International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 4, Issue 4, 2013, pp 533-541
http://www.bipublication.com

CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *Calotropis gigantea* Linn. FLOWER ESSENTIAL OIL COLLECTED FROM NORTHERN PLAIN OF INDIA.

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[Received-26/10/2013, Accepted-22/12/2013]

ABSTRACT:

Essential oil from flowers of *Calotropis gigantea* Linn (Family Asclepiadaceae) collected from northern plains of India mainly from Gwalior (Madhya Pradesh) and Ara (Bihar) was extracted by conventional hydro distillation process using clavenger type apparatus and was characterized by using GC and GC/MS. Essential oil extracted was analyzed by GC and GC/MS and 27 constituents were identified comprising 100% of oil. (±) Linalool comprising 46.0 % of oil were identified as prominent among all identified constituents. Alpha-terpineol (10.0 %) and Pentacosane (3.5 %) were the next major characterized constituents.

The essential oil solution having concentration of 300 ppm shown maximum antibacterial activity against *Stephylococcus aureus* (7,7,6 mm ZOI) followed by *E. Coli* (7,6,6 mm ZOI) as evaluated against standard antibacterial agent. The essential oil also shown antifungal activity at a concentration of 1000 ppm against *Rhizoctonia solani* sasakaii (maize host) (% Inhibition = 75) when compared with standard antifungal agent.

Keywords: *Calotropis gigantea* Linn, essential oil, chemical characterization, antimicrobial activity.

[I] INTRODUCTION:

Calotropis gigantea Linn. belongs to family Asclepiadaceae, generally known as giant weed (W.T Aiton) or milkweed or swallow-wort, is a ordinary wasteland weed found along degraded roadside and overgrazed pastures [1-3]. It is a large shrub growing to 4 m tall. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consists of five pointed petals and a small, elegant "crown" rising from the centre, which holds the stamens. *Calotropis* belongs to Asclepiadaceae family which includes 280 genera and 2,000 species of world-wide circulation but most profuse in the sub-tropics and tropics, and atypical in cold countries. It is inhabitant to Cambodia, Indonesia, Malaysia, Philippines, T hailand, Sri lanka, India and China [4].

It is a general medicinal plant in Indian subcontinent [5], has purgative, alexipharmic anthelmintic analgesic, anticonvulsant, anxiolytic, sedative and antipyretic effect [6] and is used as a treatment for leprosy, leucoderma, ulcers, tumours, piles and diseases of the spleen, liver and abdomen. *C.gigantea* reported the isolation of many cardenolides, cardiac glycosides [7-12], flavonoids, giganticine (a

novel nonprotein amino acid) [13] and other cytotoxic principles [14] from this plant.

This study reports for the first time the chemical composition of essential oil of flowers of *Calotropis gigantea* as per best of our knowledge, although essential oil composition and antibacterial and antifungal activity of different parts of *Calotropis* species from different origins have been studied [15-19]. It has been collected from Gwalior (Madhya Pradesh) and Ara (Bihar) in India.

The essential oil was obtained by conventional hydro distillation of flowers of *Calotropis gigantea* in Clevenger type apparatus, which yielded 0.13 % oil (w/w). GC and GC/MS analysis of oil showed that it contained ± Linalool as major constituent. Antimicrobial activity of ± Linalool has been reported, which prompted us to carry out antimicrobial evaluation and detailed GC and GC/MS characterization of oil collected from *C. gigantea* [20-26].

The antibacterial activity of oil collected from flower was evaluated against a set of human pathogenic bacterial stains and the results are given in Table 1, which shows the prominent activity of oil against the bacterial stains [27-32]. The oil was highly active against *Stephylococcus aureus* and significantly active against *E.Coli*.

The antifungal activity of the essential oil was evaluated against a set of human pathogenic fungal stains and result is given in Table 2, which showed that essential oil was active against *Rhizoctonia solani* sasakaii (maize host)[33-34].

Characterization of essential oil using GC and GC/MS enabled identification of 27 constituents representing 100 % of the oil. The qualitative and quantitative analytical result of essential oil components identified are presented in Table 3, according to their order of elution from supelco DB-1 column. ± Linalool (46.0 %) was characterized as a major constituent of the oil. This oil was also characterized by the presence of α- terpineol (10.0%), Pentacosane (3.53%), alpha terpinolene (0.05%), and ± Nerolidol (0.51 %).

This study is first to evaluate chemical composition and antimicrobial activity of *C. gigantea* essential oil using a variety of in-vitro methods. The development of essential oil based on its antibacterial and antifungal properties for use in oral treatment or for tropical application might be profitable in future, particularly if essential oil was cheaper.

[II] MATERIAL AND METHOD

2.1. Plant material collection and identification:

Calotropis gigantea plant material was collected from Gwalior (Madhya Pradesh) and Ara (Bihar) and authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen is available in the herbarium division of the NISCAIR [35]. Flowers of *C.gigantea* was collected in month of April- May.

2.2. Isolation of Volatile Oil:

The essential oil of *Calotropis gigantea* was obtained by conventional hydro distillation [37] using Clevenger type apparatus and the yield was 0.13 % (w/w). The oil was dried over sodium sulphate and kept in a sterile sample tube in the refrigerator at 4 degree Celsius until analysis.

2.3. Chemical Characterization:

2.3.1. GC analysis:

Essential oil composition of *Calotropis gigantea* was analyzed using Shimadzu GC -2010 equipped with flame ionization detector using SP 2560 (0.2 um X 0.25 mm ID X 100 m) column. Nitrogen was used as carrier gas at 234.6 KPa inlet pressure. The oven temperature was programmed from 80 ºC for 2.0 min, 3.0 ºC/min to 210 ºC for 5.0 min, 15 ºC/min to 240 ºC for 18 min. The injector and detector temperature were 250 ºC and 260 ºC respectively. The oil was injected neat with split ratio of 10.0 [38]. Duplicate analysis were performed and quantative results are the mean of the data derived from the GC analysis. Relative amount of individual components are based on GC peak area 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 [39].

2.3.2. GC-MS analysis:

GC-MS data were obtained on Shimadzu GCMS-QP2010 plus using SP 2560 (0.2 um X 0.25 mm ID X 100 m) column and helium as carrier gas. Temperature programming was 80 ºC for 2.0 min, 3.0 ºC/min to 210 ºC for 5.0 min, 15 ºC/min to 240 ºC for 18 min.

The individual peaks in GC of *C. gigantea* oil were identified by comparision of their retention indices on the SP 2560 (0.2 um X 0.25 mm ID X 100 m) column with literature values [40]. The final confirmation of constituents were made by computer matching of mass spectra of peaks with FFNSC1.3, Wiley8 and NIST05s libraries and mass spectral database and literature [41].

2.4. Antimicrobial Activity

2.4.1. Microbial stain used:

The antimicrobial activity of essential oil was analyzed using the following stains procured as microbial type culture collections (MTCC) from the Institute of Microbial Technology, Chandigarh and Indian Agriculture Research Institute, New Delhi, India. The bacterial stains used were *Klebsiella Pneuminiae* MTCC3384, *Stephylococcus aureus MTCC 3103, Salmonella enteric MTCC 3224, E. Coli MTCC 443, Aeromonass hydrophila MTCC 646 and Pseudomonas aeroginosa MTCC 424* while fungal stains used was *Rhizoctonia solani* (IARI) . 2.4.2. Determination of Antimicrobial activity:

Antimicrobial activity of essential oil were performed using agar well diffusion method [42-44] where agar plate of nutrient were prepared and autoclaved at 121 ºC for 20 min and these sterile plates were inoculated by spreading 100 µl of 0.5 McFarland standard respective overnight grown microbial culture over the media viz. *Klebsiella Pneuminiae* (K.p), *Stephylococcus aureus* (S.a), *Salmonella enteric* (S.e), *E. Coli* (E.c), *Aeromonass hydrophila* (A.h) and *Pseudomonas aeroginosa* (P.a) on the nutrient agar plate and Potato dextrose Agar was prepared for antifungal activity. PDA was poured in petridish, after solidification fungus inoculated and incubated for 48 hrs at 27 ± 2 ºC. *Rhizoctonia solani* sasakaii (R.s) on potato dextrose agar media surface was applied separately by spread plate method. The agar plates were punched with sterile cork borer to obtain three well of 4 mm diameter for antibacterial at an appropriate equal distance from each other in the centre of the plate and one well for antifungal. 25 µl of essential oil with respective control solvent were added separately to fill the wells under aseptic conditions for each test organism and then incubated all the culture plates at their respective optimal culture growth conditions. Growth of pathogens were measured with scale. The best results obtained from the essential oil were listed in Table 1 for antibacterial activity and Table 2 for antifungal activity.

Minimum Inhibatory Concentration (MIC) of essential oil of *Calotropis gigantea* were determined by true broth dilution assay [45]. Single dilution of the essential oil were prepared and 0.5ml of the essential oil dilutions were added to sterile MHB broth and PDA broth media of 2.5ml separately. The inoculums of each overnight grown microbial culture was adjusted to 0.5 McFarland standard and added 50µl inoculum of *Klebsiella Pneuminiae*, *Stephylococcus aureus*, *Salmonella enteric*, *E. Coli* , *Aeromonass hydrophila* and *Pseudomonas aeroginosa* to MHB broth, whereas 50µl inoculum of *Rhizoctonia solani* sasakaii was added to PDA broth media.

These broth samples of *Pseudomonas auriginosa* were incubated at 30ºC and the rest of the culture broth samples at 37ºC for 24hrs. *Pseudomonas auriginosa* was incubated for 48hrs. The broth cultures were examined for the macroscopic turbidity and the MIC of essential oil was determined as the lowest concentration that demonstrated no visible growth. The MIC results were shown in Table 1.

[III] RESULT AND DISCUSSION

Extraction of flowers of *Calotropis gigantea* gave 0.13% w/w essential oil. The GC and GC-MS analysis of *C.gigantea* oil collected from Gwalior

(Madhya Pradesh) and Ara (Bihar) were done for identification and quantification of oil components. The analysis resulted in identification of 27 compounds with ± linalool as major constituent. This oil was also characterized by the presence of α- terpineol (10.0%), Pentacosane (3.53%), alpha terpinolene (0.05%), and ± Nerolidol (0.51 %).

From Table 1, the consolidated results of antibacterial activity of *Calotropis gigantea* in terms of inhibition zones indicate the higher activity on *Staphylococcus aureus* followed by *E. Coli, Aeromonass hydrophila* and the lowest by *Pseudomonas aeroginosa* by essential oil extracted from dried flower of Calotropis gigantea.

[IV] CONCLUSION

Following conclusions can be withdrawn from the present study:

1) *Calotropis gigantea* flower have quantifiable quantity of essential oil.
2) Essential oil extracted was found to have ± linalool as major compound.
3) Essential oil extracted was found to have activity against human pathogens.

ACKNOWLEDGEMENT

The author is thankful to Sayied Ovais and Pooja Sharma , research scholar, chemistry department Jamia hamdard for their cooperation during the experiment work.

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Table 1: **Antibacterial activity of essential oil of flowers of *Calotropis gigantea* Linn**

Bacterial strains	**MIC** (in µl/ml)	**ZOI** (in mm)
MTCC3384 *Klebsiella Pneuminiae*	300	ND
MTCC3103 *Stephylococcus aureus*	300	7,7,6
MTCC3224 *Salmonella enteric*	300	ND
MTCC443 *E. Coli*	300	7,6,6
MTCC646 *Aeromonass hydrophila*	300	5,5,5
MTCC424 *Pseudomonas aeroginosa*	300	4,4,4

Note: N - No inhibition zone observed; * the net inhibition zones = (Sample Inhibition zone - Control inhibition zone) + Well diameter (4mm).

Table 2: **Antifungal activity of essential oil of flowers of *Calotropis gigantea* Linn**

Fungal strains	**MIC** (in µl/ml)	**% Inhibition**
IARI *Rhizoctonia solani*	1000	72

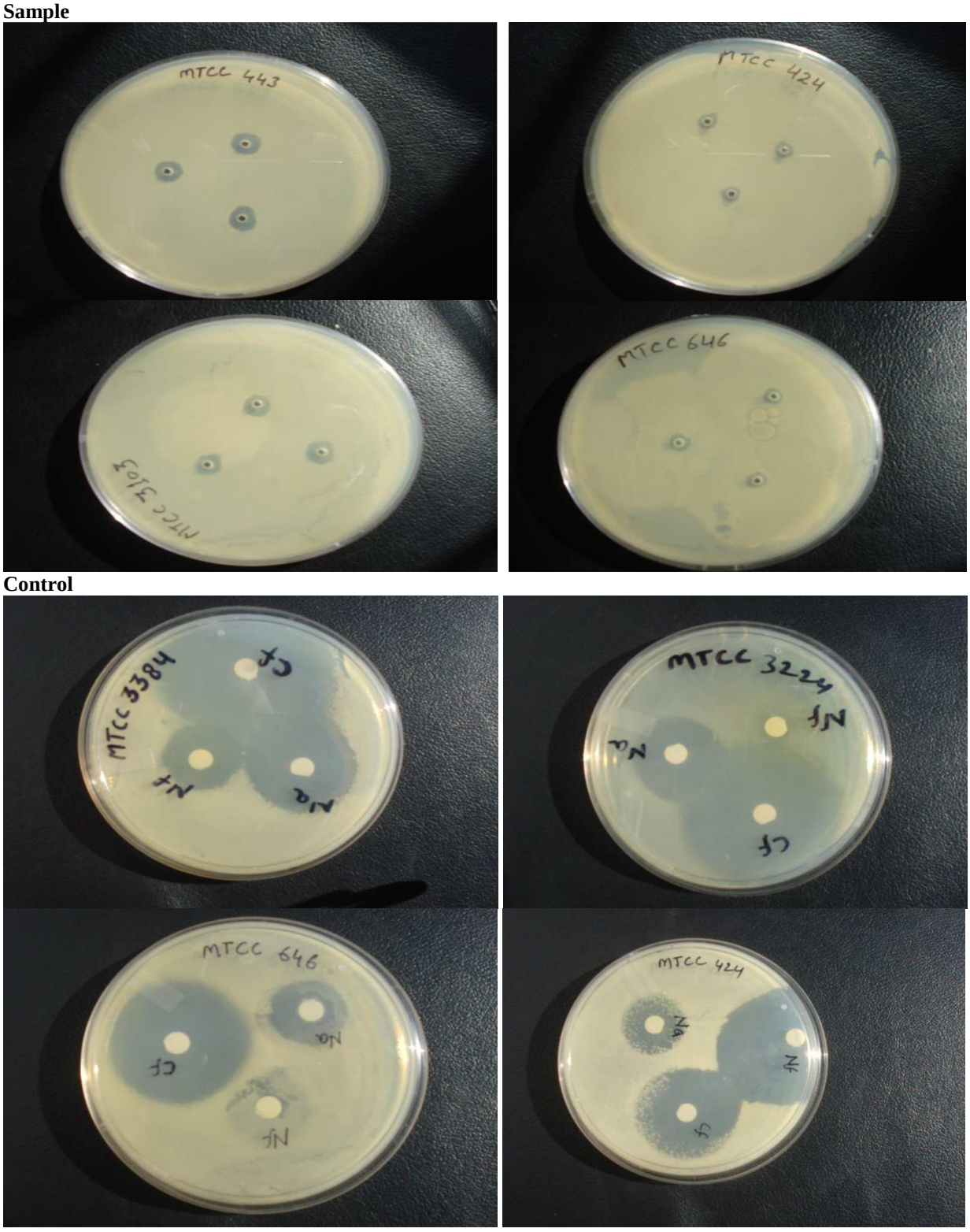
Note: % Inhibition = [(control-treatment)/Control]x100%

Table 3: **Chemical composition of essential oil of flowers of *Calotropis gigantea* Linn.**

S.No.	**Compound**	**Area %**	**RI**
1	n-Hexanal	1.118	1326
2	2-pentyl Furan	1.839	1349
3	Alpha-Terpinolene	0.527	1372
4	Heptanal	3.036	1407
5	Cinnamene	0.228	1454
6	Pelargonaldehyde	0.501	1495
7	Nonanal	4.454	1589
8	Trans-Linalool oxide	0.404	1624
9	(±)Linalool	46.012	1683
10	Capraldehyde	0.379	1693
11	Alpha-Furole	5.233	1746
12	Hotrienol	1.375	1790
13	Benzaldehyde	7.441	1817
14	Lilac aldehyde B	0.422	1849
15	Alpha-Terpineol	9.998	1891
16	Benzyl Acetate	0.928	1988
17	Nerylacetone	0.300	2090
18	Ethyl maltol	0.254	2160
19	beta-Ionone	0.698	2259
20	(±)Nerolidol	0.571	2355
21	Cis-methyl Cinnamate	1.505	2456
22	Docosane	2.680	2575

23	Farnesyl Acetone	0.185	2606
24	Pentacosane	3.530	2756
25	3-Ethyl-1-heptyn-3-ol	2.921	2854
26	Alpha-Undecene	0.461	2881
27	Eicosane	3.001	2914

Fig 1: Antibacterial activity of *Calotropis gigantea* essential oil.



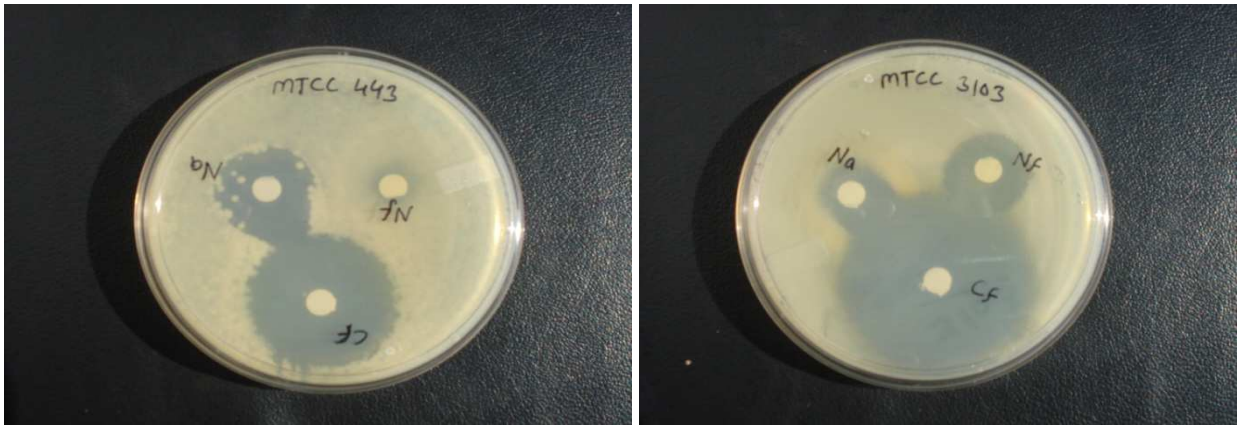


Fig 2 : Antifungal activity of Calotropis gigantean essential oil.

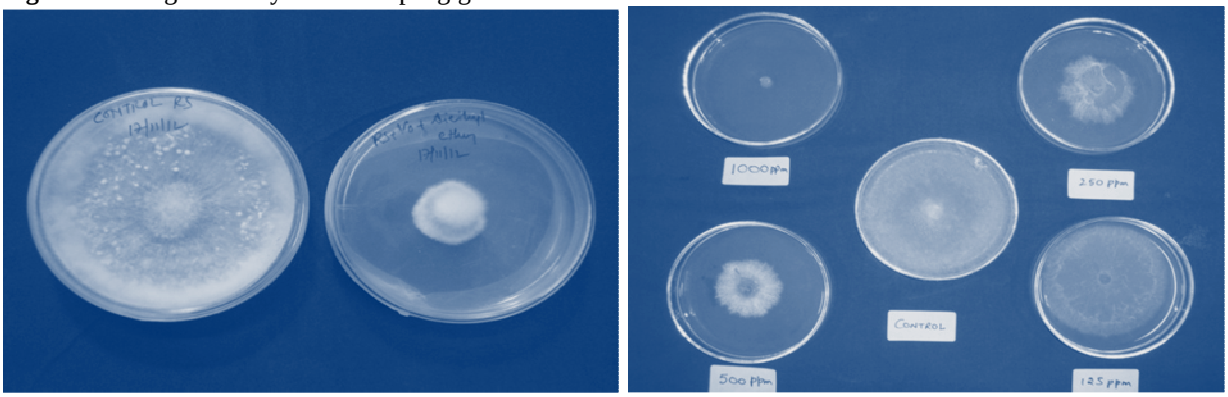


Fig 3: Chromatogram of GC showing peaks of essential oil of Calotropis gigantea

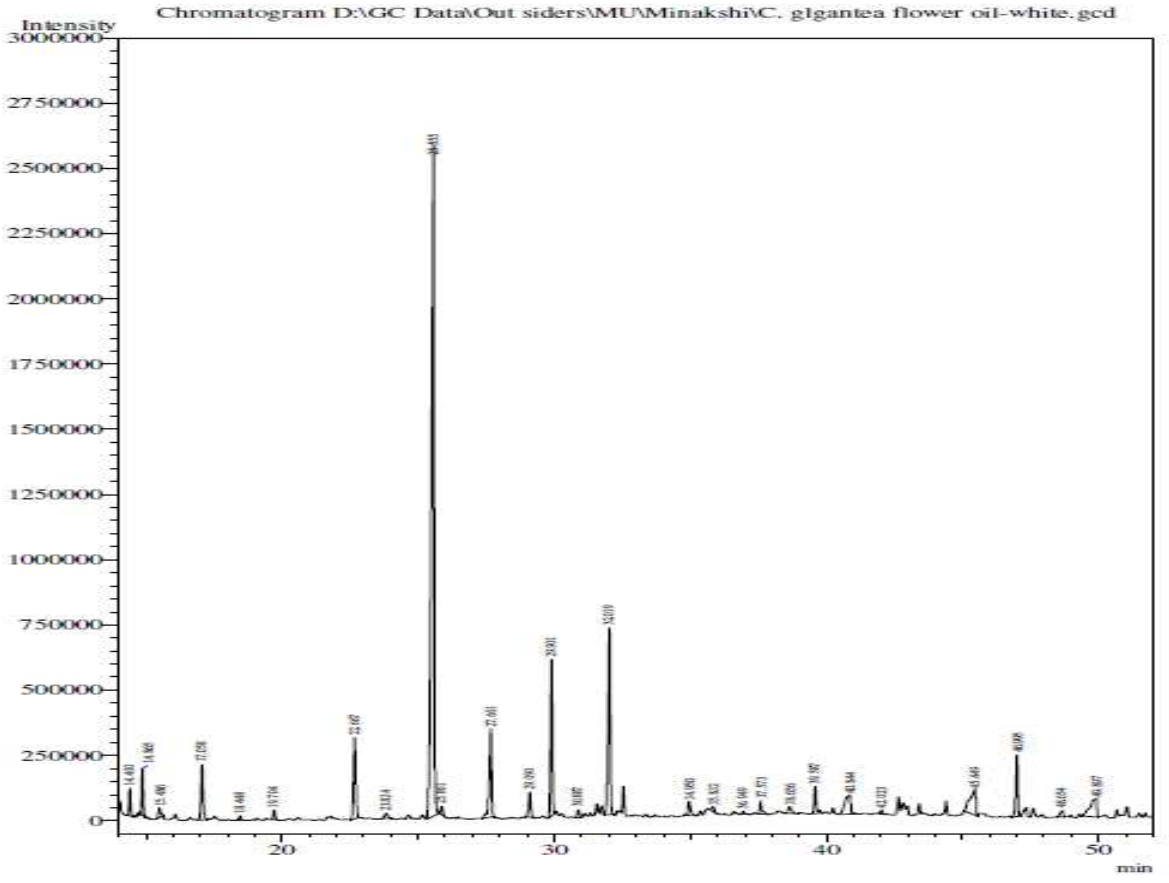


Fig 4: Chromatogram of GC-MS showing peaks of essential oil of Calotropis gigantean

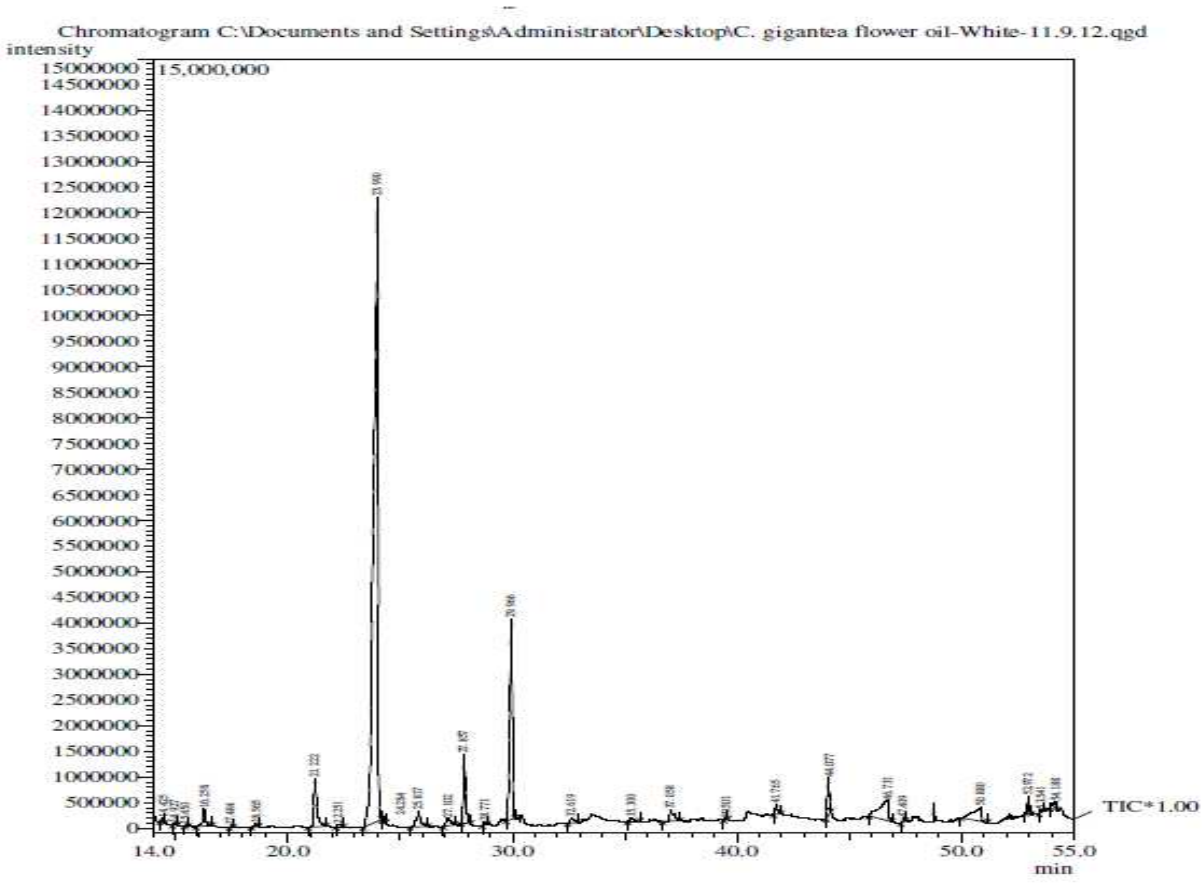


Fig 5: Chromatrogram showing peaks of standard n-alkanes mixture.