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GC- MS based chemical profiling of *Alpinia manii* Rhizome – An endemic and endangered plant from Andaman Islands, India

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Abstract
Alpinia manii Roxb. is endangered and endemic to Andaman and Nicobar Islands, India. The present work aimed at phytochemical profiling of fresh rhizome by GC- MS method to find medicinally important constituents. GC- MS by Head- space and n- hexane extract of fresh rhizome was carried out to identify major volatile constituents. A total of 37 compounds were identified by both methods. Terpinoids are the major constituents. Major compounds identified by head space method are, β- Pinene (11.78%), β -Selinene (27.45%), Limonene-6-ol pivalate (15.79%), β Bisabolene (11.36) etc. Phenol 3, 5-bis (1 1-dimethylethyl) (24.74%) and β-Selinene (11.87%) are the major compounds identified in the n-hexane extract. GC- MS analysis of *A. manii* revealed the presence of many valuable and variable bioactive components like β- pinene, caryophyllene, α Humulene, β- Bisabolene etc which have potential role in treating various disease condition.

Keywords: *Alpinia manii*, Zingiberaceae, GC- MS, Head-space, n- Hexane extract

Introduction
Zingiberaceae are the largest family among the order Zingiberales. Members of this family are used as dyes, perfumes, medicines, ornamentals, spices and condiments. *Alpinia* is the largest genus in the family Zingiberaceae with over 230 species worldwide. In India the genus represented by 15 species [1]. Some members of the genus are well known for ethno medicinal importance. *A. calcarata* 'Rasna' in Sanskrit forms a major ingredient for many Ayurvedic medicines. It is used for the treatment of indigestion, impurities of blood, throat inflammation and for voice improvement [2]. *A. galanga* is also used in traditional medicinal systems like Ayurveda, Unani, Chinese and Thai folk medicines [3, 4]. *A. malaccensis* is an active ingredient in cosmetics [5, 6]. Other earlier reports on chemical composition of genus *Alpinia* include, analysis of chemical constituents of essential oil of *Alpinia galanga* [7, 8], identification of volatile constituents from fresh leaves of *A. mutica* [9] and comparative study on rhizome essential oil of *A. galanga* and *A. calcarata* [10].
Alpinia manii is an aromatic medium sized plant which is endangered and endemic to Andaman and Nicobar Islands, India. There are no reports on GC- MS analysis of rhizome of this plant so far. This is the first report on chemical profiling based on GC- MS by head- space and n- hexane extract of fresh rhizome, to find the chemical constituents and its medicinal value.

Materials and Methods
The plants of *A. manii* with rhizome were collected from Saddle peak, Andaman & Nicobar Islands, India (Collection no. 73316) cultivated in Calicut University Botanical Garden and voucher specimens were deposited at CALI. Rhizomes from cultivated plants at CUBG were subjected to GCMS analysis.
Gas chromatographic analysis was performed using GCMS- TQ8030 SHIMADZU. Two grams (2g) of fresh crushed rhizome was transferred to head space vials and incubated at 50° C for 15 minutes. The analyte desorbed into the injector of GC equipped with a MS and a medium polar capillary column Rxi- 5Sil MS, (30m X 0.25mm I. D., 0.25µm). The samples were injected in the splitless mode. The ion energy used for the electron impact ionization (EI) mode was 70 eV. The detector temperature and injection temperature was 250° C, helium was used as the carrier gas with purity 99.999% at a flow rate of 1mL/ min. The oven programme had an initial temperature of 60° C for 2 minutes, increased to 200^0 C for 2 minutes at the rate of 5^0 C/ min followed by the temperature was increased to 220^0 C

For 1 minute at the rate of 3^0 C/ min. Finally temperature was increased to 250^0 C at the rate of 6^0 C/ min for 7 minutes. Total run time was 50 minutes.

For manual injection, cold extract was prepared by about Two grams (2g) of fresh rhizome were crushed and soaked in 25 ml of n- hexane for 24 hrs. 1µL of extract was injected on to a GC equipped with a MS. The equipment and its conditions are same used in both head space analysis and manual injection method.

The essential chemical constituents of both methods were identified by matching mass spectra with spectra of reference compounds in mass spectral library of NIST and WILEY. The relative amounts of individual components were expressed as percent peak areas relative to total peak area. Retention indices were determined by using standard C9-C26 straight chain hydrocarbons.

Results and Discussions

The compounds identified in the *A. manii* rhizome are summarised in Table 1 and the chromatograms provided in Figure 1. Total 37 compounds were identified in both methods. 22 compounds were identified in each head- space method and manual injection method.

Major constituents identified by headspace methods are β pinene (11.78). β bisabolene (11.36%) β Selinene (27.45%), Limonene 6-ol pivalate (15.79%), β elemene(6.75%), Eucalyptol (4.35%), 2-pinene (3.72%), 4(10) Thujene (2.5%), 1- pentadecene (6.2%) etc.

By manual injection method presence of Phenol 3,5-bis(1,1-dimethyl ethyl) (24.74%), Trans 2- tridecenal (8.62%), β Selinene (11.87%). 1-pentadecene (9.67%), 1-nonadecene (9.02%), Alloaromadendrene oxide (7.54%), Octadecane, 1-Chloro (7.76%), 8- heptadecene (3.26%), E14 Hexadecenal (3.96%) were also identified. Presence of β Selinene, 4-Chromanol, E14 Hexadecenal etc. were identified by both methods.

Alpinia manii, characterised by presence of many volatile compounds having potential bioactivities. Compounds β pinene and Caryophyllene have antioxidant, anticancer, antitumour and antimicrobial activities. Whereas α- humulene is anti-inflammatory [11] in action. Limonene-6-ol pivalate possess anti- oxidant and anti- inflammatory activity. β-Bisabolene is anti-ulcer in action. I-(+)- ascorbic acid 2,6-dihexadecanoate having anti- oxidant, cardio protective, cancer preventive, flavour and anti- fertility activities [12].

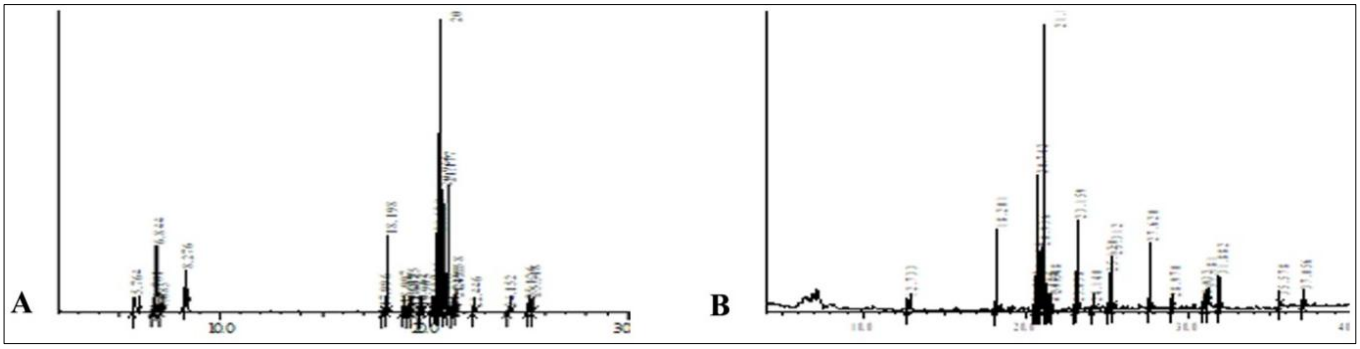

Fig 1: GC- MS chromatogram A) Head- Space B) n- Hexane extract.

Table 1: Compounds identified in Head space and n- Hexane extract coupled with GC – MS Analysis.

S. No.	RI	Compounds	Area percentage (%)	
			HS	Extract
1.	922	2- Pinene	3.72	-
2.	952	4(10)- Thujene	2.5	-
3.	957	β- Pinene	11.78	-
4.	964	β- Myrcene	0.38	-
5.	1002	Eucalyptol	4.35	-
6.	1114	1-Dodecene	-	2.18
7.	1237	β- Elemene	6.75	-
8.	1242	Trans- 2- Tridecenal	-	8.62
9.	1259	Caryophyllene	0.82	-
10.	1263	γ- Elemene	0.41	-
11.	1265	α- Bergamotene	0.16	-
12.	1267	7- Isopropenyl 1,4 dimethyl 1,2,3,4,5,6,7,8 octahydroazulene	0.69	-
13.	1275	β- Bisabolene	11.36	-
14.	1277	α- Humulene	0.36	
15.	1291	8-Isopropyl-1-methyl-5-methylene-1,6 cyclodecadiene	1.15	0.42
16.	1292	1-Pentadecene	6.2	9.67
17.	1295	β- Selinene	27.45	11.87
18.	1299	Octadecane, 1-chloro	-	7.76
19.	1299	Limonene-6-ol pivalate	15.79	-
20.	1304	Phenol 3, 5-bis(1, 1-dimethylethyl)-	-	24.74
21.	1312	Cyclobut(c)indene,1,2,2a,3,4,4a,5,6, octahydro 2,2,4a,8 tetramethyl	1.44	0.52
22.	1313	β- Sesquiphellandrene	1.47	0.93
23.	1334	β- Germacrene	0.21	-
24.	1347	Diethyl phthalate	-	0.89
25.	1373	4- Chromanol	0.51	1.16
26.	1396	8-Heptadecene	-	3.26

27.	1396	3- Hepatadecene (Z)	0.99	-
28.	1400	E14-Hexadecenal	1.14	3.96
29.	1456	1- Nonadecene	-	9.02
30.	1488	Diisobutyl phthalate	-	0.87
31.	1538	Dibutyl phtalate	-	0.79
32.	1544	I-(+)-ascorbic acid 2 6-dihexadecanoate	-	2.51
33.	1664	Bis (2-ethylhexyl)maleate	-	0.72
34.	1698	1- Heptacosonol	-	2.7
35.	1815	Alloaromadendrene oxide	-	2.54
36.	1926	Bis (2-ethylhexyl)phthaleate	-	2.99
37.	2077	1,4, Benzenedicarboxylic acid, 1,4 bis (2-ethylhexyl) ester	-	1.25

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