Biochemical evaluation of Flowers of Calotropis gigantea L. via GC-MS technology

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Abstract: The phyto-components of Calotropis gigantea Linn. flowers were screened by gas chromatography-mass spectroscopy (GC-MS) analysis. Methanolic extract was prepared by soxhlet exract from the flowers of C. gigantea . GC-MS running time for methanol extract of flowers of C. gigantea was 45 min. The total number of compounds identified in methanolic extract was 39. The major phytoconstituents present in methanolic extract were Lupenol(20.13), alpha-Selinene(15.62), Methyl commate A(12.63), Palmitic acid (12.02) and (9E,12E)-9,12-Octadecadienoic Acid (7.26).Many phytosterols were also present such as Beta –sitosterol(0.74), Campesterol(0.60), Stigmasterol(0.41), Stigmasterol acetate(0.34). It is interesting that Alpha-Tocopherol (Vitamine-E) (0.28) and Cholecalciferol (VitD3) (0.22) are reported first time in flowers of C. gigantea.

Key words: Phyto-components, GC-MS, Calotropis gigantea, Methanolic extract

1. Introduction

Calotropis gigantea belongs to *Asclepiadaceae* or Milkweed or Aak family, commonly known as "Sweta Arka". *C. gigantea* is native to Cambodia, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, India and China. It is growing widely throughout the tropical and subtropical regions of Asia and Africa[1]. *C. gigantea* is found mostly under cultivated conditions near temples in Jaipur, Bharatpur, Udaipur, Bhilwara, Banswara division with relatively moderate climatic conditions [2]. *C. gigantea* is a xerophytic, erect shrub[3]. It is a weed of roadsides and watercourses and commonly invades old cultivated land and heavily grazed areas. Traditionally *Calotropis* is used alone or with other medicines[4] to treat common disease such as fevers, jaundice, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea [5]. The plant is poisonous can lead to blindness if its juice is put in to the eyes. The milky exudate from the plant is a corrosive. Plant is also using as a source of methane, through anaerobic fermentation for bio fuel production[6] .Chemical investigations of *C. gigantea* report isolation of different types of phytochemicals such as flavonoids, glycosides, steroids, triterpenoids, cardiac glycosides, calotropin, calotoxin, syriogenin, proceroside, calctin. Calotrposide A, calotroposide, calotropin D1 and D2, procerosterol, taraxsterol *etc*.[7]. Flowers show antimicrobial activity[8], anti-inflammatory activity[9], antitumor[10], hepatoprotective, anticonvulsant, antiasthmatic and analgesic[11].Therefore, in the present study the major flower constituents were separated and identified through GC-MS analysis.

2. Material and Methods

2.1 Plant Material

Flowers of *C. gigantea* were collected from local area of Jaipur, Rajasthan, India. They were authenticated from Department of Botany, University of Rajasthan, Jaipur. Voucher spaciman no. 9145 was deposited in the university.

2.2 Extraction

The fresh flowers were subjected to shade drying (22ºC) for two weeks and then processed at laboratory mill. Air dried coarse powder thus obtained (1 kg) was extracted with methyl alcohol in soxhlet extractor by continued successive hot extraction method. Finally the marc was collected and concentrated.

2.3 Parameters of GC-MS Analysis

GC-MS model: Perkin Elmer Autosystem XL with Turbomass, Column type: PE-5MS, Column Material: 5% Phenyl polysiloxane, Column Length: 30 meters, Column inner diameter: 0.250 mm, Flow rate (N_2): 1 ml/min, Temperature of injector: 250℃, Temperature of detector: 280℃, Temperature of source: 280℃, Temperature of transfer: 280℃, Programming rate: Starting from 78℃ for 5min. Increasing temperature with rate 10℃/min up to 280℃ and hold for 20min. Retention time: 45min.

3. Result and Discussion

GC-MS running time for Methanol extract of flowers of *C. gigantea* was 45 min. The total number of compounds identified in methanolic extract was 39. The GC-MS retention time (RT) and percentage peak of the individual compounds were presented in Table 1, Fig.1. The major phytoconstituents present in methanolic extract were Lupenol(20.13), alpha- Selinene(15.62), Methyl commate A(12.63), Palmitic acid (12.02) and (9E,12E)-9,12-Octadecadienoic acid (7.26)(Fig.2-6).Many phytosterols were also present such as Campesterol(0.60), Stigmasterol(0.41), Stigmasterol acetate(0.34), Beta–sitosterol (0.74). Alpha-Tocopherol (Vitamine-E) (0.28) and Cholecalciferol ($VitD_3$) (0.22) are reported as new phytoconstitutes in flowers of *C. gigantea*.

4. Conclusion and Significance

The results reveal that the extracts have a quite number of chemical constituents, which may be responsible for many pharmacological activities. For instance, Lupenol shows anti-inflammatory, anti-arthritic activity and wound healing activity[12], anti-cancer activity[13]. Methyl Commate A shows antimicrobial and anti inflammatory activity[14]. Palmitic acid-induced apoptosis in pancreatic β-cells is increased by liver X receptor agonist and attenuated by eicosapentaenoate. Further studies are needed on these extracts in order to isolate, identify, characterize and elucidate the structure of these compounds.

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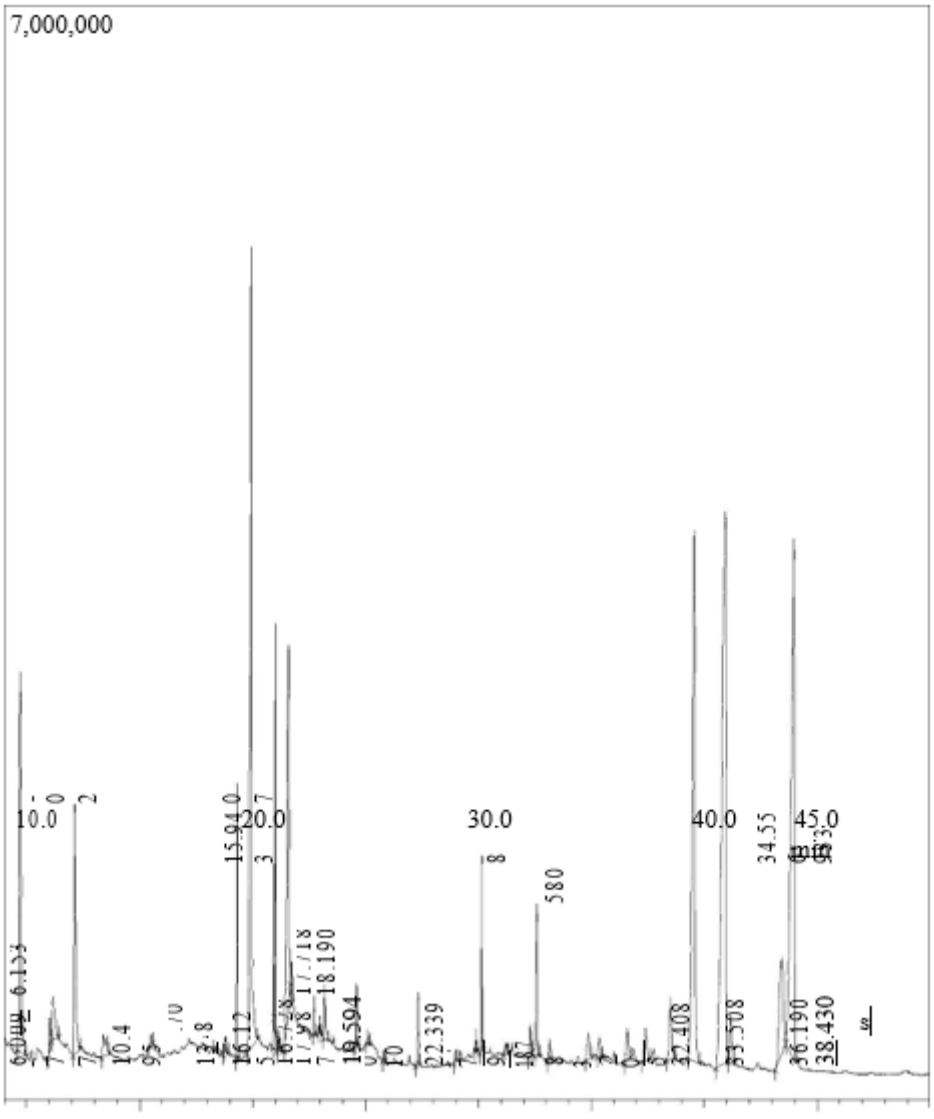
Table 1.:Chemical constituents present in the methanolic extract using GC-MS analysis.

Peak number	**RT (min.)**	**Conc. (%)**	**Name of the compound**	**Mol. Formula**	**Mol Weight**
1	4.696	6.55	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144
2	6.009	0.55	BENZOFURAN	C_8H_8O	120
3	6.153	1.11	Hydroxymethyl furfurole	$C_6H_6O_3$	126
4	7.102	3.74	Venyl guaiacol	$C_9H_{10}O_2$	150
5	8.377	0.68	Glutaminic acid Hydroxychloride	$C_6H_9NO_3$	143
6	10.495	0.12	Butyric acid	$C_{19}H_{34}O_2$	294
7	13.708	0.07	Propenoic acid 3 (2,3dimethoxy phenyl)	$C_{19}H_{36}O_2$	296
8	13.814	0.15	n- Pentadecylic acid	$C_{20}H_{40}O$	296
9	14.307	1.44	Hexadecanoic acid, methyl ester	$C_{16}H_{30}O$	238
10	14.933	**12.02**	Palmitic acid	$C_{16}H_{32}O_2$	256
11	15.943	0.82	Methyl Linolelaidate	$C_{19}H_{34}O_2$	294
12	16.003	2.10	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	269
13	16.125	0.17	Phytol	$C_{20}H_{40}O$	296
14	16.586	**7.26**	(9E,12E)-9,12-OCTADECADIENOIC ACID	$C_{18}H_{32}O_2$	280
15	16.728	0.19	Stearic acid	$C_{18}H_{36}O_2$	284
16	17.718	0.26	Trtradecane	$C_{14}H_{30}$	198
17	17.987	0.11	Isotetradecane	$C_{14}H_{30}$	198
18	18.190	0.53	Icosyl cyclohexane	$C_{26}H_{52}$	364
19	19.594	0.45	Tetracosane	$C_{24}H_{50}$	338
20	20.100	0.13	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390
21	20.810	0.10	DOCOSANE	$C_{22}H_{46}$	310
22	22.339	0.77	Octacosane	$C_{28}H_{58}$	394
23	23.977	0.11	Nonadecane	$C_{19}H_{40}$	268

24	24.899	0.15	1-DOCOSANOL	$C_{22}H_{46}O$	326
25	25.148	1.43	Icosane	$C_{20}H_{42}$	282
26	26.187	0.22	Cholecalciferol(VitD3)	$C_{27}H_{44}O$	384
27	27.298	0.34	Stigmasterol acetate	$C_{31}H_{50}O_2$	454
28	27.580	1.63	Nonacosane	$C_{29}H_{60}$	408
29	28.143	0.28	Alpha-Tocopherol(Vit. E)	$C_{29}H_{50}O_2$	430
30	29.862	0.60	Campesterol	$C_{28}H_{48}O$	400
31	30.350	0.41	Stigmasterol	$C_{29}H_{48}O$	412
32	31.585	0.74	Beta-sitosterol	$C_{29}H_{50}O$	414
33	32.408	0.86	Methyl commate C	$C_{31}H_{50}O_4$	486
34	33.508	1.40	Lupeol	$C_{30}H_{50}O$	426
35	34.556	**12.19**	Methyl Commate A	$C_{30}H_{48}O$	424
36	35.933	**20.13**	Lupenol	$C_{30}H_{50}O$	426
37	36.190	0.92	Lupenyl acetate	$C_{32}H_{52}O_2$	468
38	38.430	3.64	Thunbergol	$C_{20}H_{34}O$	290
39	38.966	**15.62**	Alpha-Selinene	$C_{15}H_{24}$	204



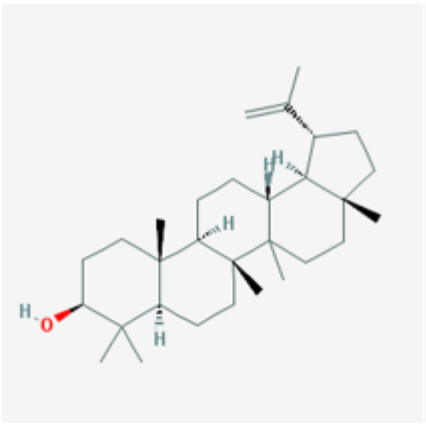


Figure 2: Lupenol

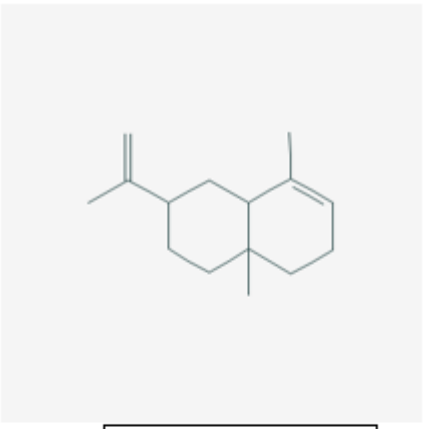


Figure 3: alpha- Selinene

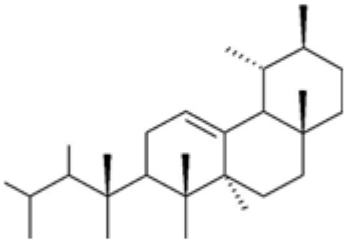


Fig.4 Methyl Commate A

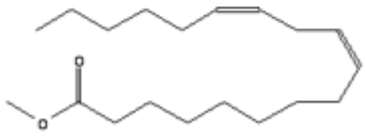


Fig.5: (9E,12E)-9,12-Octadecadienoic Acid

Fig. 6: Palmitic acid