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Original Article

GC-MS analysis of ethanol extract of stem of *Nothapodytes nimmoniana* (Graham) Mabb

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ABSTRACT

The investigation was carried out to determine the phytocompounds of ethanol extract of *Nothapodytes nimmoniana* stem. GC-MS analysis of ethanol extract was performed using a Perkin-Elmer GC clarus 500 system and Gas Chromatograph interfaced to a Mass Spectometer (GC-MS) equipped with a Elite-1,fused silica capillary column(30mm×0.25mm 10×1µMdf, composed of 100% Di methyl poly siloxene). Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute standard and Technology (NIST). Fourteen compounds were identified. The prevailing compounds were Acetic acid, 3-hydroxy-6-isopropenyl- 4, 8a-dimethyl-1, 2, 3, 5, 6, 7, 8, 8a- octahydronaphthalen-2-yl ester (30.79%), á-D-Glucopyranose, 4-O-á-D-galactopyranosyl- (23.96%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (9.99%), Dodecanoic acid, 3-hydroxy- (5.93%), Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- (5.64%), Isopropyl Palmitate (4.84%), n-Hexadecanoic acid (4.26%), 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- (3.28%), Tetradecanoic acid (3.29%) and E-2-Tetradecen-1-ol (1.59%).

Key words: *Nothapodytes nimmoniana*, n-Hexadecanoic acid, Phytol, Vitamin E.

INTRODUCTION

Phytochemicals are chemical compounds formed during the plants normal metabolic process. These chemicals are often referred to as "Secondary metabolites"of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [1]. In addition to these substances, plants contain other chemical compounds.

GC-MS is a technique used for screening /identification/ quantification of many susceptible compounds in plant extracts. Gas chromatography (GC) is used to separate drugs that might be present in the sample. The retention time (RT) is an identifying characteristic of a drug. The combination of speed, sensitivity and a high resolving power in gas chromatography provides a very adequate technique for the separation of complex samples. Moreover, the coupling to spectrometric methods such as mass spectrometry (MS) direct identification of unknown compounds is easy to establish [2]. In recent years GC-MS studies have been increasing applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar compounds and essential oil, fatty acid, lipids and alkaloids [3].

Nothapodytes nimmoniana (Graham) Mabb. formerly known as *Nothopodytes foetida* Sleymer and *Mappia foetida* Miers (Icacinaceae), is a small tree, naturally distributed in many parts of the Western Ghats, South India, some parts of the Himalayan foot hills, Srilanka, Myanmar and Thailand [4]. This tree is a rich source of camptothecin, isoquinoline alkaloid, which is currently being used for treating colorectral and ovarian cancer [5,6,7]. The objective of the present study is to identify the possible phytoconstituents present in the ethanol extract of *Nothapodytes nimmoniana* stem using GC-MS study.

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MATERIALS AND METHODS

Collection of plant sample: Stem of *N. nimmoniana* (Graham) Mabb. was collected from Kollankodu, Kanyakumari, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical survey of India, Southern circle, Coimbatore, Tamil Nadu. The voucher specimens preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

Plant sample extraction: Stems were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask and treated with ethanol until the powder is fully immersed. The flask 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 obtained was then subjected to GC- MS analysis.

GC - MS Analysis: GC - MS analysis of stem extracts were performed using a Perkin - Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectometer (GC - MS) equipped with a Elite - 1, fused silica capillary column (30 mm x 0.25 mm 10 x 1 µMdF, composed of 100% Di methyl poly siloxene). For GC - MS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µ 1 was employed (split ratio of 10:1); injector temperature 250^0 C; ion-source temperature 280^0 C. The oven temperature 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 450Da, 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.

Interpretation on mass spectrum GC - MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 paterns. 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 GC-MS chromatogram (Figure-1) showed 18 peaks indicating the presence of eighteen phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the 18 phytoconstituents were characterized and identified, which are listed with their retention time (RT), molecular formula, molecular weight and mass spectrum in table 1. The prevailing compounds were Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester (30.79%), á-D-Glucopyranose, 4-O-á-D-galactopyranosyl- (23.96%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (9.99%), Dodecanoic acid, 3-hydroxy- (5.93%), Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- (5.64%), Isopropyl Palmitate (4.84%), n-Hexadecanoic acid (4.26%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.28%), Tetradecanoic acid (3.29%) and E-2-Tetradecen-1-ol (1.59%). Table 2 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the stem of *N. nimmoniana*.

DISCUSSION

In the present study, 18 compounds have been identified from ethanol extract of the stem of *N. nimmoniana* by Gas Chromatography- Mass spectroscopy (GC-MS) analysis.

Among the identified phytochemical n-Hexadecanoic acid and squalene have the property of antioxidant acid, 9,12-Octadecadienoic acid (Z,Z) have the property of antiinflammatory and antiarthritic as reported by the earlier worker [8]. Recently squalene possesses chemopreventive activity against colon carcinogenesis [9]. Vitamin E is thought to be important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution and immunocompetence, membrane and DNA repair and decreasing oxidative DNA damage [10]. *In vitro* studies showed that vitamin E can prevent oxidation of DNA by inhibiting activated neutrophils. Vitamin E can protect the conjugated double bond of β-carotene from oxidation [11]. 1, 2 Benzenedicarboxylic acid diisoctyl ester also known as diisooctyl phthalate (DIOP). It is reported on Human health Hazard Assessment on DIOP, the bulk of labelled phthalates ingested by humans were eliminated in urine within the first 24 hours and there was no significant tissue accumulation of DIOP [12]. This constituent may therefore not be injurious to health. Phytol was also 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 rheumatoid arthritis and possibly other chronic inflammatory diseases [13]. Phytol was observes to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membrane as a result there is a leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamin E and K1. It is used along with simple or corn syrup as a hardener in candies [14, 15]. Phytol acts as effective adjuvants and also increases the titers of all major Immunoglobulin G (IgG) subclass and is also capable of inducing specific cytotoxic effector T cell responses [16].

The above said components found in the stem of *N. nimmoniana* which are being used for the pharmacological work. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant [17].

CONCLUSION

The current study suggests that 18 active compounds were present in the ethanol extract of *N. nimmoniana* stem. It is concluded that the ethanol can be used for extracting active compounds from plants and incorporating into medicinal/ food products. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity and animal study to evaluate the dosage of the identified chemical compounds.

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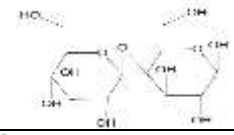
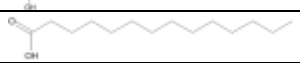
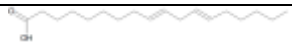
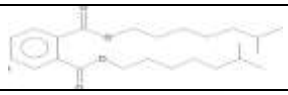
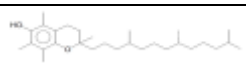
Table 1 : Phytocomponents detected in *N. nimmoniana* Stem

RT	Name of the compound	Molecular Formula	MW	Peak Area %	Structure
10.28	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	5.93	
10.75	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	1.59	
12.08	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	23.96	
12.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.26	
12.44	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.29	
12.59	Isopropyl Palmitate	$C_{19}H_{38}O_2$	298	4.84	
13.83	Phytol	$C_{20}H_{40}O$	296	0.61	
14.42	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.94	
14.49	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	3.28	
19.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	9.99	
20.15	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	0.23	
23.02	Squalene	$C_{30}H_{50}$	410	0.46	
26.28	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$	222	0.23	
27.15	Vitamin E	$C_{29}H_{50}O_2$	430	0.55	

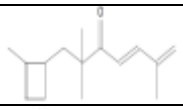
30.35	2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	$C_{15}H_{22}O$	218	1.33	
31.23	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	1.07	
31.99	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	5.64	
33.11	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	278	30.79	

Table.2 Activity of phytocomponents identified in the ethanol extract of *N. nimmoniana* stem

RT	Name of the compound	Molecular Formula	Compound Nature	**Activity
10.28	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	Lauric acid compound	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide
12.08	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	Sugar moiety	Preservative
12.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
12.44	Tetradecanoic acid	C14H28O2	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
13.83	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic
14.42	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
14.49	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	Linolenic acid	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
19.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Plasticizer compound	Antimicrobial Anti-fouling

20.15	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	C9H16O2S	Sulfur compound	Antimicrobial
23.02	Squalene	$C_{30}H_{50}$	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
26.28	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$	Sesquiterpene alcohol	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
27.15	Vitamin E	$C_{29}H_{50}O_2$	Vitamin compound	Antiageing, Analgesic Antidiabetic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
33.11	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	Acetic acid compound	Antimicrobial

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

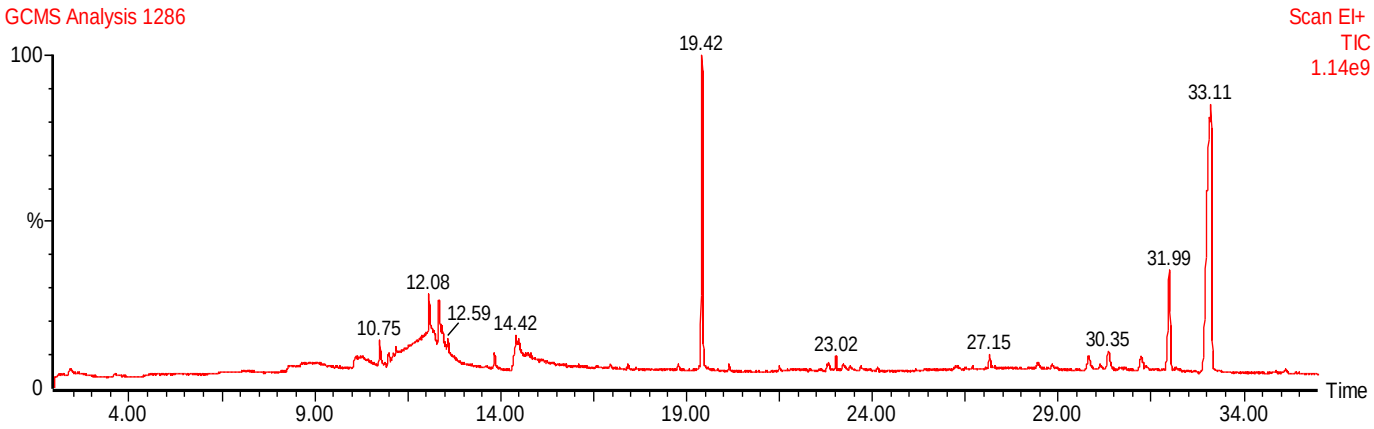


Fig -1: GC-MS chromatogram of the ethanol extract of *N. nimmoniana* stem

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