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Molecular Composition For Identification Of Bioactive Compounds Against Human Breast Cancer MCF-7 Of *Acmella Calva* (DC) R. K. Jansen

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ABSTRACT
Acmella calva (DC) R. K. Jansen is a medicinal herb widely used by different communities as a traditional cuisine and food ingredient. Different parts of this toothache plant are used as medicine to treat stomatitis, mouth ulcers, and characteristically for toothaches. The current investigation was carried out in search of bioactive compounds of plant parts (stem, leaves, and inflorescence) extracted in ethanol. The elements identified were specifically Caryophyllene 13.45%, Neophytadiene 21.08%, and N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide 25.30 % and peak area was found as a major compound in stem, leaf, and inflorescence respectively by GC-MS. The anticancer activity was evaluated against MCF-7 Human breast cancer cell line at the doses of 10 to 100 µg/ml extract for stem, leaves, and inflorescence. A significant reduction in mobility of the cell line was demonstrated by the stem, leaves, and inflorescence as an exposure of MCF7. The bioactive compounds collectively act on cancer cells to exert significant cytotoxicity and apoptotic activity on the MCF-7 cell line. It was drastically reduce the mobility of cells, elucidating *A. calva* as a promising agent against MCF7 that can be further purified and utilized in anticancer studies.

Key words- *A. calva,* Bioactive compounds, Anticancer, cytotoxicity

Introduction

The medicinal herbs are the major source of therapeutic remedies for the treatment of chronic human diseases including cancer, diabetes, cardiovascular complications and many more. Nowadays, plant secondary metabolites play a crucial role in drug discovery for life-threatening diseases [1]. Natural drugs derived from herbal medicinal plants are considered as effective and safe compared to modern chemical drugs, considering their long history of use by its humans as a medicine and food [2].

A. calva (DC.) R. K. Jansen is a southeast Asian indigenous to tropical and sub-tropical perennial herb around the world. It is found to be associated with swap, damp, and roadside weed [3]. The leaves and flowers of this medicinal herb have a pungent taste and are accompanied by a tingling

sensation and numbness when it come in contact with the skin and tongue [4]. It also possesses antidepressant, anti-diarrheal, analgesic, and hypoglycemic properties [5]. Ranjan and Puthur (2022) [6] isolated toxicological compounds from *A. calva* and *Xanthimum strumarium*. The different extracts of *A. calva* are known to have potent medicinal values to treat and cure stammering in children, leucorrhoea, flu, cough, rabies, and tuberculosis [7]. The leaves are used in articular rheumatism and as a remedial agent for snakebite [8]. It has also been used as local anaesthetic purpose [9]. Flowers are known to have antifungal properties [10], fast-acting muscle relaxant and antiwrinkle agent [11,12], antibacterials and antimalarials [13]. The flower is mainly used for toothache and dysentery [14], and whole plant extracts are used as bio-insecticides [15,16], A study carried out by Revathi and Parimelazhghan (2010) [17] has found that *A. calva* is helpful in cases of tuberculosis. Similarly, other studies have found that test plant could be used as digestive [18,19] and sold as medicinal species [20]. Flowers chewed for toothache in Nagaland, and also used against snakebites and as appetizer [21].

Breast cancer is one of the most popular cancer types observed worldwide in women, accounting for the highest number of deaths related to cancer [22]. Currently, chemotherapy is the preferred method, and combined with surgical operations has serious side effects that reduce patients' quality life [23]. There is a need to develop direct integrative and novel therapies that will lead to improve disease outcome and quality life of patients [24]. The advancement in research on the bioactive chemicals of herbal remedies products proved that plant secondary metabolites are used as the most prevalent source for the treatment of cancer. To overcome synthetic drug therapeutic molecules, the natural source is being bioremedial and most effective in cancer treatment [25]. Till date, no reports were found on the anticancer activity and molecular characterization of *A. calva* and hence, the present investigation focused on the identification of different secondary metabolites by GC-MS analysis of the stem, leaves, and inflorescence exacts of *A. calva* and further, it was evaluated for cytotoxicity effects against MCF-7 cell lines.

Materials and methods

1. Pant material

The healthy and fresh plant of *A. calva* was collected from the Kolhapur (MS.) India. Collected plant parts were separated as stem, leaves, and inflorescence and washed with tap water and shade dried at room temperature (32 ± 2^0c). The samples were grounded using the mechanical grinder and stored for further use.

2. Extraction

The grounded powder of stem, leaves, and inflorescence were used for extraction. 10g of powdered samples were taken for extraction using ethanol (100ml) as a solvent in Soxhlet assembly to obtain 10% w/v concentration until complete extraction.

3. GC-MS analysis

The ethanolic extracts of the stem, leaves, and inflorescence of the test plant were used for the study of molecular constituents using GC-MS (TQ8050). The method adopted for GC-MS analysis was conducted as the instrument was built with a capillary column of 30 m length, 0.25 mm diameter, 0.25 µm film thickness, column oven temperature 50 ^{0}C, and injection temperature 250.0 ^{0}C, column flow rate 1.01mL/min. The chemical constituents were identified by matching the mass spectra and retention periods with the spectra from NBS/NIST and Wiley Libraries.

4. MTT assay for anticancer activity

We acquired MCF-7 breast cancer cell lines from NCCS in Pune, India. All cell lines were cultured and kept alive in DMEM (E) supplemented with NEAA and FBS, which was an appropriate medium.

After being trypsinated, all cells were subjected to five different doses (10, 20, 40, 80, and 100 µg/ml) of plant extracts of *A. calva.* These were incubated for eight hours, the 96-well plates containing material solutions and a cell culture with 1X 103 cells per well in a CO_2 incubator at 37°C. Following the medication exposure period, 20 µL of MTT solution was added to each well and the aluminium foil-wrapped plates, plates were then incubated for a further 4 hours at 37 °C in a CO_2 incubator. The culture medium was taken out, and added the MTT-formazan crystals in plates allowed to dissolved in the wells.

The Cell viability for extracts exposure by MTT assay was calculated as,

$$\text{Viability (\%)} = \frac{\text{Mean OD of test material}}{\text{Mean OD of control}} \times 100$$

Result and discussion

1. GC-MS analysis

In the present investigation, molecular characterization of ethanolic extracts of stem, leaves, and inflorescence were screened using the GC-MS analysis method for the first time of *A. calva.* The detailed composition of identified phytochemicals along with their details is presented in Table no. 1, 2, and 3 of stem, leaves, and inflorescence respectively. The obtained chromatograms by GC-MS of different parts of *A. calva* is presented in fig. no. 1, 2, and 3 accordingly. The result of GC-MS analysis showed the presence of 42 different types of compounds in three parts of test plant. Among them, 6 compounds found in all three parts of *A. calva* viz. beta.-Bisabolene, Caryophyllene, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-, Dibutyl phthalate, Humulene, and Neophytadiene. Three compounds commonly found in stem and inflorescence viz. (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene, and 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha .,7.beta.,7a.beta.,7b.alpha.)]- , and Silane, diethoxydimethyl-. One compound found in both the leaves and inflorescence 1,8,11-Heptadecatriene, (Z,Z)- rest of the 33 different types of compounds characteristically found specifically either only in stem, leaves, or inflorescence. The key elements identified were specifically Caryophyllen 13.45%, Neophytadiene 21.08%, and N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide 25.30 % known as spilanthol which is characteristic compound of the genus *Spilanthus* and *Acmella* with the highest peak area found as a major compound in stem, leaves, and inflorescence respectively. The maximum number of bioactive compounds were found to be souluble in ethanolic extract of individual plants [26,27,28,29,30].

2. MTT assay for cell cytotoxicity

The cytotoxicity of stem, leaves, and inflorescence extracts of *A. calva* were assessed by screening cell viability (%) by using MTT assay. The five different concentrations of test extracts viz. 10, 20, 40, 80, and 100 µg/ml were taken under consideration. The stem extract exhibited significantly preferential targeting high toxicity on MCF7 cells, 10 µg/ml showed 56.847± 8.512 %; 20 µg/ml showed 47.667 ± 2.147 %; 40 µg/ml showed 46.155 ± 1.753 %; 80 µg/ml showed 37.538 ± 11.1 90 % and 100 µg/ml showed 34.425 ± 1.197 % cell viability p=0.000. Corresponding to leaves 54.315 ± 2.808> 53.067 ± 0.775> 49.358 ± 0.979 > 49.156 ± 0.701> 39.749 ± 0.899 % cell viability of 10, 20, 40, 80, and 100 µg/ml concentration respectively. Whereas 95.576 ± 1.047 % of 10 µg/ml; 93.273 ± 6.994 % of 20 µg/ml; 81.681 ± 0.953 % of 40 µg/ml; 62.947 ± 6.244 % of 80 µg/ml and 57.405 ± 3.209 % of 100 µg/ml recorded for inflorescence extract Table no. 4. However, in the control cytotoxic effects was found absent. In general, cytotoxic effect of stem,

leaves, and inflorescence extract was found to be enhanced with an increase in the concentration of ethanolic extract. The IC_{50} values for these three extracts was also determined as 25.13, 50.89, and 110 µg/ml respectively against MCF-7 breast cancer cell line.

Overall, Stem, Leaves, and Inflorescences extract consist of a concentration-dependent decrease in MCF7 cell viability. A statistically positive significant cytotoxicity was shown by 100 µg/ml concentration of Stem compared to leaves and the lowest of inflorescence. The toxic activity of stem, leaves, and inflorescences on MCF7 at higher concentrations compared to control reveals the cytotoxic efficacy of *A. calva* as an anticancer plant.

The bioactive compound β-bisabolene found in all three parts were of *A. calva* effective in reducing the growth of transplanted 4T1 mammary tumor in vivo and said to be have tumor -specific pro apoptotic properties [31]. Similarly, MCF7 cell line significantly arrested at G2/M phase by Caryophyllene oxide and it showed apoptotic profile elevated early and late apoptosis and necrosis [32]. Neophytadiene the major compound observed in leaves with 21.08% peak area it inhibits the three receptors that are essential for the survival of cancer: the human LRH1 receptor, the human A2a receptor at the adenosine binding site, and the hERG K+ channel. These prevent cancer from proliferating and spreading, and they may also serve as a viable apoptotic inducer in silico studies [33].

Conclusion

In conclusion, our data revealed that *A. calva* could be induce cytotoxic effect cell death via apoptosis. Stem, leaves, and inflorescence extracts could be used as a potential alternative for the development of bioactive leads in the treatment of cancer. *A. calva* is a promising agent against MCF7 that can be further purified and used in anticancer studies because of the bioactive compounds combined effects on cancer cells, which significantly reduce cell motility and exert cytotoxicity and apoptotic activity on the MCF7 cell line. Further, extensive investigations are required to isolate and purify the bioactive compounds from *A. calva* to evaluate responsibilities for management and drug development in therapeutic actions.

Table no. 1: Bioactive compound detected from stem ethanolic extract of *A. calva*

Peak	R.Time	Area%	Name	Formula	Mole. Weight
1	16.749	5.47	2,6-Octadiene, 2,6-dimethyl-	$C_{10}H_{18}$	138
2	20.188	2.47	(1R,3aS,5aS,8aR)-1,3a,4,5a-Tetramethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene	$C_{15}H_{24}$	204
3	20.855	13.45	Caryophyllene	$C_{15}H_{24}$	204
4	21.415	10.14	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene	$C_{15}H_{24}$	204
5	21.803	2.02	Humulene	$C_{15}H_{24}$	204
6	22.264	5.44	cis-7-Tetradecen-1-ol	$C_{14}H_{28}O$	212
7	22.600	6.89	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	204
8	22.750	7.33	n-Pentadecanol	$C_{15}H_{32}O$	228

9	23.055	0.96	1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-,[1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	$C_{15}H_{24}$	204
10	23.193	12.75	.beta.-Bisabolene	$C_{15}H_{24}$	204
11	26.493	7.74	.tau.-Cadinol	$C_{15}H_{26}O$	222
12	26.758	1.22	1,8,11-Heptadecatriene, (Z,Z)-	$C_{17}H_{30}$	234
13	30.982	2.98	Neophytadiene	$C_{20}H_{38}$	278
14	34.058	13.78	Dibutyl phthalate	$C_{16}H_{22}O_4$	278
15	36.692	7.34	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	$C_{12}H_{38}O_5Si_6$	430

Table no. 2: Bioactive compound detected from leaves ethanolic extract of *A. calva*

Peak	R.Time	Area%	Name	Formula	Mol. weight
1	3.158	1.67	Silane, diethoxydimethyl-	$C_6H_{16}O_2Si$	148
2	20.202	1.09	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	$C_{15}H_{24}$	204
3	21.004	16.44	Caryophyllene	$C_{15}H_{24}$	204
4	21.928	1.46	Humulene	$C_{15}H_{24}$	204
5	22.398	0.77	.gamma.-Muurolene	$C_{15}H_{24}$	204
6	22.571	13.33	.beta.-copaene	$C_{15}H_{24}$	204
7	22.933	1.04	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	$C_{15}H_{24}$	204
8	23.208	1.91	.beta.-Bisabolene	$C_{15}H_{24}$	204
9	23.602	1.57	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-	$C_{15}H_{24}$	204
10	24.739	0.60	(1S,3aR,4R,8R,8aS)-1-Isopropyl-3a-methyl-7-methylenedecahydro-4,8-epoxyazulene	$C_{15}H_{24}O$	220
11	25.092	10.78	Caryophyllene oxide	$C_{15}H_{24}O$	220
12	25.344	-0.50	Salvial-4(14)-en-1-one	$C_{15}H_{24}O$	220
13	26.314	1.12	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}O$	220
14	30.993	21.08	Neophytadiene	$C_{20}H_{38}$	278
15	32.099	5.87	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
16	34.063	19.34	Dibutyl phthalate	$C_{16}H_{22}O_4$	278

17	35.535	1.14	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-,(E,E)-	$C_{20}H_{34}O$	290
18	44.880	1.30	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390

Table no. 3: Bioactive compound detected from inflorescence ethanolic extract of *A. calva*

Peak	R.Time	Area%	Name	Formula	Mole weight
1	3.156	0.75	Silane, diethoxydimethyl-	$C_6H_{16}O_2Si$	148
2	20.209	2.02	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	$C_{15}H_{24}$	204
3	21.007	6.70	Caryophyllene	$C_{15}H_{24}$	204
4	21.304	0.54	cis-.alpha.-Bergamotene	$C_{15}H_{24}$	204
5	21.930	0.65	Humulene	$C_{15}H_{24}$	204
6	22.588	12.49	Germacrene D	$C_{15}H_{24}$	204
7	22.936	-0.81	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	$C_{15}H_{24}$	204
8	23.202	2.81	.beta.-Bisabolene	$C_{15}H_{24}$	204
9	23.600	29.89	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-	$C_{15}H_{24}$	204
10	25.025	0.90	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}O$	220
11	26.770	0.30	1,8,11-Heptadecatriene, (Z,Z)-	$C_{17}H_{30}$	234
12	31.002	1.98	Neophytadiene	$C_{20}H_{38}$	278
13	32.100	0.61	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
14	34.062	4.37	Dibutyl phthalate	$C_{16}H_{22}O_4$	278
15	39.299	25.30	N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	$C_{16}H_{25}NO$	247
16	40.904	3.73	Tetracontane	$C_{40}H_{82}$	562
17	44.283	6.29	Dotriacontane	$C_{32}H_{66}$	450
18	44.879	-0.01	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390
19	48.009	1.47	Tetracosane	$C_{24}H_{50}$	338

Table no. 4. Cytotoxicity (cell viability %) by MTT assay

Sr. no	Doses (µg/ml)	Stem		leaves		Inflorescence	
		Absorbance (OD)	Cell viability (%)	Absorbance (OD)	Cell viability (%)	Absorbance (OD)	Cell viability (%)
1	Control	1.177 ± 0.006^c		1.177 ±		1.177 ±	

				0.006^{d}		0.006^{c}	
2	**10**	0.668±0.098^{b}	56.847± 8.512^{b}	0.639±0.035^{c}	54.315 ± 2.808^{c}	1.125 ± 0.016^{c}	95.576 ± 1.047^{b}
3	**20**	0.561±0.027^{ab}	47.667 ± 2.147^{ab}	0.624±0.006^{bc}	53.067 ± 0.775^{bc}	1.098 ± 0.088^{bc}	93.273 ± 6.994^{b}
4	**40**	0.543±0.020^{ab}	46.155 ± 1.753^{ab}	0.581±0.009^{b}	49.358 ± 0.979^{b}	0.961 ± 0.007^{b}	81.681 ± 0.953^{b}
5	**80**	0.443±0.134^{ab}	37.538 ± 11.190^{ab}	0.578± 0.006^{b}	49.156 ± 0.701^{b}	0.740 ± 0.072^{a}	62.947 ± 6.244^{a}
6	**100**	0.405±0.013^{a}	34.425 ± 1.197^{a}	0.468 ± 0.010^{a}	39.749 ± 0.899^{a}	0.676 ± 0.039^{a}	57.405 ± 3.209^{a}
IC_{50} (µg/ml)		25.13		50.89		110.59	
P-value		0.000	0.00	0.000	0.000	0.000	0.000
S.E. (Mean)		0.0669	3.2266	0.0560	1.4732	0.0497	4.4829
S.E. (diff)		0.0980	9.0948	0.0225	2.0733	0.0703	6.3310
C.D. at 5%		0.2136	20.2644	0.0490	4.6196	0.1531	14.1062
CV %		44.8189	28.0652	35.0429	11.6139	21.8979	22.2091

Fig. 1. GC-MS chromatogram of stem ethanolic extract of *A. calva*

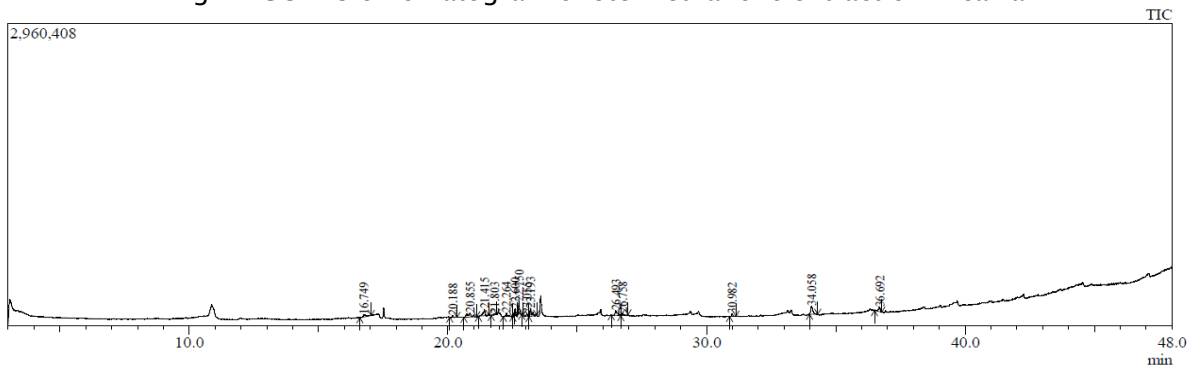


Fig. 2. GC-MS chromatogram of leaves ethanolic extract from *A. calva*

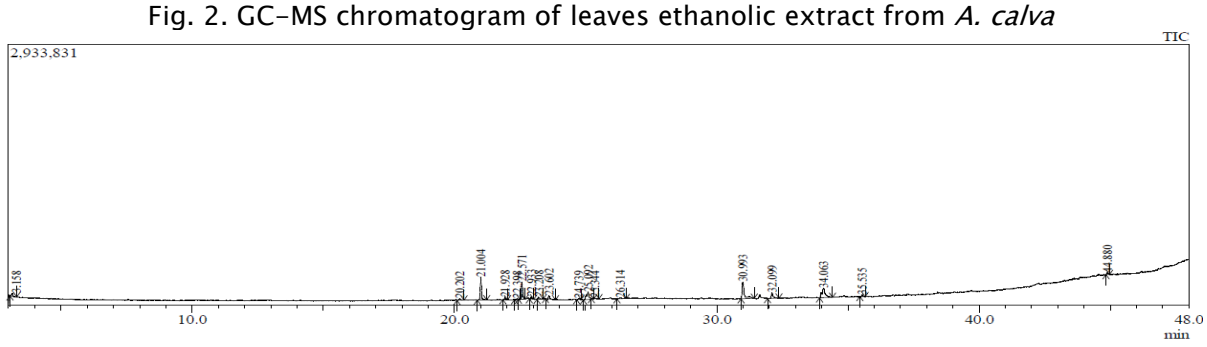
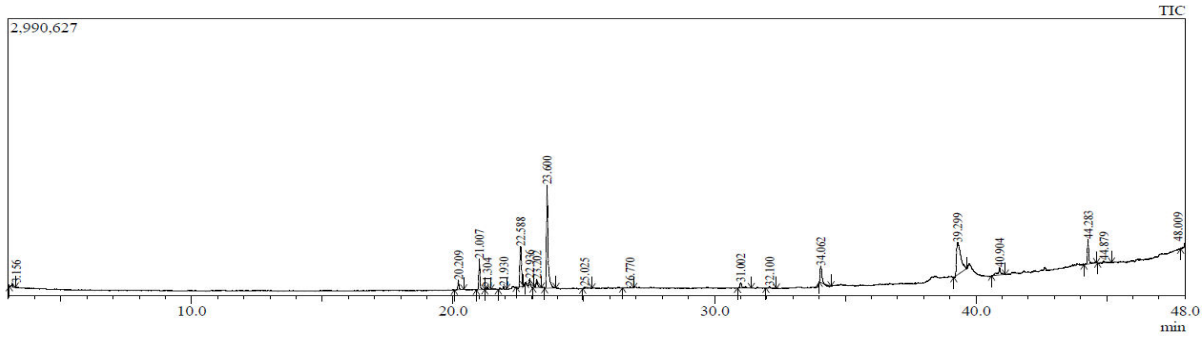


Fig. 3. GC-MS chromatogram of inflorescences ethanolic extract of *A. calva*



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