INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 3: Issue-1: Jan - Mar-2012 *ISSN 0976-4550*

Accepted: Dec-2011 *Research Article*

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF *RAUWOLFIA DENSIFLORA* (Wall) BENTH. ex HK.f

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ABSTRACT : *Rauwolfia densiflora* (Wall) Benth. ex HK.f known to the Kanikkars as "Paarisirunila" is one of the medicinally important plants belonging to the family Apocynaceae. Kanikkar tribes, inhabitants of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu, India use the paste prepared from the fresh leaves and flowers to treat rheumatic complaints. This study was carried out to analyse the active constituents present in the whole plant of said plant. Seven compounds were identified in the ethanol extract of *Rauwolfia densiflora* by Gas Chromatography - Mass Spectrometry (GC-MS) analysis.

Key words: GC-MS analysis, *Rauwolfia densiflora*, Phytol.

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can prtotect humans against diseases. Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO), more than 80% of the world's population, mostly in developing countries depend on traditional plant based medicines for their primary healthcare needs[1]. *Rauwolfia densiflora* (Wall) Benth. ex HK.f is an erect herb with a smooth stem. *R.densiflora*, known to the Kanikkars as "Paarisirunila", is an important medicinal plant. The Kanikkar tribe, inhabitants of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu, India use the paste prepared from the fresh leaves and flowers to treat rheumatic complaints (Lalitharani *et al*., 2011). Taking into consideration the medicinal importance of *R.densiflora*, the ethanol extract of the above said whole plant were analysed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value.

MATERIALS AND METHODS

Whole plant of *Rauwolfia densiflora* (Wall) Benth. ex HK.f were collected from Karaiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The samples were air-dried and powdered. Required quantity of powder was weighted and transferred to stoppard flask and treated with the ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts was carried out by following the method of Hema *et al*. (2010). GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-I fused silica capillary column (30m×0.25mm ID × 1µdf), composed of 100% Dimethyl polysiloxane). 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 2µl was employed split ratio of 10:1 injector temperature 250^{0}C; ion-source temperature 280^{0}C. The oven temperature was programmed from 110^{0}C (isothermal for 2 min) with an increase of 10^{0}C/min to 200^{0}C, then 5^{0}C/min to 280^{0}C, ending with a 9 min isothermal at 280^{0}C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds 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 Turbo mass.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62000 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.

RESULT AND DISCUSSION

The compounds present in the ethanol extract of *R.densiflora* were identified by GC-MS analysis (Fig.1). The active principles with a retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of *R.densiflora* are presented in Table 1. Seven compounds were identified in ethanol extract by GC-MS. The major components present in the whole plant of *R.densiflora* were propanoic acid, anhydride, 1,10-Decanediol, Phytol and 3-Pentanol,2,4-dimethyl-. Fig.2,3, 4 and 5 show a mass spectrum and structure of Propanoic acid, anhydride, 1,10-Decanediol, Phytol and 3-Pentanol,2,4-dimethyl-. Phytol is detected in whole plant of *R.densiflora* 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 promising novel class of pharmaceuticals for the treatment of rheumoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009). Table 2 listed the major phytocomponents and its beneficial activities obtained through GC-MS study of whole plant of *R.densiflora*.

Table 1: Components detected in the ethanol extract of whole plant of *R.densiflora* by GC-MS

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	7.37	Propanoic acid, anhydride	$C_6H_{10}O_3$	130	10.81
2.	11.60	1,10-Decanediol	$C_{10}H_{22}O_2$	174	21.62
3.	14.92	Phytol	$C_{20}H_{40}O$	296	5.41
4.	24.62	1,5-Heptadiene, 2,6-dimethyl-	C_9H_{16}	124	8.11
5.	28.50	3-Pentanol, 2,4-dimethyl-	$C_7H_{16}O$	116	10.81
6.	30.99	3,4-Dimethyl-5-hexen-3-ol	$C_8H_{16}O$	128	21.62
7.	32.24	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	$C_{18}H_{34}O_4$	314	21.62

Table 2: Activity of phytocomponents identified in the ethanol extract of whole plant of *R.densiflora* by GC-MS

RT	Name of the compound	Molecular	Compound nature	**Activity
7.37	Propanoic acid, anhydride	$C_6H_{10}O_3$	Acidic compound	Antimicrobial
11.60	1,10-Decanediol	$C_{10}H_{22}O_2$	Alcoholic compound	Antimicrobial
14.92	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Anticancer AntioxidantDiuretic
28.50	3-Pentanol, 2,4-dimethyl-	$C_7H_{16}O$	Alcoholic compound	Antimicrobial

**Activity Source: Dr.Duke's Phytochemical and Ethnobotanical databases

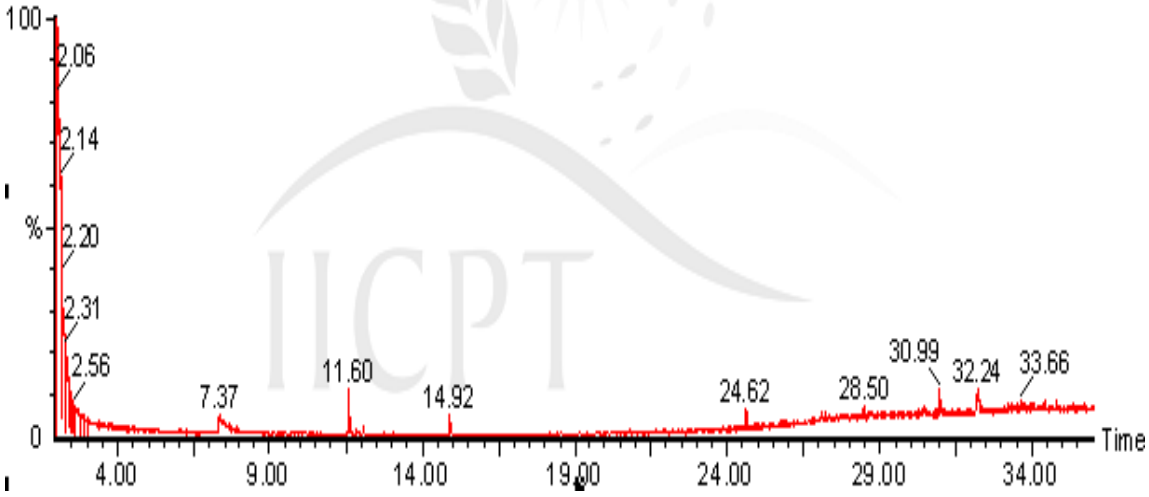

Fig.1: GC-MS chromatogram of the ethanol extract of whole plant of *R.densiflora*

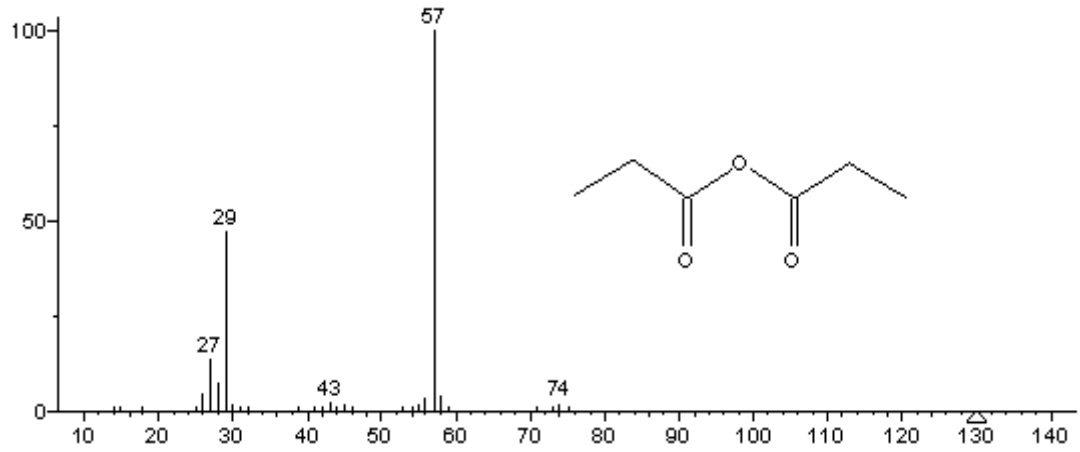

Fig.2: Mass spectrum of Propanoic acid, anhydride

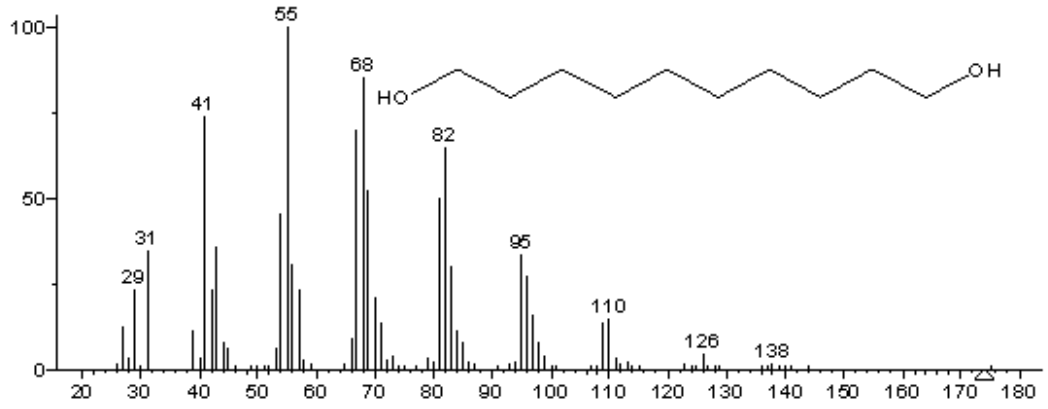

Fig.3: Mass spectrum of 1,10-Decanediol

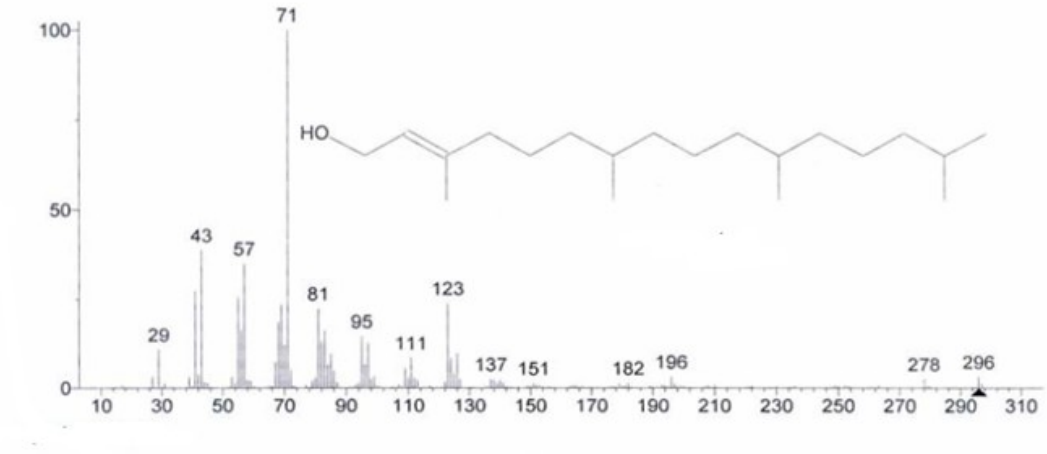

Fig.4: Mass spectrum of Phytol

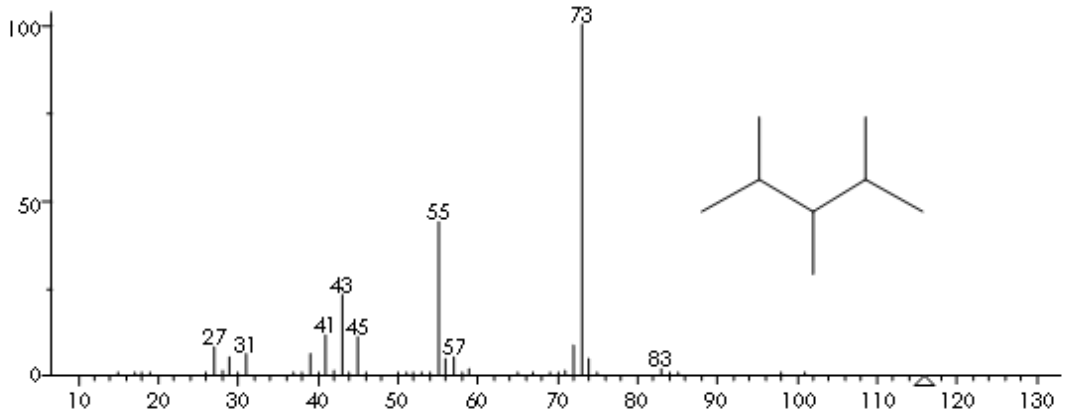

Fig.5: Mass spectrum of 3-Pentanol, 2,4-dimethyl-

In the present study, 7 compounds have been identified from the ethanol extract of whole plant of *R.densiflora* by Gas Chromatography – Mass Spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constitiuents and subjecting it to pharmacological activity will definitely give fruitful results.

Acknowledgement

We would like to thank Mr. S.Kumaravel, Senior Scientist, Indian Institute of Crop Processing Industries, Government of India, Tanjavore, Tamil Nadu for providing all the facilities and support used to carry out the work.

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