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Research article

GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF ARISTOLOCHIA BRACTEATA RETZ (ARISTOLOCHIACEAE)

Jegadeeswari P¹, Nishanthini A¹, Muthukumarasamy S² and Mohan VR^{1*}

¹Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, 628 008, Tamil Nadu, India.

²Department of Botany, Sri K.G.S. Arts College, Srivaikuntam, Tamil Nadu, India.

*Corresponding author V.R.mohan, Ethnopharmacology Unit Research Department of Botany, V.O.Chidambaram College, Tuticorin 628008. Tamil Nadu, India. <u>Tel:+919487279902</u> Email <u>vrmohanvoc@gmail.com</u>

ABSTRACT: The investigation was carried out to determine the possible bioactive components of whole plant of *Aristolochia bracteata* using GC-MS. The chemical compositions of the ethanol extract of whole plant of *Aristolochia bracteata* were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry while the Mass Spectra of the compounds found in the extract was matched with the National Institute of Standard and Technology (NIST) Library. Eleven compounds were identified; α -D-glucose (27.88%) was found to be major component followed by 3-O-Methyl-d-glucose (24.54%), Linolenic acid, trimethylsilyl ester (17.81%), Hexadecanoic acid, trimethylsilyl ester (9.46%) etc. **Keywords:** *Aristolochia bracteata*, GC-MS, α -D-glucose.

INTRODUCTION

Aristolochia bracteata is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al.*, 2003). This plant belongs to the family Aristolochiaceae. It has been found most commonly in ancient texts for important medicinal properties including anthelmentic, fever, purgative and painful joints. Recently, it has been reported for hypotensive, hypothermia, antioxidant and anti-inflammatory properties (Chitme *et al.*, 2009). Taking into consideration of the medicinal importance of Aristolochia bracteata, in the present study, GC-MS analysis of ethanol extract of whole plant of Aristolochia bracteata has been evaluated. This work will help to identify the compounds of therapeutic value.

MATERIALS AND METHODS

The whole plant of *Aristolochia bracteata* Sivaranjan and Pradeep were collected from vadavalli Coimbatore, Tamil Nadu, India. The plant was identified with help of local flora and authenticated in Government of India, Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 μ Mdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 μ l was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C.

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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 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % 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.

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 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

Eleven compounds were identified in *Aristolochia bracteata* whole plant by GC-MS analysis. The active principles with their Retention time (RT), Molecular formula (MF), Molecular weight (MW) and Concentration (%) are presented in Table 1 and Figure 1. The prevailing compounds were α -D-glucose (27.88%), 3-O-Methyl-d-glucose (24.54%), Linolenic acid, trimethylsilyl ester (17.81%), Hexadecanoic acid, trimethylsilyl ester (9.46%), 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,5,6,7,9,10,10a-dedecahydro-1,4a-dimethyl-7-(1-methylethylidene)-methylester, [IR-(1a,4aa',4ba'10aa)]-[synonyms: Neoabietic acid, methyl ester] (8.96%), β -sitosterol (3.73%), 3,7,11,15-Tetramethyl-2-hexadecan-1-ol (3.00%) and Vitamin E (2.00%). Figure 2,3,4 and 5 shows the mass spectrum and structures of important constituents of *Aristolochia bracteata* whole plant viz: Phytol, 9,12,15- Octadecatrienric acid, methyl ester,(Z,Z,Z)-, Vitamin E and β -sitosterol respectively. Table 2 listed the various phytochemical constituents which contribute to the medicinal activity of ethanol extract of *Aristolochia bracteata* whole plant.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	10.26	α-D-Glucose	C ₆ H ₁₂ O ₆	180	27.88
2	10.73	3-O-Methyl-d-glucose	C7H14O6	194	24.54
3	11.43	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	3.00
4	13.23	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.27
5	13.83	Hexadecanoic acid, trimethylsilyl ester	C ₁₉ H ₄₀ O ₂ Si	328	9.46
6	14.72	Phytol	C ₂₀ H ₄₀ O	296	1.27
7	15.45	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	1.08
8	15.99	Linolenic acid, trimethylsilyl ester	C ₂₁ H ₃₈ O ₂ Si	350	17.81
9	25.88	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a-dimethyl-7- (1-methylethylidene)-, methyl ester, [1R-(1à,4aá,4bà,10aà)]- [Synonyms: Neoabietic acid, methyl ester]	C ₂₁ H ₃₂ O ₂	316	8.96
10	28.61	Vitamin E	C ₂₉ H ₅₀ O ₂	430	2.00
11	31.80	ß-Sitosterol	С ₂₉ Н ₅₀ О	414	3.73

Table.1 Components detected in the whole plant ethanol extract of Aristolochia bracteata

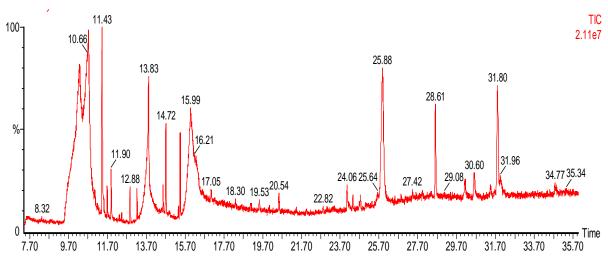
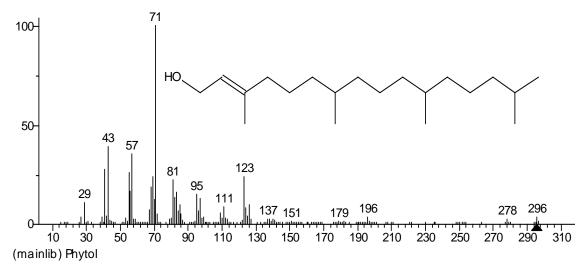
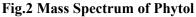


Fig.1GC-MS Chromotogram of the ethanol extract of whole plant of Aristolochia bracteata

Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity (Sutha *et al.*, 2012). 9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z) have the property of anti-inflammatory and anti arthritic as reported by earlier workers(Lalitharani *et al.*, 2009;Maruthupandian *et al.*, 2011). Omega-3-fatty acid have been found to be essential for normal growth and development and may play an important role in the prevention and treatment of coronay artery disease, hypertension, diabetes and arthritis, other inflammatory and autoimmune disorders and cancer (Simopoulos, 1991; Simopoulos, 1997; Simopoulos, 2004). Phytol is detected in *Aristolochia bracteata* whole plant which was also found to be effective at different stages of the arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promosing novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al.*, 2009). Beta-sitosterol limits the amount of cholesterol entering the body by inhibiting cholesterol absorption in the intestines, therefore decreasing the levels of cholesterol in the body. It is helpful with benign prostatic hyperplasia (BPH), due to its anti-inflammatory effects and it's ability to improve urinary symptoms and flow.





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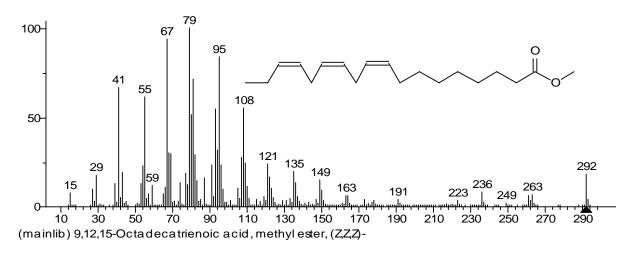


Fig.3 Mass Spectrum of 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-

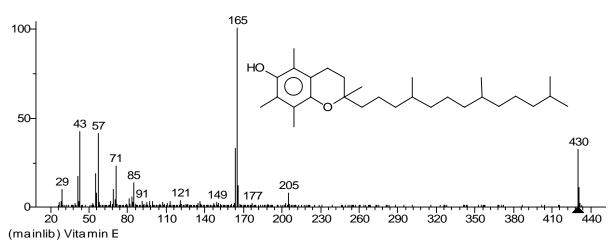


Fig.4 Mass Spectrum of Vitamin E

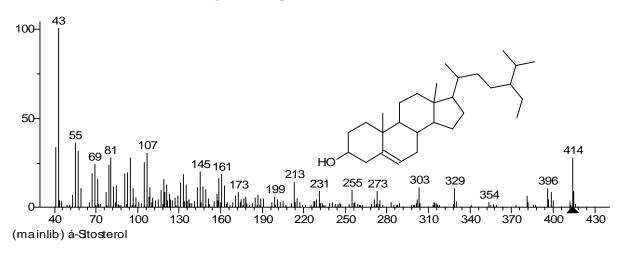


Fig.5 Mass Spectrum of β-Sitosterol

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No	Name of the compound	Molecular formula	Compound Nature	**Activity
1	α-D-Glucose	C ₆ H ₁₂ O ₆	Sugar content	Preservative
2	3-O-Methyl-d-glucose	C7H14O6	Sugar moiety	Preservative
3	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	С20Н40О	Terpene alcohol	Antimicrobial Antiinflammatory
4	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	Palmitic acid ester	
5	Phytol	C ₂₀ H ₄₀ O	Diterpene	Anticancer Antioxidant Antiinflammatory Diuretic
6	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	Linolenic acid ester	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
7	Vitamin E	C29H50O2	Vitamin compound	Antiageing, Analgesic, Antidiabatic Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic,Anticoronary
8	ß-Sitosterol	C ₂₉ H ₅₀ O	Steroid	Antimicrobial Antioxidant Antiinflammatory Antiarthritic, Antiasthma Diuretic

Table.2 Activity of Phyto-components identified in the ethanol extract of whole plant of Aristolochia bracteata

**Source: Dr.Duke's: Phytochemical and Ethnobotanical Databases

In the present study, eleven chemical constituents have been identified from ethanol extract of the whole plant of *Aristolochia bracteata* by GC-MS analysis. The presence of various bioactive compounds justifies the use of the whole plants for various ailments by traditional practitioners. So it is recommended as a plant of pharmaceutical importance. However, further studies will need to be undertaken to as certain fully its bioactivity.

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