

## SUPPLEMENTARY MATERIAL

### **Thermal desorption modulation based detection of volatile constituents of *Alpinia galanga* by two dimensional gas chromatography and time of flight mass spectrometry.**

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#### **Abstract**

*Alpinia galanga* Wild.(L.) is well known for its aromatic constituents. Though the aromatic composition is already known, but lots of constituents which contributing overall aroma of the oil are still unknown due to the co-eluting factor of single column in GC-MS. Thus the current study aims to characterise maximum volatile constituents present in the essential oil of *A. galanga* using thermal desorption modulator of two-dimensional gas chromatography and time-of-flight mass spectrometry. The 102 compounds with good match and high probability value were identified out of which 42 were identified for the first time. The total identified compounds include 47 hydrocarbons 25 alcohols, 7 ketones, 7 esters, 3 aldehyde, 4 ethers and 9 other classified aromatic compounds. It was further categorised into Monoterpene Hydrocarbons, Oxygenated Monoterpenes, Sesquiterpene Hydrocarbons and Oxygenated Sesquiterpenes. The major constituent also varies with respect to area percentage. The in-depth characterisation will help in its qualitative analysis.

**Keywords:** *Alpinia galanga*, thermal desorption modulator, GC×GC-TOFMS, essential oil.

## Experimental

### *Essential oil extraction*

Rhizomes of *Alpinia galanga* were collected from the district Khurda, Odisha in the month of January 2018 and was authenticated by Dr P.C Panda, Principal Scientist, (Taxonomy and Conservation Division, RPRC, Bhubaneswar, India) and was deposited in the herbarium of Regional Plant Resource Centre, Bhubaneswar with the voucher specimen no. 10688. Dried rhizomes of *A. galanga* (100 g) was subjected to hydro-distillation for a period of 4 hours using a Clevenger-type apparatus. Essential oil obtained was treated with anhydrous sodium sulfate for trace water removal and stored in amber glass vials at 4°C until further analysis. Oil yield was calculated based on volume of oil divided by fresh weight of sample.

### *GCxGC-TOFMS analysis*

Essential oil of *Alpinia galanga* rhizome was analysed by using two-dimensional gas chromatography and time-of-flight mass spectrometer (GCxGC-TOFMS) of Leco Pegasus 4D Time of Flight Mass Spectrometer comprising Agilent 7890A gas chromatograph equipped with Gerstel automatic multi-sampler, quad jetdual stage thermal modulator, and a secondary oven. The first dimension column used was Rxi-5Sil MS (30mx0.25mm I.Dx0.25µm film thickness) and second dimension column used was Rxi- 17Sil MS (2mx0.25mm I.Dx0. 25µm film thickness). The injection port temperature was set to 290°C and the sample was injected in split less mode with the injection volume of 0.1µl. Helium was used as a carrier gas with the flow rate of 1mL/min.

The primary oven temperature program was kept isothermal at 50°C for 1 min, heated at 5°C/min to 230°C at 5 min hold, finally raised at 15°C/min to 260°C with a 1 min hold. The secondary oven and modulator temperatures were programmed at 10°C and 15°C relative to the primary oven temperature and secondary oven temperature, respectively. The modulation period was 5s with 0.9s hot pulse and 1.6s cool pulse.

During the chromatographic run, the Mass Spectrometer was run in Electron Ionization mode with the ionization voltage of 70eV and spectra were recorded in the mass range of 40-700 with the acquisition rate of 100 spectra/s at acquisition voltage of 1600V. Solvent delay of 300s was given. The ion source and transfer line temperature was 250°C and 280°C, respectively.

ChromaTOF software (LECO Corporation) was used for data acquisition and processing. Manual peak identification was done using the contour plots. Individual compound identification was done using in house NIST library. The linear temperature programmed retention index (LTPRI) were calculated using homologous n-alkane (C<sub>8</sub>–C<sub>20</sub>) series (Adams 2007; Quijano et al. 2007) as external references. LTPRI was determined by Van den Dooland Kratz equation. The minimum similarity of 80% (800/1000) with the library was the criteria to identify the compound.

TABLE

Table S1. Volatile compounds identified in the rhizome oil of *Alpinia galanga* using GCxGC-TOF-MS.

No	Compound	$t^1_{R(m)}$	$t^2_{R(s)}$	LTPRI <sub>exp</sub> <sup>a</sup>	LTPRI <sub>exp</sub> <sup>b</sup>	Ref RI <sub>lit</sub> <sup>c</sup>	Compounds reported first time (cross marked )	Percentage in oil (%) <sup>e</sup>
	<b>Monoterpenic compounds</b> <i>Hydrocarbon-type</i>							
1	Tricyclene	6.16	2.72	923	921	Adams (2007)		1.32
2	$\alpha$ -Pinene	7.1	2.36	930	932	Adams (2007)		1.76
3	$\alpha$ - Fenchene	7.1	2.91	940	945	Adams (2007)	×	0.87
4	Camphene	7.41	2.72	948	946	Adams (2007)		0.48
5	Verbenene	7.83	3.46	956	961	Adams (2007)	×	0.15
6	Sabinene	8.03	2.78	970	969	Adams (2007)		1.35
7	$\beta$ -Pinene	8.61	2.86	979	974	Adams (2007)		2.44
8	$\alpha$ -Myrcene	8.5	2.8	987	988	Adams (2007)		0.26
9	$\alpha$ -Phellandrene	9.08	2.86	1000	1002	Adams (2007)		0.12
10	$\delta$ 3-carene	9.08	3.16	1006	1008	Adams (2007)	×	0.27
11	$\alpha$ -Terpinene	9.03	2.9	1008	1014	Adams (2007)		0.33
12	$\rho$ -Cymene	9.5	3.3	1021	1020	Adams (2007)		1.80
13	Limonene	9.75	2.88	1022	1024	Adams (2007)		4.26
14	$\beta$ - ocimene	9.83	3.45	1040	1044	Adams (2007)		0.10
15	$\gamma$ -Terpinene	10.5	3.17	1049	1054	Adams (2007)		0.68
16	$\rho$ -mentha-3,8-diene	10.91	3.53	1066	1068	Adams (2007)	×	0.22
17	Terpinolene	11.33	3.74	1082	1086	Adams (2007)		0.27
	<i>Aldehyde-type</i>							
18	$\alpha$ -Campholenal	12.5	3.86	1119	1122	Adams (2007)	×	0.33
19	Cumin aldehyde	15.83	2.36	1230	1238	Adams (2007)	×	0.1
20	(E)-o-Methoxy cinnamaldehyde	23.1	3.41	1526	1527	Adams (2007)	×	0.22
	<i>Ketone-type</i>							
21	Camphenilone	11.25	4.42	1079	1078	Adams (2007)	×	0.28

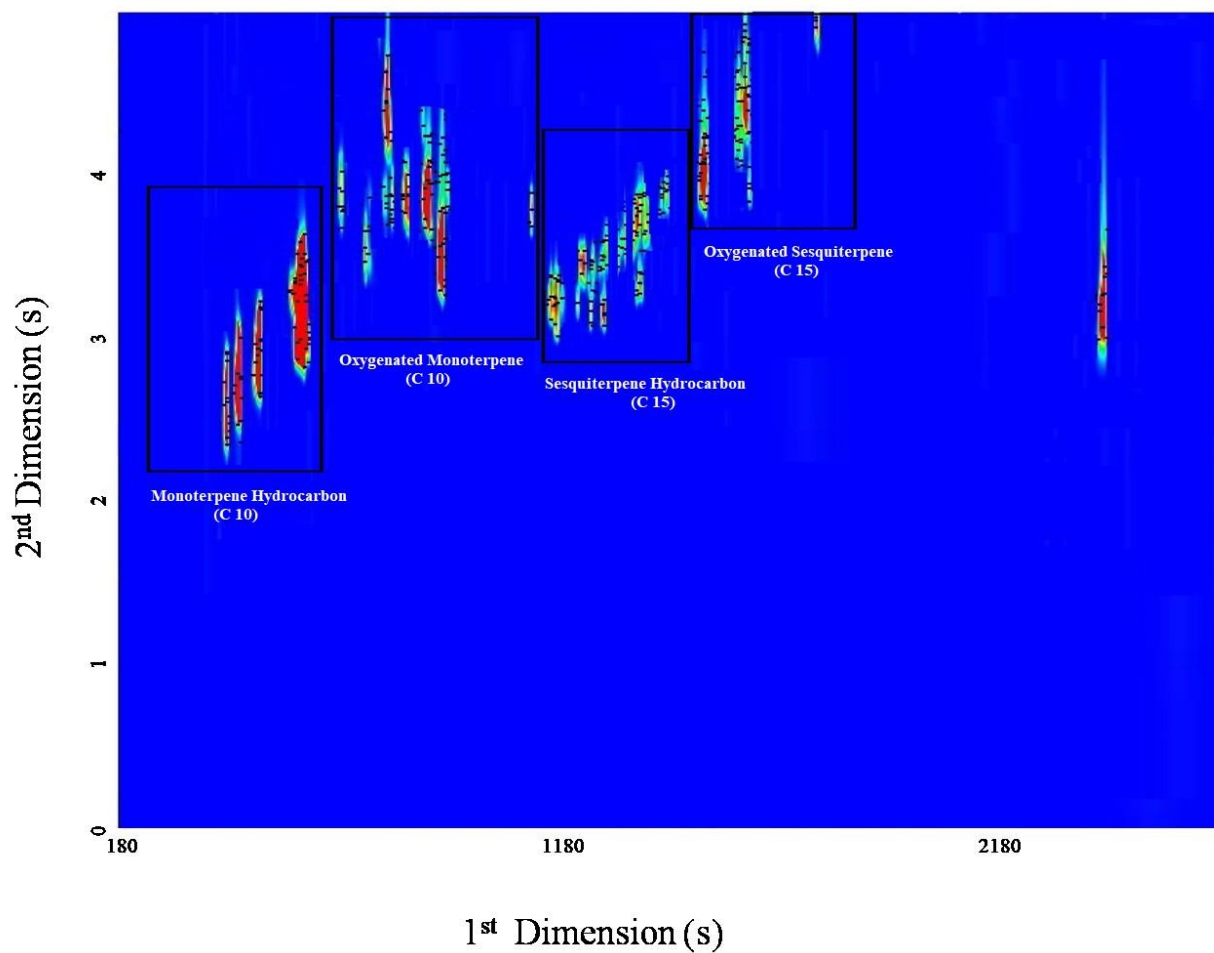
22	Fenchone	11.33	3.23	1080	1083	Adams (2007)		0.76
23	Camphenone	11.53	4.15	1094	1095	Adams (2007)	×	0.26
24	(+)-Nopinone	12.83	4.88	1134	1135	Adams (2007)	×	0.38
25	Camphor	13.08	4.45	1143	1141	Adams (2007)		4.44
26	Pinocarvone	13.5	4.48	1159	1160	Adams (2007)		0.53
27	(-)-Carvone	15.83	4.64	1238	1239	Adams (2007)		0.35
	<b><i>Ether-type</i></b>							
28	Dehydrocineole	8.58	3.12	989	988	Adams (2007)	×	0.26
29	Eucalyptol	9.75	3.23	1026	1026	Adams (2007)		6.70
30	Thymol, methyl ether	15.5	4	1228	1232	Adams (2007)		0.44
	<b><i>Alcohol- type</i></b>							
31	Linalool	11.66	3.29	1093	1095	Adams (2007)		1.9
32	exo-Fenchol	12	3.02	1116	1118	Adams (2007)		2.1
33	trans sabinol	13.08	3.87	1134	1137	Adams (2007)	×	0.73
34	Camphene hydrate	13.33	3.87	1146	1145	Adams (2007)	×	0.48
35	Borneol	13.91	3.88	1165	1165	Adams (2007)		2.58
36	Nonanol	13.91	4.38	1167	1165	Adams (2007)	×	0.30
37	Terpinen-4-ol	14.16	3.68	1169	1174	Adams (2007)		0.47
38	p-Cymen-8-ol	14.5	4.3	1181	1179	Adams (2007)		0.70
39	$\alpha$ -Terpineol	14.58	3.92	1186	1186	Adams (2007)		2.43
40	Nerol	15.25	3.54	1220	1227	Adams (2007)	×	0.21
41	Geraniol	16.58	4.94	1250	1249	Adams (2007)		0.15
	<b><i>Ester-type</i></b>							
42	2-Methylbutyl isovalerate	11.83	3	1100	1103	Adams (2007)	×	0.28
43	Fenchyl acetate	15.08	3.5	1218	1218	Adams (2007)		1.71
44	Bornyl acetate	16.91	3.75	1252	1254	Adams (2007)		0.33
45	$\alpha$ -Terpinyl acetate	18.58	3.79	1345	1346	Adams (2007)		0.73
46	Thymol acetate	18.66	3.2	1346	1349	Adams (2007)		0.38
47	Methyl cinnamate <(E)->	19.33	3.26	1378	1376	Adams (2007)		0.29
48	Geranyl acetate	19.5	3.37	1380	1379	Adams (2007)		0.80
	<b><i>Others</i></b>							
49	Benzaldehyde	7.5	2.79	951	952	Adams (2007)	×	1.23

50	Ethyl-(3E)-hexenoate	9.08	3.16	1002	1017	Adams (2007)	×	0.27
51	(E)-Methyl isoeugenol	22.16	3.61	1490	1491	Adams (2007)	×	0.48
52	Dihydro Eugenol acetate	23.41	3.2	1535	1536	Adams (2007)		0.16
	<b>Sesquiterpenic Compound</b> <i>Hydrocarbon type</i>							
53	$\alpha$ -Copaene	19.25	3.24	1372	1374	Adams (2007)		0.65
54	$\beta$ -Patchoulene	19.41	3.2	1374	1379	Adams (2007)		0.42
55	Daucene	19.5	3.25	1381	1380	Adams (2007)	×	0.22
56	$\beta$ -Cubebene	19.85	3.22	1382	1387	Adams (2007)	×	0.26
57	$\beta$ -elemene	19.75	3.25	1390	1389	Adams (2007)		0.14
58	$\alpha$ -Gurjunene	19.91	3.2	1410	1409	Adams (2007)		0.53
59	cis- $\alpha$ -Bergamotene	20.3	3.22	1414	1411	Adams (2007)	×	0.52
60	E-Caryophyllene	20.75	3.34	1417	1417	Adams (2007)		0.14
61	trans- $\alpha$ -Bergamotene	20.83	3.17	1430	1432	Adams (2007)		0.89
62	$\beta$ -Humulene	21.16	3.42	1435	1436	Adams (2007)	×	0.38
63	$\alpha$ -Guaiene	21.25	3.48	1436	1437	Adams (2007)		0.19
64	(Z)- $\beta$ -Farnesene	21..33	3.15	1439	1440	Adams (2007)		0.61
65	Germacrene D	21.33	3.5	1480	1484	Adams (2007)		1.00
66	$\gamma$ -Curcumene	22	3.61	1484	1481	Adams (2007)	×	0.21
67	$\alpha$ -Selinene	22.25	3.63	1499	1498	Adams (2007)		0.74
68	$\beta$ -Himachalene	22.5	3.66	1500	1500	Adams (2007)	×	0.12
69	Isodaucene	22.58	3.75	1502	1500	Adams (2007)	×	0.31
70	$\alpha$ -Farnesene	22.58	3.27	1507	1505	Adams (2007)		0.42
71	$\beta$ -Bisabolene	22.66	3.34	1509	1505	Adams (2007)		0.14
72	$\beta$ -Curcumene	22.75	3.4	1515	1514	Adams (2007)	×	0.19
73	(Z)- $\gamma$ -Bisabolene	22.83	3.89	1517	1514	Adams (2007)	×	0.12
74	Cubebol	22.91	3.66	1519	1514	Adams (2007)	×	1.32
75	7 epi-a-Selinene	23	3.76	1521	1520	Adams (2007)		0.40
76	Calamenene	23	4.01	1524	1521	Adams (2007)	×	0.14
77	$\beta$ -Sesquiphellandrene	23.08	3.43	1527	1521	Adams (2007)		0.15
78	(E)- $\gamma$ -Bisabolene	23.1	3.82	1530	1529	Adams (2007)	×	0.11
79	Dauca-4(11),8-diene	23.25	3.68	1532	1530	Adams (2007)	×	0.13

80	$\alpha$ -Calacorene	23.5	4.26	1540	1544	Adams (2007)	×	0.15
81	Selina-3,7(11)-diene	23.58	3.77	1544	1545	Adams (2007)	×	0.16
82	$\beta$ -Germacrene	24	3.88	1560	1559	Adams (2007)		0.26
	<b><i>Alcohol-type</i></b>							
83	Elemol	23.66	3.9	1546	1548	Adams (2007)		0.27
84	Nerolidol	24	3.45	1555	1561	Adams (2007)		0.37
85	Viridiflorol	25	4.07	1590	1592	Adams (2007)		0.41
86	Carotol	25.08	4.08	1591	1594	Adams (2007)		4.71
87	Guaiol	25.16	4.07	1598	1600	Adams (2007)		1.25
88	5-epi-7-epi- $\alpha$ -Eudesmol	25.33	3.25	1601	1607	Adams (2007)	×	0.58
89	Isolongifolan-7 $\alpha$ -ol	25.5	4.13	1615	1618	Adams (2007)	×	0.11
90	$\beta$ -Eudesmol	26.25	4.27	1645	1649	Adams (2007)		1.99
91	Pogostol	26.33	4.28	1648	1651	Adams (2007)	×	0.41
92	Intermedeol	26.41	4.55	1660	1665	Adams (2007)	×	0.50
93	$\alpha$ -Bisabolol	26.83	3.92	1682	1685	Adams (2007)		0.54
94	(Z)- $\alpha$ -trans- Bergamotol,	27.66	3.99	1692	1690	Adams (2007)		0.14
95	trans-Farnesol	28	3.61	1700	1698	Adams (2007)		0.18
96	2E,6E-Farnesol	29.33	4.96	1745	1742	Adams (2007)		0.62
	<b><i>Ether-type</i></b>							
97	Daucol	26	4.33	1639	1641	Adams (2007)	×	1.45
	<b><i>Others</i></b>							
98	Caryophyllene oxide	24.5	4.31	1580	1582	Adams (2007)		0.16
99	n-Hexadecanoic acid	32.5	3.32	1956	1959	Adams (2007)		0.19
100	(Z)-Isoeugenyl benzyl ether	38.83	3.38	2075	2072	Adams (2007)	×	0.66
101	(E)-Isoeugenyl benzyl ether	37.5	2.36	2130	2125	Adams (2007)	×	0.53
102	Linoleic acid	40.25	3.28	2139	2132	Adams (2007)	×	1.02

**Note:** t1R(min): first dimension retention time in minutes, t2R(s): second dimension retention time in seconds. a: Retention index obtained through the modulated chromatogram. b: Retention index reported for 5% phenyl polysilphenylene-siloxane GC column or equivalents reported in the literature. c: Retention index found in the literature for 5% phenyl polysilphenylene-siloxane GC column or equivalents. d: Terpenic constituents previously reported in the literature in the oil of *A. galanga*: Pooter et al. 1985; Charles et al. 1992; Kubota et al. 1999; Raina et al. 2002; Mallavarapu et al. 2002; Jirovetz et al. 2003; Menon 2006; Tonwitawat 2007; De Padalia et al. 2010; Raj et al. 2012; Raina et al. 2014; Wu et al. 2014; Abdullah et al. 2015; Nampoothiri et al. 2016. Cross mark (×) represents not identified earlier as per literature. e: Percentage of each component in the oil is calculated as peak area of analyte divided by total peak area.

## FIGURES

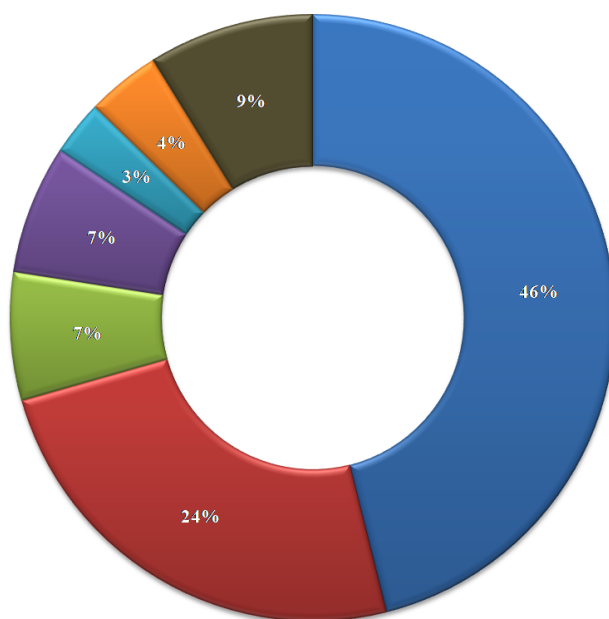


**Fig S1-** GCxGC-TOF-MS extracted ion chromatogram contour plot of chromatographic spaces corresponding to mono (C10) and sesquiterpenic compounds (C15) of *A. galanga* are highlighted.

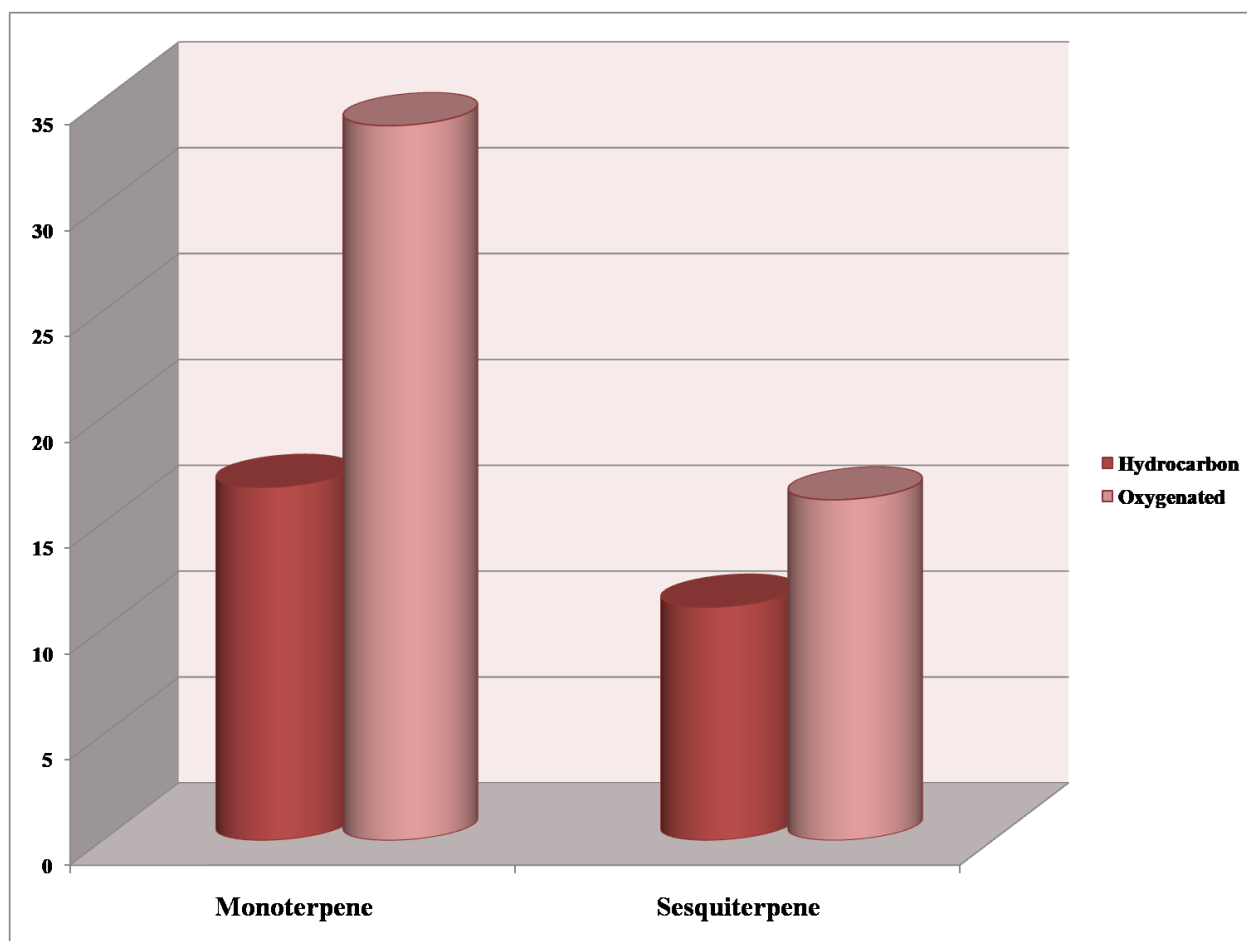


## CLASSIFICATION

■ Hydrocarbons ■ Alcohols ■ Ketones ■ Esters ■ Aldehydes ■ Ethers ■ Others



**Fig S2-** Classification of 102 constituents based on their different derivatives.



**Fig S3**-Classification of 102 constituents on the basis of class of terpenes and their area percentage.

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