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GC-MS analysis and qualitative phytochemical screening of *Aristolochia assamica*, a newly discovered rare medicinal plant species of India

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Aristolochia assamica D. Borah & T.V. Do, is an ethnomedicinal plant and a new record to Indian flora. Through this study, the presence or absence of secondary metabolites was screened and the presence of alkaloids, glycosides, flavonoids, phenolic compounds, terpenoids and tannin was established. The chromatograph of the GC-MS analysis of the methanolic leave extract showed the presence of various compounds mainly 2-Octylcyclopropene-1-heptanol, α-ylangene, 6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-, (Z)-7-Hexadecenal, 1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-, 3-Hexadecyne with highest area percentage. These compounds are recorded for the first time in this plant. Further researches related to pharmacological activities and isolation of the phytocompounds from *A. assamica* may help in the novel drug designing and development.

Keywords: *Aristolochia assamica*, GC-MS analysis, Medicinal plants, Phytochemistry, Phytocompounds.

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Introduction

The first line of defence for human health care and management is achieved through plant-derived medicines. Thousand years back, people from all over the world have been utilizing medicinal plants and plant products for formulating conventional herbal drugs. The trending demand of using herbal medicines instead of allopathic one is due to the cost-effectiveness, cultural, and ecological acceptability with lesser side effects for why more than eighty per cent population of the globe to rely tremendously on them and pharmaceutical industries to construct new drugs from potential medicinal plants through proper scientific enumeration[1-3]. The fundamental sources of drugs and magic behind the pharmacological values of medicinal plants are attributed by the secondary metabolites such as polyphenols, flavonoids, lignin, alkaloids, terpenoids etc[4]. Native people of Indian subcontinent are well acquainted of utilizing numerous endemic plants for nutritional diet and healthcare purposes on regular basis[1,5]. It has been noted that around two thousand and five hundred medicinal plants are used by native traditional healers of India though thousands of plant species with potential medicinal and pharmacological abilities are still to be explored and clinically tested[6].

Aristolochia is the largest and most significant genus of the family Aristolochiaceae and a total of twenty species of this genus have been found in India. *Aristolochia assamica* D. Borah & T.V. Do, is a new species reported to be found in two North Eastern states, Arunachal Pradesh and Assam[7]. The ethnobotanical studies of this plant revealed that the roots and leaves of the plant is used in several ailments like fever, malaria, stomach pain, dysentery, high blood pressure, body pain, urinary tract infections, headache and cough etc. by the ethnic tribes of Assam and Arunachal Pradesh through the chemical profiling have not done yet[8,9]. *Aristolochia indica*, *A. albida*, *A. tagala*, *A. brevipes*, *A. bracteolata* are some known medicinal plants that possess antibacterial, antifungal, anti-inflammatory, abortifacient, anti-implantation activity and nephrotoxic activity etc. and frequently used in Chinese traditional medicines[10]. The valuable phytoconstituent aristolochic acid presents in a number of species of genus *Aristolochia* has utmost importance as it is found to cause renal diseases and

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urothelial malignancy[11]. Chemical investigation of each single species of *Aristolochia* is an emergent need. Following this aim, the preliminary phytochemical constituents and the GC-MS were analysed to ascertain the phytoconstituents present.

Materials and Methods

Plant collection and identification

The leaves of *A. assamica* were collected from the Behali Reserve Forest of Biswanath district of Assam in the month of September 2021 at a latitude of 26.867509º and longitude of 93.255691º. Permission was obtained from the Principal Chief Conservator of Forest & Head of Forest Force, Government of Assam, India. Collection no. for the plant specimen was PunamJB-021 (Fig. 1) and identification of the plant was done by the author himself through the holotype[7]. Herbarium sheet of the plant specimen (Accession no. 98880) was submitted to the ASSAM herbarium, Botanical Survey of India, Eastern Regional Centre.

Extract preparation

The collected fresh leaves were washed in the running tap water and allowed to dry in shade for 2-3 weeks. Thereafter, they were grounded. The coarsely powdered plant materials were extracted with solvents; Petroleum ether, Methanol, Acetone, and Water. A total of 10 g plant sample was treated with 250 mL of different solvents separately and using a Soxhlet apparatus were continuously extracted for 24 hours until the solvent became colourless. The aqueous extraction was made through the Hot water extraction method. Evaporation of the solvents were done in the hot water bath in 30 to 40ºC then the dried crude plant extracts were stored in the refrigerator at 4ºC and applied in the qualitative phytochemical tests.

Fig. 1 — Flower of *Aristolochia assamica*.

Qualitative phytochemical analysis

To confirm the occurrence of different groups of phytocompounds, preliminary qualitative tests were conducted using different crude extracts of *A. assamica* leaves. The presence or absence of alkaloids, carbohydrates and glycosides, proteins and amino acids, phenolic compounds, tannins, flavonoids, terpenoids and saponins were investigated following the standard protocols[12-14].

Test for alkaloids

Mayer's Test- One mL of extract was mixed with 2 mL of 1% HCl. After heating the mixture, few drops of Mayer's reagent were added. The formation of turbidity of the resulting precipitate confirmed the presence of alkaloid.

Wagner's Test- Few drops of Wagner's reagent were added to the mixture of 1 mL of extract with 2 mL of 1% HCl and heated. The resulted precipitate showing turbidity confirmed the occurrence of alkaloid.

Test for carbohydrates and glycosides

Fehling's Test- One mL of Fehling's A and Fehling's B reagent were mixed and added in one mL of extract. The solution was boiled in a water bath for few minutes. Appearance of red coloured precipitate indicated the test as positive.

Molish's Test- Few mL of extract was mixed with few drops of Molish's reagent. The mixture was then shaken properly. Then 2 mL of concentrated H_2SO_4 was added carefully from the side of the test tube. Purple or violet coloured ring indicated the presence of carbohydrate.

Iodine Test- A 2 mL of iodine solution added to the crude extract resulting in purple colour indicated the presence of carbohydrates.

Test for proteins and amino acids

Ninhydrin Test- A 2 mL of extract was mixed with 2 mL of 0.2% solution of ninhydrin and boiled.

Appearance of violet colour indicated the presence of amino acids and proteins.

Millon's Test- A 2 mL of extract was mixed with 2 mL of Millon's reagent. Appearance of white precipitate which turned into red after heating confirmed the presence of proteins.

Test for phenolic compounds and tannin

Ferric chloride Test- Two mL of leaf extract when treated with $FeCl_3$ droplets resulting in bluish-green colour indicate the presence of phenol.

Lead acetate Test- A 1 mL of 10% lead acetate solution was mixed in few drops of extract. A bulky white precipitate confirmed the phenolic compounds.

Test for flavonoids

Shinoda Test- Two mL of crude extract was mixed with few fragments of magnesium ribbon and few drops of concentrated HCl were added. After a few minutes formation of pink colour appeared and confirmed the test as positive.

Alkaline reagent Test- Two mL of 2% NaOH was mixed with plant extract. A few drops of diluted HCl were added and colour of the mixture turned colourless which indicated the presence of flavonoids.

Test for saponins

A few mL of extracts was mixed with few mL of distilled water and shaken vigorously. Foam development indicated the presence of saponin.

Test for terpenoids and steroids

Few mL of extract was treated with few drops of chloroform and concentrated sulfuric acid. The mixture was shaken well. After sometimes, red colour formation in the lower layer confirmed the presence of sterol, while yellow colour formation indicated the terpenoid.

GC-MS Analysis

GC-MS analysis of methanolic leaf extracts of *A. assamica* was performed in a Shimadzu make GC-MS 2010 model, Plus/TQ8030 with a 30.0 m length, 0.25 mm diameter and 0.25 µM thick column DB-5-MS. Helium was the carrier gas passed through a pressure of 65.9 kPa at a flow rate of 1 mL/min. Volume of 1 µL of the sample was injected into the GC-MS where the column oven temperature was 80ºC and injection temperature and ion source temperature were ensured as 260 and 200ºC respectively. Injector mode was split less (split ratio 20.0); mass scan range was covered from 50 to 1000 *m/z*. The details of programmed temperature set were as follows-starting column temperature was 80ºC held for 2 min; increased by 5ºC raised to 230ºC, held for 5 min; and further increased by 3ºC elevated to 280ºC, held for 1 min. The total running time program of the GC-MS was 54.67 min.

Acquired data were processed through GC-MS Software post-run analysis. For the identification and detection of the probable compound present in the extract were employed in the National Institute Standard and Technology (NIST 11) library and PESTEI_3 library. The spectrum of the known compounds from these libraries were compared to the unknown compounds of the extracts of *A. assamica* to interpret the data and to ascertain the molecular names of the phytocompounds. The average peak area obtained by particular compound was compared to the total areas to calculate the relative percentage amount.

Results and Discussion

The species of *Aristolochia* have been used extensively in the Chinese traditional medicines. The major groups reported to be present in various *Aristolochia* species are aristolochic acids, aristolactams, aporphines, protoberberines, isoquinolines, benzylisoquinolines, amides, flavonoids, lignans, biphenyl ethers, coumarins, tetralones, terpenoids, benzenoids, and steroids[15]. The preliminary investigation of the occurrence of these secondary metabolites of different leaf extracts of *A. assamica* through this study, we have confirmed the presence of alkaloids, carbohydrates and glycosides, phenolic compounds, tannins, flavonoids, terpenoids, and saponins. All extracts of different solvents while tested for protein and amino acids have showed negative result as shown in Table 1.

The GC-MS analysis of the methanolic leave extract of *A. assamica* had accounted for 101 phytocompounds as represented in Table 2 with their respective retention time, *m/z* value, area and height percentage. The chromatogram showed the peaks with their retention time are presented in Fig. 2.

The major compound recorded from the GC-MS analysis of *A. assamica* are 2-Octylcyclopropene-1-heptanol (area percentage = 12.17%; retention time= 38.328) >α-ylangene (area percentage = 7.04%; retention time= 26.891) > 6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)- (area percentage = 6.19%; retention time= 42.128)> (Z)-7-Hexadecenal (area percentage = 5.98%; retention time = 35.099) > 1-

Table 1 — Qualitative phytochemical analysis in various extracts of *A. Assamica*

S. No	Secondary metabolites	Tests for qualitative analysis	Methanol extract	Aqueous extract	Petroleum ether extract	Acetone extract
1	Alkaloids	Mayer's Test	+	+	-	+
		Wagner's Test	+	+	+	+
2	Carbohydrates & Glycosides	Fehling's Test	+	+	-	+
		Molish's Test	+	+	+	+
		Benedict's Test	-	+	-	-
		Iodine Test	+	+	+	+
3	Proteins and Amino acids	Ninhydrin Test	-	-	-	-
		Millon's Test	-	-	-	-
4	Phenolic compounds & Tannins	Ferric Chloride Test	+	+	-	-
		Lead Acetate Test	+	+	+	-
5	Flavonoids	Shinoda Test	+	+	-	-
		Alkaline Reagent Test	+	-	-	+
6	Saponin	Test for Saponins	-	+	-	+
7	Terpenoids	Test for Terpenoids	+	+	-	-

Here, '+' indicates Positive and '-' Negative

Table 2 — Phytocompounds of *A. assamica*

Peaks	Retention time	Compound name	Area percentage	Height percentage	m/z
1	14.795	Octadecane, 6-methyl-	1.11	0.93	57.10
2	15.069	1-Undecyne	1.58	1.29	67.05
3	19.814	Heptadecane, 2-methyl-	0.71	0.72	57.05
4	21.012	Neoisolongifolene, 8-bromo-	2.66	2.67	203.10
5	23.883	N-(5-Chloro-2-hydroxyphenyl) dodecanamide	0.56	0.42	55.15
6	24.020	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-	4.32	4.17	109.05
7	25.156	3-Hexadecyne	3.82	4.64	67.00
8	25.672	3-Octyne, 7-methyl-	0.91	0.87	67.05
9	25.808	17-Oxo-4-nor-3,5-seco-5-androsten-3-oic acid, methyl ester	2.53	1.98	217.05
10	25.900	Fenretinide	0.13	0.26	57.10
11	26.064	4-Tetradecyne	1.46	1.64	67.00
12	26.291	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	0.48	0.62	147.00
13	26.470	Butanal, 3-hydroxy-2-methyl-4-[4-t-butyl]-	2.33	2.09	201.10
14	26.550	2-[2-(7-Chloro-7-norcaranyl) ethynyl] thiophene	0.06	0.23	166.90
15	26.891	alpha-ylangene	7.04	5.27	109.10
16	26.999	1,1'-Butadiynylenedicyclohexanol	2.19	1.60	55.10
17	27.136	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	3.17	3.15	147.00
18	30.000	Bis[di(trimethylsiloxy) phenylsiloxy] trimethylsiloxyphenylsiloxane	0.65	0.76	135.15
19	30.374	Levomenthol	3.50	2.52	71.05
20	30.495	Cyclohexanol, 2-[(2-hydroxyethyl) thio]-	0.19	0.27	81.05
21	30.605	4-[3-(Benzotriazol-1-yl) propyl-1-(2-methoxyphenyl) piperazine	0.08	0.22	53.15
22	30.669	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,5.beta.)]-	3.26	3.22	71.05
23	30.875	Bicyclo[4.1.0]heptane-7-methanol, 7-bromo-.alpha.-ethyl-, (1.alpha.,6.alpha.,7.alpha.)-	0.15	0.26	55.00
24	30.920	Acetic acid, 2-(2-t-butyl-4-methyl-6-oxo [1,3] dioxan-4-yl)-1-phenyl-ethyl ester	1.14	0.69	77.15
25	31.019	(5a.alpha.,9a.beta.,9b.beta.)-5,5a,6,7,8,9,9a,9b-octahydro-6,6,9a-trimethylnaphtho[1,2-c]furan-1-(3H)-one (drimenin)	0.97	1.04	109.05
26	31.065	4-Hexyl-1-(7-methoxycarbonylheptyl) bicyclo[4.4.0]deca-2,5,7-triene	0.27	0.56	91.05
27	31.554	cis-Z-.alpha.-Bisabolene epoxide	1.81	1.86	109.10
28	33.214	Cyclohexanone, 2,6-bis(2-methylpropylidene)-	0.85	0.66	151.15
29	33.426	Butanal, 3-hydroxy-2-methyl-4-[4-t-butyl]-	3.01	3.25	201.10

(*contd.*)

Table 2 — Phytocompounds of *A. assamica (contd.)*

Peaks	Retention time	Compound name	Area percentage	Height percentage	m/z
30	33.786	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha	0.80	0.82	148.85
					Contd.
31	34.162	Dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocycl	0.30	0.28	77.00
32	34.870	26-Dehydroxy-dihydropseudoprogenin-25-ene	0.61	0.31	81.10
33	35.099	7-Hexadecenal, (Z)-	5.98	4.37	81.00
34	35.188	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	1.50	1.59	134.05
35	37.325	11-Methyldodecanol	1.58	1.30	55.10
36	38.003	6 beta-Hydroxy-17-oxo-4,5-secoandrostan-4-oic acid	0.67	0.85	217.05
37	38.328	2-Octylcyclopropene-1-heptanol	12.17	11.44	55.00
38	38.465	1-Oxaspiro [2.2] pentane, 5-isopropylidene-2,2,4,4-tetramethyl-	0.48	0.55	148.80
39	38.505	3-Hydroxypropanoic acid, 3-(2,2,6-trimethylbicyclo [4.1.0] hept-1-yl)-, ethyl ester	0.16	0.31	208.10
40	38.983	8,10-Dioxaheptadecane	0.66	0.69	57.05
41	39.675	Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	0.39	0.27	73.10
42	39.825	l-Methionine, N-(5-chlorovaleryl)-, methyl ester	0.34	0.38	207.05
43	40.212	Eudesma-5,11(13)-dien-8,12-olide	1.87	0.96	217.05
44	40.454	2-(Bromomethyl)benzyl alcohol, trimethylsilyl ether	0.11	0.29	207.00
45	40.535	17 beta -Acetoxy-1',1'-dicarboethoxy-1beta, 2 beta-dihydro-17.alpha.-methyl-3'H-cycloprop[1,2]-5.alpha.-androst-1-en-3-one	0.18	0.25	266.75
46	40.835	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	0.35	0.29	207.05
47	41.345	Methoxymethyl(triethyl)stannane	0.17	0.41	355.00
48	42.128	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	6.19	6.16	81.05
49	42.397	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	0.11	0.29	280.90
50	42.590	Phenol, 2,4-dichloro-6-nitro-	0.07	0.19	207.00
51	42.935	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	0.47	0.46	206.90
52	43.035	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	0.09	0.31	207.10
53	43.365	Stannane, diethylbis(phenylmethyl)-	0.24	0.22	207.00
54	43.430	3.alpha.,4.alpha.,9.beta.,11-Diepoxymuurolan-10-ol	0.09	0.33	282.00
55	43.455	Pentasiloxane, dodecamethyl-	0.46	0.34	280.90
56	44.265	Cyclodecasiloxane, eicosamethyl-	0.21	0.28	354.80
57	44.351	9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-	0.19	0.38	207.00
58	44.605	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	0.33	0.47	281.00
59	44.711	6H-Furo [2',3':4,5] oxazolo[3,2-a] pyrimidin-6-one, 2-[(acetyloxy)methyl]-2,3,3a,9a-tetrahydro-3-[(trimethylsilyl)oxy]-, [2R-(2.al	0.35	0.28	72.95
60	44.885	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	0.22	0.53	207.00
61	44.950	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	0.37	0.32	207.00
62	45.010	Tetrasiloxane, decamethyl-	0.37	0.43	207.00
63	45.100	5,7-Dipropyl-1,3-diazaadamantan-6-one	0.24	0.15	207.00
64	45.175	p-Benzoquinone, 2,5-bis[(2-hydroxypropyl) amino] -3,6-dimethyl-	0.28	0.33	281.95
65	45.234	Benzestrol di-TMS derivative	0.15	0.45	207.00
66	45.255	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	0.37	0.39	207.05
67	45.342	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	0.34	0.56	206.95
68	45.390	Tartronic acid, 4-(dimethylethylsilyl)phenyl-, dimethyl ester	0.25	0.50	281.85
69	45.445	Urocanic acid, N,O-bis(trimethylsilyl)-	0.23	0.27	72.95
70	45.490	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate	0.40	0.33	282.00
71	45.586	Silicic acid, diethyl bis(trimethylsilyl) ester	0.29	0.39	206.95
72	45.783	Chlorthiophos	0.17	0.31	225.00
73	46.285	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	0.30	0.28	207.00
74	46.365	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol	0.22	0.49	207.00
75	46.395	Phenol, 2,6-dichloro-4-nitro-	0.48	0.56	207.05
					(*contd.*)

Table 2 — Phytocompounds of *A. assamica contd.*

Peaks	Retention time	Compound name	Area percentage	Height percentage	m/z
76	46.459	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	0.31	0.67	207.00
77	46.492	Butyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilahexadecan-1-oate	0.27	0.62	265.80
78	46.525	Benzo(a)heptalen-9(5H)-one, 6,7-dihydro-10-hydroxy-1,2,3-trimethoxy-7-(methylamino)-, (S)-	0.41	0.62	207.05
79	46.600	2-{4-[2-(4-Methoxymethylphenyl)vinyl]phenyl}propan-2-ol	0.43	0.41	282.00
80	46.678	Acetic acid, [bis[(trimethylsilyl)oxy] phosphinyl]-, trimethylsilyl ester	0.14	0.37	207.05
81	46.819	1,2-Bis(trimethylsilyl)benzene	0.55	0.40	207.00
82	46.865	1,2-Bis(trimethylsilyl)benzene	0.21	0.37	207.10
83	47.106	Stannane, tributylmethyl-	0.15	0.32	282.95
84	47.205	Phenol, 2-(6-bromoquinolin-8-yl)iminomethyl-	0.32	0.38	206.90
85	47.490	Diethyl 3-chloro-2-hydroxypropylmalonate	0.22	0.52	207.00
86	47.520	Butyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilahexadecan-1-oate	0.50	0.49	207.00
87	47.726	Ethyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilatetradecan-1-oate	0.74	0.52	207.00
88	47.780	3,3-Di-p-tolyl-thietan-2-one	0.19	0.53	280.90
89	47.805	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, tris-(O-trimethylsilyl)-	0.19	0.42	266.95
90	48.095	1,2-Bis(trimethylsilyl)benzene	0.23	0.29	191.95
91	48.144	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	0.22	0.66	206.95
92	48.364	4,6-Dihydroxy-2-[3,4,5-trimethoxybenzyl] pyrimidine	0.08	0.32	341.00
93	48.500	tert-Butyl 2-aminophenylcarbamate	0.28	0.27	190.90
94	48.580	1-Phenazinecarboxylic acid, 6-[1-[(1-oxooctyl)oxy]ethyl]-, (.+-.)-	0.50	0.45	190.95
95	48.650	Thiophene, 2-[2-(3-thienyl) ethenyl]-	0.10	0.38	190.95
96	48.705	Pentasiloxane, dodecamethyl-	0.27	0.25	264.85
97	48.799	Silanol, trimethyl-phosphite	0.10	0.33	207.05
98	48.875	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	0.09	0.22	207.00
99	49.120	Silicic acid, diethyl bis(trimethylsilyl) ester	0.32	0.35	207.05
100	49.360	Pentasiloxane, dodecamethyl-	0.10	0.25	281.00
101	49.409	Hexasiloxane, tetradecamethyl-	0.42	0.41	73.00

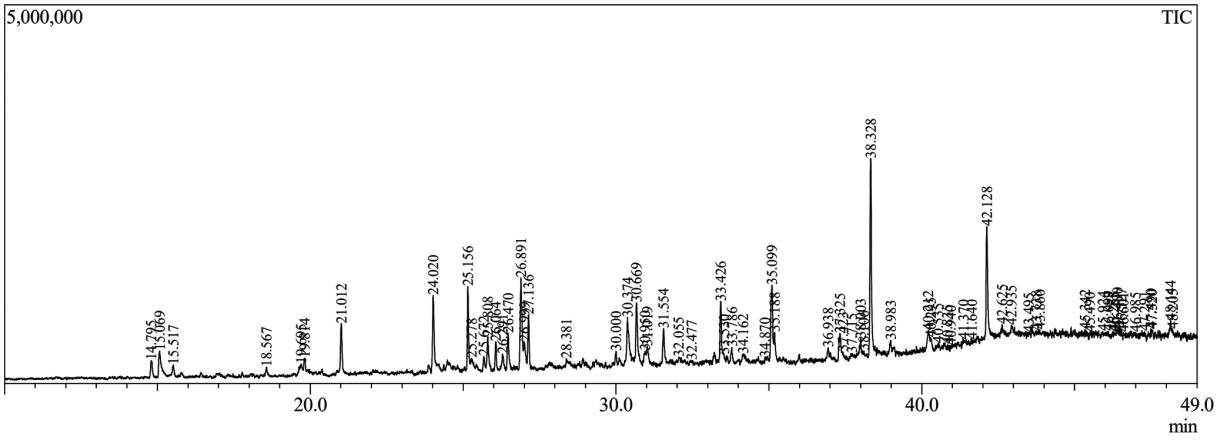

Fig. 2 — Chromatogram of methanolic leaf extract of *A. assamica*.

Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl- (area percentage = 4.32%; retention time = 24.020)> 3-Hexadecyne (area percentage = 3.82%; retention time = 25.156).

α-ylangene is a Sesquiterpenoid; has antioxidant and antimicrobial properties reported earlier from the essential oil of *Ballota hispanica* (L.) Benth.[16]. (Z)-7-Hexadecenal, which is a fatty aldehyde showed antiviral activity and used as organic fertilizer also accounted from GC-MS analysis of *Syzygium jambos* (L.) methanolic leaf extract[17] whereas 6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-

hexamethyl-, (all-E)- is a triterpene; produced naturally in human body and important for synthesis of cholesterol, steroid hormones and vitamin D, is found to be present in another potent medicinal plant *Premna tomentosa*[18]. Similarly, the GC-MS analysis of the ethanolic leaf extract of *Neibuhria apetala* Dunn. resulted to contain 3-Hexadecyne at the highest percentage[19]. Hence, the crude extract of *A. assamica* also contains phytocompounds of effective biological value which are responsible for therapeutic effects.

Conclusion

Medicinal plants are storehouses of secondary metabolites with various medicinal properties. The primary step to confirm the phytochemical compounds and their nature present in a medicinal plant is the GC-MS analysis. Our study is a first approach towards the phytochemical investigation of *A. assamica* and it has revealed the presence of various terpenes responsible for preventing diseases and promotes pharmacological activities. The isolation and further investigation of these phytocompounds and their biological activities may light up the discovery of novel drugs.

Conflict of interest

We declare that there is no conflict of interest.

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