ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

GC-MS Determination of Bioactive Components of *Alstonia venenata* R.Br

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ABSTRACT

Alstonia venenata R.Br. (Apocynaceae) known to the Kanikkar as "Malaivaathamudakki'' is an important medicinal plant. The Kanikkar tribe, inhabitants of KMTR, Western Ghats, Tamil Nadu use this plant to get relief from rheumatic pain. The present investigation deals with GC-MS analysis of ethanol extract of the above said plant. Twenty three compounds were identified.

Keywords: *Alstonia venenata*, GC-MS analysis, Mass spectrum.

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ISSN: 0975-8585

INTRODUCTION

The genus Alstonia finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of Alstonia in the treatment of various human ailments [1-4]. Kanikkar tribals of Kalakad-Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled the fresh leaves of *Alstonia venenata* in neem oil over a low flame until the oil extracts the complete essence of the drug. The affected part is massaged with the lukewarm oil, specifically, in the direction from the limbs to the upper portion applying soft presence. Hot water bath is administered after few hours, until there is relief from the rheumatic complaints [5]. The fruits are stated to posses tonic and anthelmintic properties and are reported to used as a remedy for impure blood, syphilis, insanity and epilepsy [6]. Taking into consideration of the medicinal importance of this plant, the ethanol extract of leaves of *Alstonia venenata* were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the best techniques to identify the bioactive constituents of long chain branched chain hydrocarbons, alcohols, acids, ester etc.

MATERIALS AND METHODS

The leaves of *Alstonia venenata* R.Br. were collected from the well grown plant found in the natural forest of Kalakad-Mundanthurai Tiger Reserve Forest, Western Ghats, Tirunelveli, Tamil Nadu, India. The leaf of *Alstonia venenata* R.Br were cut into small pieces, cleaned, shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask, and treated with 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 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 µMdf, 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 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°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 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.

ISSN: 0975-8585

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

The components present in the ethanol extract of leaves of *Alstonia venenata* were identified GC-MS analysis (Figure 1. a, b, c). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of leaves of *Alstonia venenata* are presented in Table 1. Twenty three compounds were identified in ethanol extracts of *Alstonia venenata* leaf. The prevailing compounds were 3-0-methyl D glucose (87.64%), hexanedioic acid, bis (2-ethylhexyl) ester (1.87%), n-Hexadecanoic acid (1.70%) and Vitamin E (1.70%) figure 2, 3 and 4 shows the mass spectrum and structure of n-Hexadecanoic acid, Vitamin E and Squalene respectively.

TABLE 1: COMPONENTS DETECTED IN *ALSTONIA VENENATA* LEAF EXTRACT

Sl. No.	R.T	Name	Formula	Peak area %	M.W
1	3.17	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	0.26	180
2	3.54	2-Butanone, 3-methoxy-3-methyl-	$C_6H_{12}O_2$	0.09	116
3	4.11	Glycerin	$C_3H_8O_3$	0.04	92
4	4.28	2-Propenal	C_3H_4O	0.13	56
5	4.34	2-Propen-1-ol	C_3H_6O	0.13	58
6	6.14	Thymine	$C_5H_6N_2O_2$	0.09	126
7	7.70	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	Trace	144
8	8.01	Tetrahydropyran Z-10-dodecenoate	$C_{17}H_{30}O_3$	0.13	282
9	12.77	Phenol, 4-propyl-	$C_9H_{12}O$	0.70	136
10	17.60	Pentanoic acid, 2 –hydroxy -, ethyl ester	$C_7H_{14}O_3$	0.04	146
11	17.71	Methyl β-D-arabinopyranoside	$C_6H_{12}O_5$	0.09	164
12	18.99	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	0.78	194
13	19.67	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	87.64	194
14	23.64	Z-2-Dodecenol	$C_{12}H_{24}O$	1.31	184
15	23.99	Didodecyl phthalate	$C_{32}H_{54}O_4$	0.22	502
16	25.32	Decanoic acid, 2-methyl-	$C_{11}H_{22}O_2$	0.30	186
17	26.06	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.70	256
18	28.64	4-Dodecanol	$C_{12}H_{26}O$	0.26	186
19	33.63	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	1.87	370
20	34.89	1-Cyclohexylnonene	$C_{15}H_{28}$	1.48	208

ISSN: 0975-8585

21	35.98	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	0.70	278
22	37.88	Vitamin E	$C_{29}H_{50}O_2$	1.70	430
23	41.65	Squalene	$C_{30}H_{50}$	0.35	410

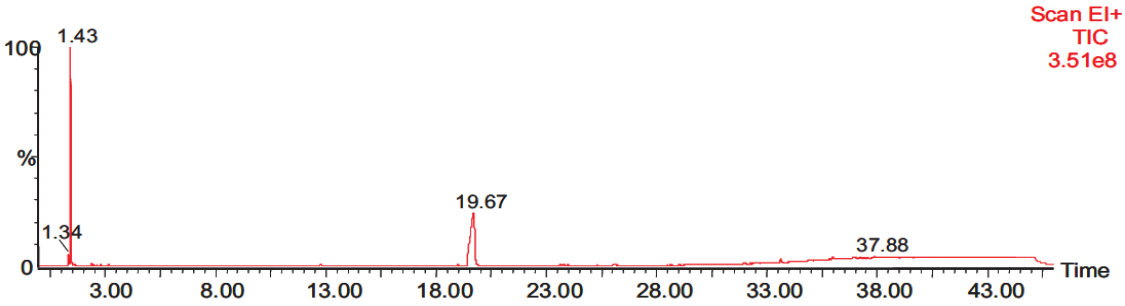

Fig. 1a: GC-MS Chromatogram of the ethanol extract of *Alstonia venenata* leaf

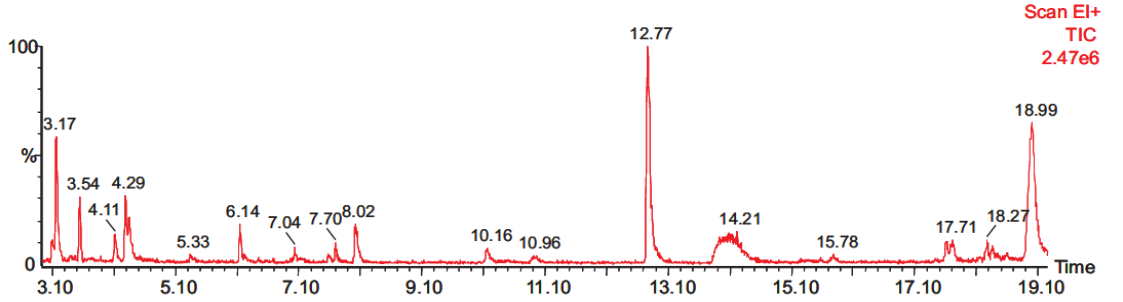

Fig. 1b: GC-MS Chromatogram of the ethanol extract of *Alstonia venenata* leaf - enlarged version I

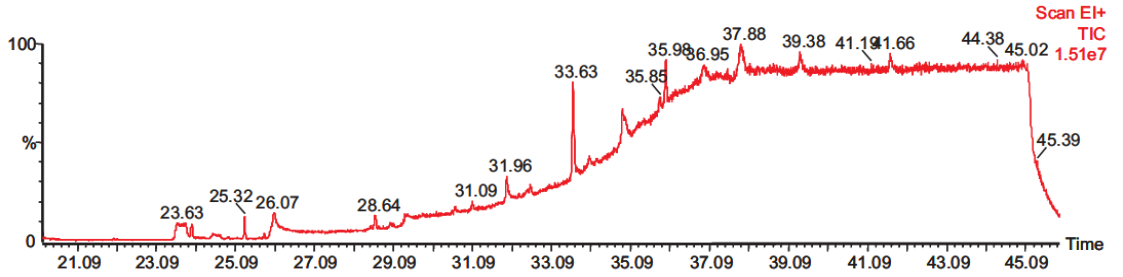

Fig. 1c: GC-MS Chromatogram of the ethanol extract of *Alstonia venenata* leaf - enlarged version II

DISCUSSION

In the present study, 23 compounds have been identified from ethanol extract of the leaves of *Alstonia venenata* by Gas Chromatography-Mass spectrometry (GC-MS) analysis. Among the identified phytochemicals, n-Hexadecanoic acid squalene has the property of antioxidant activity [7]. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

ISSN: 0975-8585

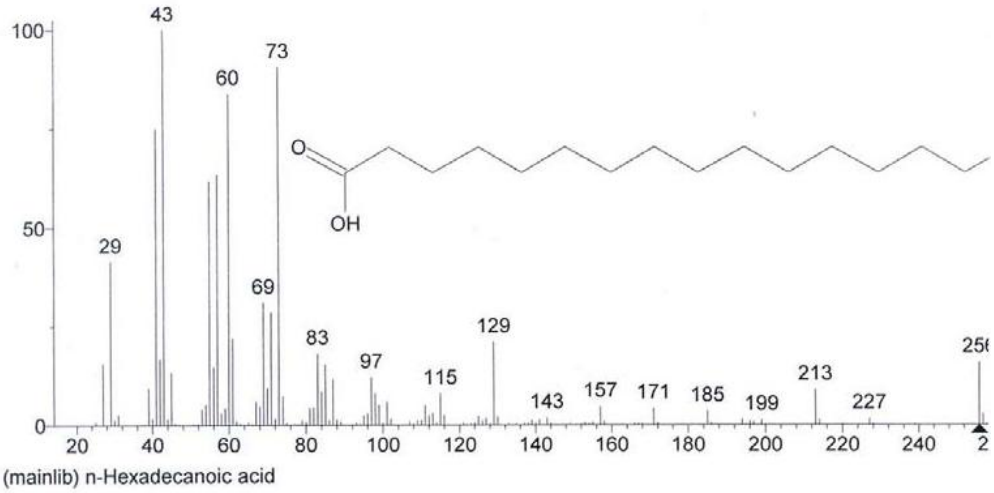

Fig. 2: Mass spectrum of n-Hexadecanoic acid

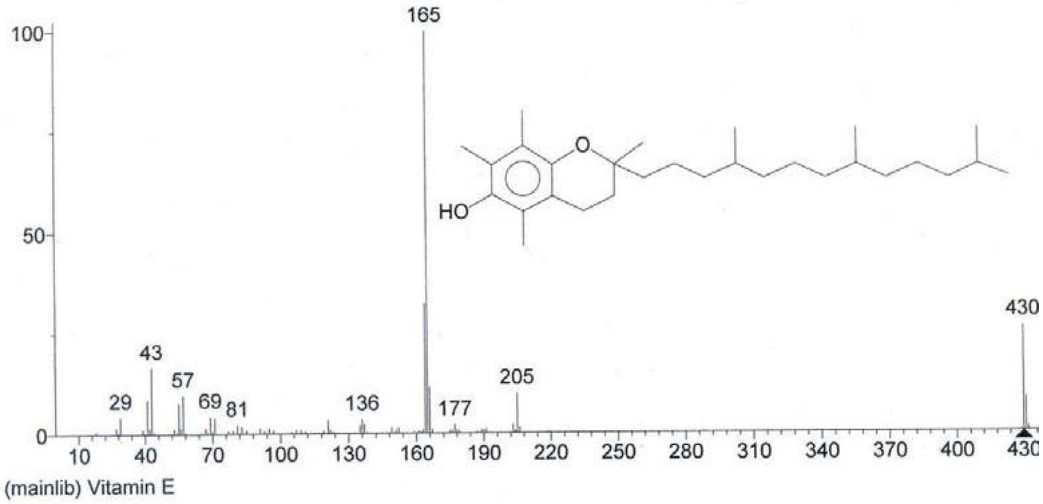

Fig. 3: Mass spectrum of Vitamin E

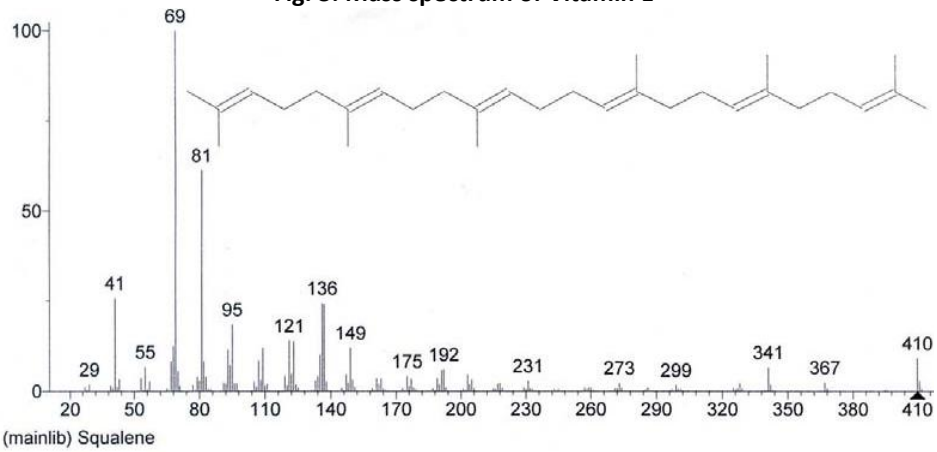

Fig. 4: Mass spectrum of Squalene

ISSN: 0975-8585

ACKNOWLEDGEMENT

We would like to thank Mr. S. Kumaravel, senior scientist, India Institute of Crop Processing Technology (Ministry of Food 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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