COMPARATIVE STUDY OF CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA* FLOWER, LEAF AND FRUIT ESSENTIAL OIL

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Keywords: *Calotropis gigantean;* benzyl alcohol; 4-vinylguaiacol; ± linalool; α- terpineol.

Essential oil from flowers, leaves and fruits of *Calotropis gigantea* (L), was extracted by hydrodistillation method. Total 21 components in leaves, 43 components in flowers and 21 components in fruits were identified. The extracted oil was characterized by GC and GC-MS techniques. Constituents common to flower, leaves and fruit were 3-hexen-1-ol, benzaldehyde, benzyl alcohol, ± linalool, oct-3-en-2-ol, phenethyl alcohol, α- terpineol, 2,4-dimethylacetophenone, 4-vinylguaiacol and n-tetradecane.

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INTRODUCTION

Calotropis a genus belongs to family of Asclepiadaceae in plant kingdom, consist of six species of perennial shrubs distributed in tropical and subtropical Asia, and Africa.[1] *Calotropis gigantea* is found in Indian subcontinent and have great economic importance.[2] It has great medicinal importance, various parts of this plant is reported to have multiple therapeutic activity. The root bark is used to treat dysentery and elephantiasis, the flowers in small doses gives relief in colds, coughs, asthma, and indigestion[1]. Reported as a indigenous medicine the latex and roots are used in birth control[3] In agriculture leaf extracts are used to protect *Oryza sativa* from pathogenic fungus,[4,5] and in material science to synthesize nanomaterials.[6] Interestingly, as traditional biopesticides the plant is used in controlling mollusks and mosquitoes.[7,8] *Calotropis gigantea* invites attention of the researchers worldwide for its pharmacological activities. Latex contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin and uscharidin; gigantin and aqueous extract has reported activities such as anti diabetic, antitoxin, antihepatotoxin, antioxidant and wound healing activity. Moreover, variety of chemical groups including cardenolides, steroids, flavonoids, terpenoids, cardiac glycosides, resins, fatty acids and non-protein amino acids etc. have also been identified and reported in *C. gigantea*,[9] which leads to various applications in food, flavour and pharma industries as well as chemical industries to transformed into promising biologically active molecules for chemicals and drugs synthesis. For example, eugenol and guaiacol one of the major constituents of *Calotropis* essential oil, which is recently being explored as a substitute of fossil based chemicals for making biobased materials and green chemicals.[10,11]

Essential oils (EO) are proficient “green” alternative in the pharmaceutical, food and agriculture industries due to the presence of promising chemicals[10,11,15] and effective biological activity.[16]

In the present study, we investigate the essential oil extract of C. gigantea flower, leaf and fruit chemical profiling and comparative evaluation of the chemicals identified by gas chromatography with flame ionisation detection (GC-FID) and gas chromatography with mass spectrometry (GC-MS). This study includes identification of about 21 compounds from the hydrodistilled extract of flower, 43 compounds from leaf and 21 compound from fruit of *C. gigantea*.

MATERIALS AND METHOD

Source of the plant

Fresh flower, leaves and fruit of *Calotropis gigantea* were collected during flowering season from the roadsides (April 2014), dry wasteland area adjuscent to Muzaffarnagar (29.4723° N, 77.7089° E) having weather (29 °C, wind at 6 km h^{-1}, 80 % humidity), Uttar Pradesh state, India. Collected plant materials were submitted for identification at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen is available in the herbarium division of the NISCAIR.[20]

Isolation of essential oil

Flower, leaves and fruit were washed properly three times with fresh water and dried in shade in properly ventilated room under fan in ambient conditions (27 °C) to prevent loss of essential oil. Dried flower, leaf and fruit were crushed, weighed and subjected to hydro-distillation using distilled water in Clevenger type apparatus for 4-5 h. The distillated product was further solvent extracted with n-hexane and passed through anhydrous sodium sulphate to remove residual water. The yield of essential oil obtained was found to be 0.13 % w/w from flower, 0.11 % w/w from leaf and 0.23 % w/w from fruit.

Essential oil composition analysis by Gas Chromatography (GC)/ Mass Spectrometry (MS)

Essential oil composition of *Calotropis gigantea* was analyzed using Shimadzu GC -2010 equipped with flame ionization detector using Rtx-5MS (30 m x 0.25 mm ID x 0.25 μm) column.

Table 1. Comparative evaluation of components of *Calotropis gigantea* flower, leaves and fruit.

RT	Component	Flower (Area %)	Leaf (Area %)	Fruit (Area %)
6.02	Furfural	2.39	-	-
6.07	2-Furancarboxaldehyde	-	2.50	-
6.80	3-Hexen-1-ol	0.53	-	0.30
6.92	4-Methyl-3-penten-1-ol	-	3.83	-
8.45	2-Hexyn-1-ol	-	2.86	-
9.68	Gentanol	-	2.90	-
10.80	Benzaldehyde	0.85	1.88	-
12.22	2-Methyl-6-hepten-1-ol	0.57	-	-
12.27	6-Methyl-5-hepten-2-ol,	-	-	2.89
13.43	(+)-β-citronellene	-	1.39	-
13.67	4-Methyl-1-heptanol	-	4.98	-
14.21	cis- Linalool oxide	-	-	3.29
14.30	Benzyl alcohol	42.89	4.10	-
14.72	Phenylacetaldehyde	-	9.16	-
15.53	trans-Linalool oxide	-	-	0.37
16.86	Guaiacol	-	0.86	-
17.29	3,7-Dimethyl-1,6-octadien-3-ol,	2.53	-	-
17.30	± Linalool	-	0.49	0.34
17.50	Oct-3-en-2-ol	-	1.24	0.50
17.51	3-Thiophenemethanol	0.68	-	-
17.90	Phenethyl alcohol	3.56	2.52	3.41
17.93	2,2,6-Trimethyl-1,4-cyclohexanedione,	-	-	1.95
19.03	Gardenol	-	0.64	-
19.82	(-)-Cis-Myrtanol	-	0.72	-
20.21	α-Terpinene	-	0.84	-
20.78	DL-Menthol	-	0.28	-
21.30	Nerol	-	-	0.83
21.60	α- Terpineol	0.80	0.63	0.14
23.08	2,3-Dihydro-benzofuran	1.94	-	-
23.15	n-Undecane	-	1.65	-
23.35	2,3-epoxygeranyl acetate,	-	-	0.38
23.60	β-Cyclocitral	-	0.55	-
23.91	p-Cymen-7-ol	-	-	0.95
24.00	Isocyclogeraniol	-	0.25	-
24.40	2,4-dimethyl-Acetophenone	1.84	-	3.72
24.41	Ethanone, 1-(2,4,6-trimethylphenyl)-	-	-	2.74
24.57	n-Tridecane	-	-	0.75
24.60	(E)- 3,7-dimethyl-2,6-octadien-1-ol	0.80	-	-
25.70	Dill ether	-	0.46	-
26.97	(E)-Cinnamyl alcohol	0.35	-	-
27.28	2,4-Di-tert-butylphenol	-	-	2.43
27.30	4-vinylguaiacol	15.56	-	3.86
27.82	α-Citral	-	0.06	-
27.95	Heptylidene acetone	-	0.48	-
28.54	Pinocampheol	0.95	-	-
28.60	Artemisia alcohol	-	1.16	-
29.03	Eugenol	-	0.30	-
29.24	2-Methoxy-4-(2-propenyl)phenol,	0.96	-	-
29.46	(Z)-Amylcinnamaldehyde	-	0.96	-
30.22	Spathulenol	-	0.39	-
30.33	3-Phenyl-2-propenoic acid, methyl ester	0.80	-	-

30.50	n-Tetradecane	0.76	0.30	-
31.04	Nerylacetone	-	0.53	-
34.60	Phthalic acid, bis(7-methyloctyl) ester	-	-	0.71
34.68	(E)-β-Ionone	-	1.84	-
34.86	5-Methyl-2-phenylhex-2-enal	-	2.47	-
35.68	Dodecanoic acid, trimethylsilyl ester	-	-	1.07
36.02	Cyclohexyl ketone	-	0.97	-
38.13	Methyl jasmonate	-	0.38	-
38.92	Ribitol, TMS	-	-	1.25
38.98	Trimethylsilyl laurate	-	1.05	-
42.17	5-Methyl-2,4-diisopropylphenol	1.64	-	-
43.45	Phytone	-	0.30	-
45.00	n-Eicosane	0.65	-	-
45.04	Tetradecanoic acid, trimethylsilyl ester	-	-	1.24
46.19	Diisobutyl phthalate	-	-	0.58
48.52	Stearic acid	-	0.67	-
48.74	n-Tetracosane	-	0.71	-
52.29	Phytol	-	17.94	-
52.86	n-Docosane	0.18	-	-

Nitrogen was used as carrier gas at 234.6 kPa inlet pressure. Oven temperature was programmed from 50 ºC for 3.0 minutes, 3.0 ºC min^{-1} to 200 ºC for 2.0 min, 10 ºC min^{-1} to 280 ºC for 7 min. The injector and detector temperature were 250 ºC and 260 ºC respectively. The oil was injected neat with split ratio of 10. Relative amount of individual components are based on GC peak area percentage obtained without FID response factor correction. The retention Indices were obtained from GC by logarithmic interpolation between bracketing n-alkanes. The homogenous series of n-alkanes (C8-C22, Poly Science, Niles, USA) were used as standard.

GC-MS data were obtained on Shimadzu GCMS-QP2010 plus using same chromatographic conditions and column used for GC-FID i.e. Rtx-5MS (30 m x 0.25 mm ID x 0.2 µm column and helium as carrier gas. Temperature programming was 50 ºC for 3.0 mins, 3.0 °C min^{-1} to 200ºC for 2.0 minutes, 10ºC/minutes to 280ºC for 7 minutes.

Identification of constituents

The individual peaks in GC of *Calotropis gigantea* flower, leaf and fruit oil were identified by comparison of their retention indices on the SP Rtx-5MS (30 m x 0.25 mm ID x 0.25 µm) column with literature values[5] and were reported based on its area % response by GC-FID. The essential oil constituents were confirmed by matching of mass spectra of peaks with FFNSC2, NIST08 and Wiley8 library. Identification was done by GC-MS while RI and quantification done by GC-FID.

RESULTS AND DISCUSSION

Chemical investigation of essential oil of traditionally useful *Calotropis gigantea* which is reported for the first time in the literature and identified based on total ion chromatogram (TIC) of oil identified 21 compounds from flower, 43 compounds from leaf and 21 compounds from fruit corresponding to 81.28 %, 81.9 % and 29.9 % total area percentage for flower, leaf and fruit respectively. The yield of essential oil obtained by hydro distillation from *C. gigantea* was 0.13 % (w/w) for flower, 0.11% (w/w) for leaf and 0.23% for fruit oil with respect to their total dry mass. The identified peak comparison is reported in Table 1.

The chemical profiles of the EO reveals the dominance of oxygenated diterpenes (phytol), aromatic alcohol (benzyl alcohol) and linear chain alcohol (4-methyl-1-heptanol) as shown in the Table 1. Among major constituents of essential oil, the major peaks was dominated with benzyl alcohol (42.89 %) and 4-vinylguaiacol (15.56 %) in flower, phytol (17.94 %) in leaf and 4-vinylguaiacol (3.86 %) in fruit. In present study we have identified phenylethyl alcohol (3.56 %, 2.52 %, 3.41 %) and α-terpineol (0.80 %, 0.63 %, 0.14 %) as major constituents in flower, leaf and fruit oil. Benzaldehyde (0.85 %, 1.88 %), benzyl alcohol (42.89 %, 4.10 %) and n-tetradecane (0.76 %, 0.30 %) were identified in flower and leaf oil. ± linalool (0.49 %, 0.34 %) and oct-3-en-2-ol (1.24 %, 0.50 %) were identified in leaf and fruit oil. 4-vinylguaiacol (15.56 %, 3.86 %), 3-hexen-1-ol (0.53 %, 0.30 %) and 2,4-dimethylacetophenone (1.84 %, 3.72 %) were identified in flower and fruit oil.

Phytol and 4-vinylguaiacol are important fragrant ingredient used in many house-hold products including cosmetics, fine fragrances, shampoos, toilet soaps and detergents.[23,24,25] In addition to that, the derivatives of phytol shows crucial pharmacological effects in humans and other animals,[23] while 4-vinylguaiacol can acts as a green source to produce acetovanillone and ethyl guaiacol (used in perfumery) as well as biodegradable polystyrine.[25]

Moreover, a number of other odor-active compounds such as ± linalool (0.49 %), hexanol (0.48 %), α-citral (0.06 %), eugenol (0.30 %) etc. are identified in this study. These minor products are used extensively in chemical industries as perfumery or precursors for making value added products along with some pharmaceutical purposes.[11,26-28] In addition, compounds with strong antimicrobial activity like Benzyl alcohol, p-cresol, and guaiacol have also been identified.

These products can also shows repellent properties against some insects, and thereby being used extensively as important ingredient in insecticide formulation.

CONCLUSION

This study reports the comparative compositional analysis of essential oil in leaf, flower and fruit of *Calotropis gigantea* for the first time with high benzyl alcohol (42.89%) and 4-vinylguaiacol (15.56 %) in flower, phytol content (17.94%) in leaf and 4-vinylguaiacol (3.86 %) in fruit essential oil as attention grabbing biochemical for health scientists as well as the fuel industry. Due to the presence of many biologically active molecules the EO or isolated molecules can be applied as a natural preservative, fuel additives and drugs including pesticides and bulk chemicals. Guaiacol is important building block chemicals in the range of traditionally produced molecules from fossil resources. The production/isolation of these chemicals products from *Calotropis gigantea* could help in exploring biobased chemical production to fulfill future energy chemical demands.

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