.) Nees. (Acanthaceae) is an erect, hispid herb or under shrub, 60-120 cm height found in forest undergrowth, hedges and waste band almost throughout India. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea. *P. bicalyculata* is used by the traditional healers for curing many skin related problems; as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellant. This plant

*Corresponding author: Johnson M, Centre for Biotechnology, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India .
Tel: + 91 97 91 81 38 25
Fax: + 91 462 2561 765
E-mail: mahesh14angel@gmail.com

is also used for horse feed and ploughed into the soil as green manure. Although undocumented, the plant is used in South West Nigeria in the treatment of hypertension and other cardiovascular diseases. It was recently discovered to have hypolipidemic effects[15] and such effects are known to protect against cardiovascular diseases, including hypertension. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phytoconstituents present in *P. bicalyculat*a using GC-MS analysis.

2. Materials and methods

2.1. Collection and preparation of plant material

The fresh plants of *P. bicalyculat*a (Retz.) Nees. were collected from the natural habitats of Tiruchirappalli district, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

2.2. Plant sample extraction

The powdered plant material was analyzed using the Clarus 500 GC-MS (Perkin Elmer). Ten grams of powdered sample was extracted with 30 mL ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g) and the extract was concentrated to 1 mL by bubbling nitrogen into the solution. 2 μL of the ethanolic extract of *P. bicalyculat*a was employed for GC-MS analysis[16].

2.3. GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The 2 μL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110℃ with 2 minutes holding. The injector temperature was set at 250℃ (mass analyser).

The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200℃; Source temperature: 200℃). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST Ver. 2.0-year 2005 library.

3. Results

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract of *P. bicalyculat*a (Retz.) Nees. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of propane,1,1-diethoxy (68.89%) and (6Z)-nonen-1-ol (24.00%), 4-methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene-1 (3.56%), cyclooctyl alcohol (1.78%), oxirane,butyl- (0.89%), (2H)pyrrole-2-carbonitrile,5-amino-3,4-dihydro- (0.44%) and ethaneperoxoic acid,1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl] pentyl ester (0.44%). The spectrum profile of GC-MS confirmed the presence of seven major components with the retention time 2.82, 11.63, 11.91, 12.11, 14.98, 20.85 and 24.69, respectively (Figure 1A). The individual fragmentation patterns of the components were illustrated in Figure 1B-H. The mass spectrum of the compound with retention time 2.82 (68.89%) gave 10 major peaks (m/z) at 59.999, 87.661, 47.584, 103.456, 31.394, 29.349, 75.348, 57.221, 27.187 and 45.160 (Figure 1B). The mass spectrum of the compound with retention time 11.63 (24.00%) gave 10 major peaks (m/z) at 41.999, 67.808, 55.475, 68.404, 31.400 39.398, 27.319, 95.316, 82.306 and 29.294 (Figure 1C). The mass spectrum of the compound with retention time 11.91 (0.44%)

Table 1.
Activity of phytocomponents identified in *P. bicalyculata* by GC-MS.

Name of the compound	Retention time	Molecular formula	Molecular weight	Peak area %	Nature of compound	Activity
Propane, 1,1-diethoxy-	2.82	$C_7H_{16}O_2$	132	68.89	Ether	No activity
(6Z)-nonen-1-ol	11.63	$C_9H_{18}O$	142	24.00	Alkene	Anti-microbial
(2H)pyrrole-2-carbonitrile, 5-amino-3,4-dihydro-	11.91	$C_5H_7N_3$	109	0.44	Alkaloid	Anti-microbial, Anti-inflammatory
Cyclooctyl alcohol	12.11	$C_8H_{16}O$	128	1.78	Aromatic alcohol	Anti-microbial, Antioxidant
Oxirane, butyl-	14.98	$C_6H_{12}O$	100	0.89	Oxirane	No activity
Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl]pentyl ester	20.85	$C_{19}H_{25}NO_5$	347	0.44	Cyano compound	Anti-microbial, Insecticide
4-Methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene1	24.69	$C_{24}H_{36}O_2Si_2$	412	3.56	Aromatic ether with silicon	No activity reported

gave 10 major peaks (m/z) at 81.999, 109.904, 54.851, 82.846, 42.781, 28.482, 55.481, 43.314, 56.308 and 41.302 (Figure 1D). The mass spectrum of the compound with retention time 12.11 (1.78%) gave 10 major peaks (m/z) at 57.999, 68.435, 41.435, 67.415, 82.400, 81.332, 55.291, 44.242, 43.240 and 56.214 (Figure 1E). The mass spectrum of the compound with retention time 14.98 (0.89%) gave 10 major peaks (m/z) at 71.999, 41.685, 42.596, 29.449, 27.418, 55.411, 39.394, 58.339, 43.222 and 31.161 (Figure 1F). The mass spectrum of the compound with retention time 20.85 (0.44%) gave 9 major peaks (m/z) at 149.999, 105.280, 150.100, 151.20, 185.20, 205.20, 159.10, 161.10 and 210.10 (Figure 1G). The mass spectrum of the compound with retention time 24.69 (3.56%) gave 9 major peaks (m/z) at 207.999, 73.40, 208.21, 209.21, 74.12, 347.9, 412.7, 413.3 and 348.2 (Figure 1H).

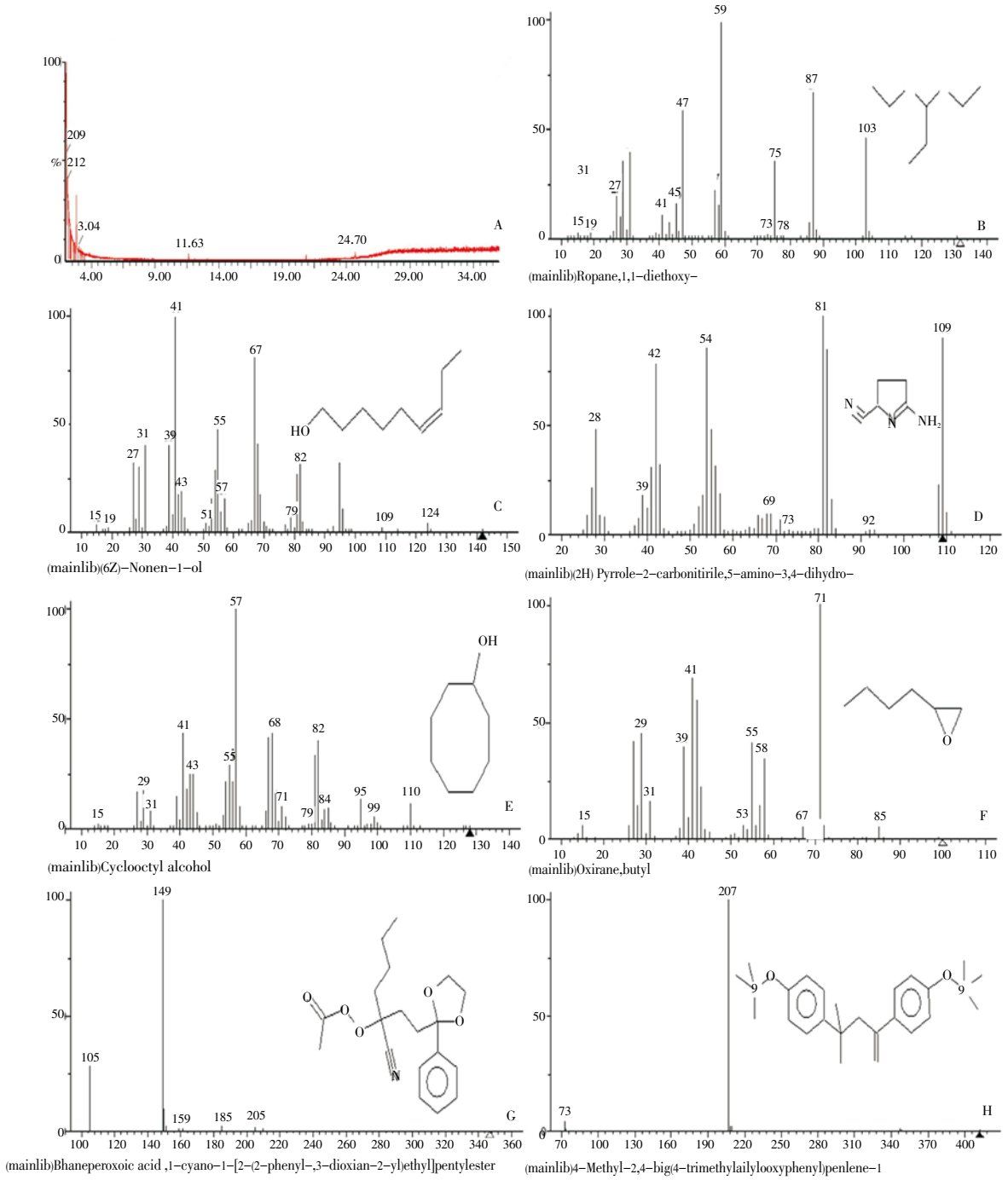


Figure 1. GC-MS analysis of *P. bicalyculata*.
A. GC-MS Chromatogram of ethanolic extract of *P. bicalyculata*; B. Mass spectra of Propane, 1,1-diethoxy-; C. Mass spectra of (6Z)-Nonen-1-ol; D. Mass spectra of (2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro-; E. Mass spectra of Cyclooctyl alcohol; F. Mass spectra of Oxirane, butyl- G. Mass spectra of Ethaneperoxoic acid, 1-cyano-1- [2-(2-phenyl-1,3-dioxolan- 2-yl)ethyl] pentyl ester; H. Mass spectra of 4-Methyl-2,4-bis (4′-trimethylsilyloxyphenyl) pentene-1

4. Discussion

(6Z)-nonen-1-ol is suggested to be an alkene compound

and it may acts as an antimicrobial agent. (2H)pyrrole-2-carbonitrile, 5-amino-3,4-dihydro- is recommended to be an alkaloid and it may used as an antimicrobial and anti-inflammatory agent. Cyclooctyl alcohol is suggested to be an aromatic alcohol and it may be employed as an antioxidant and antimicrobial agent. Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester is suggested to be an cyano compound and it may be an active antimicrobial agent and insecticide. The present study results were confirmed by the antibacterial inhibition against the selected five pathogens. The ethanolic extract showed better inhibitory activity against all the tested pathogens[17]. By interpreting these compounds, it is found that *P. bicalyculat*a possess various therapeutical application. Several phytochemical screening studies have been carried out in different parts of the world using GC-MS[18-20]. In the present study we characterized the chemical profile of *P. bicalyculat*a using GC- MS. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *P. bicalyculat*a using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the present study supported and supplemented the previous observations[18-21].

GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *P. bicalyculat*a for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to the Indian Institute of Crop Processing Technology (IICPT), Thanjavur, for providing the laboratory facilities (GC-MS).

References

[1] Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK, et al. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry* 2003; **63**: 97-104.

[2] Sumathi, Parvathi. Antimicrobial activity of some traditional medicinal plants. *J Med Plant Res* 2010; **4**: 316-321.

[3] Zaidan MRS, Rain NA, Badrul AR. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop Biomed* 2005; **22**: 165-170.

[4] Gordon DM. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. *Microbiology* 2001; **147**: 1079-1085.

[5] Wink DA, Vodovotz Y, Grisham MB, DeGraff W, Cook JC, Pacelli R, et al. Antioxidant effects of nitric oxide. *Methods Enzymol* 1999; **301**: 413-424.

[6] Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidant activity of bioreactive constituents from *Hydnophytum formicarum* Jack. *Molecules* 2008; **13**: 904-921.

[7] Nogueira JC, Diniz Mde F, Lima EO. *In vitro* antimicrobial activity of plants of acute *Otitis externa*. *Braz J Otorhinolaryngol* 2008; **74**: 118-124.

[8] Costa ES, Hiruma-LimaC A, Limo EO, Sucupira GC, Bertolin AO, Lolis SF, et al. Antimicrobial activity of some medicinal plants of Cerrado. *Brazil Phytother Res* 2008; **22**: 705-707.

[9] Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activity of different parts of *Trubulus terrestris* L. growing in Iraq. *J Zhejiang Univ Sci* 2008; **B9**: 154-159.

[10] Chen IN, Chang CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of *Zingiberaceae* plants in Taiwan. *Plant Foods Hum Nutr* 2008; **63**: 15-20.

[11] Pesewu GA, Cutler RR, Humber DP. Antibacterial activity of plants used in traditional medicine of Ghana, with particular reference to MRSA. *J Ethnopharmacol* 2008; **116**: 102-111.

[12] Turker AU, Usta C. Biological screening of some Turlish medicinal plants for antimicrobial and toxicity studies. *Nat Prod* 2008; **22**: 136-146.

[13] Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. *Immunopharmacol Immunotoxicol* 2007; **29**: 81-93.

[14] Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. *Phytother Res* 2007; **21**: 1142-1145.

[15] Abdulazeez AM, Nok AJ, Gwani H. Effect of *Peristrophe bicalyculata* on lipid profile of P-407-induced hyperlipidemic wistar rats. An unpublished thesis submitted to the Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, 2009;

[16] Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. *Pharmacognosy Res* 2009; **1**(3): 152-156.

[17] Janakiraman N. *Studies on antimicrobial and phytochemical analysis of Peristrophe bicalyculata (Retz.) Nees., a medicinal herb*. M.Sc dissertation submitted to St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India, 2011;

[18] Wu L, Gao H, Wang X, Ye J, Lu J, Liang Y. Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPLC. *J Med Plants Res* 2010; **4**(5): 421-426.

[19] Vohra A, Kaur H. Chemical investigation of medicinal plant *Ajuga bracteosa*. *J Nat Prod Plant Resour* 2011; **1**(1): 37-45.

[20] Sangeetha J, Vijayalakshmi K. Determination of bioactive components of ethyl acetate fraction of *Punica granatum* Rind extract. *Int J Pharm Sci & Drug Res* 2011; **3**(2): 116-122.

[21] Roy S, Rao K, Bhuvaneswari Ch, Giri A, Mangamoori LN. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. *World J Microbiol Biotechnol* 2010; **26**: 85-91.