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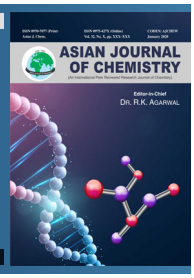

Chemometric Profile of *Calotropis gigantea* and its Antioxidant Activity through Bioactive Compounds from Latex, Leaves and Flower Extracts

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In present study, antioxidant effect was compared among latex, leaf and flower extract of *Calotropis gigantea* and is first report on comparing the chemometric profiling of various parts of *C. gigantea*. Ethanol and chloroform extracts of *C. gigantea* revealed the presence of 28 different chemotypes in latex, 27 in leaves and 32 in flowers. Major phtyochemicals present were of which fatty acid ethyl esters, fatty alcohols, terpenes, coumarins and carbocycles. *C. gigantea* leaf extract recorded 78% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and IC_{50} values of 14.44, 6.74 and 21.35 µg/mL were observed for latex, leaves and flower extracts respectively. For 2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) [$ABTS^+$] assay, IC_{50} values ranged between 6.74 and 21.25 µg/mL by various components of *C. gigantea.* The results represent free radical-scavenging activities of extracts of several parts of *C. gigantea.* The findings will definitely use in new directions in pharmacological and therapeutic investigations on *C. gigantea* latex, leaves and flowers.

Keywords: *Calotropis gigantea*, Antioxidant activity, Latex, Fatty acid esters.

INTRODUCTION

Calotropis belongs to the family Apocynaceae and the species *Calotropis gigantea* have thick oblong leaves and odorless purplish flowers. The plant is found in most parts of the world with a warm climate and is grown in dry, sandy soil. Numerous phytoconstituents were derived from *C. gigantea* [1-4], which has been used in treating a variety of ailments [5-9]. Antioxidants in the plants are responsible for the scavenging activity *via* various mechanisms [10]. Latex is a natural polymer in plants and contains alkaloids, sterols, fatty acids, carbohydrates, tannins, glycosides and enzymes. Latex is well known for its toxic as well as medicinal properties. Plant latex have been reported about their pharmacological, anticancer, antimicrobial and analgesic activities. Latex of *C. gigantea* contains glycosides, fatty acids, sterols and terpenes [11] and the antioxidant activity is due to superoxide dismutase, catalase and glutathione. Phenolics present in *C. gigantea* leaves are known to have radical scavenging activity [12]. Some of the novel compounds identified in *C. gigantea* displayed significant biological activities are cardenolides, lignan glycosides, epidioxysterols and pregnanones [13-15]. Methanolic extract of *C. gigantea* root extract possesses antitumor activity and antitumor effect of anhydrosophoradiol-3-acetate from *C. gigantea* flower is explored by Habib and Karim [16,17]. Calotroposides from root bark of the plant showed inhibitory activity against glioblastoma and prostate cancer cell lines [18]. Biosynthesized silver nanoparticles using *C. gigantea latex* can be used for therapeutic applications [19]. Leaves and roots of *C. gigantea* possess anticonvulsant sedative and muscle relaxant effect [20,21]. These characteristics properties of this species as rich and attractive source of bioactive compounds to be used in drug development.

Imbalance between free radicals generated during cell metabolism and antioxidants results in the production of reactive oxygen species (ROS) which include superoxide anion, hydroxyl radical and peroxy radicals. Generation of ROS lead to a pathological process known as oxidative stress. ROS Diseases due to cell damage through oxidative stress is common in humans and several plant metabolites are endowed with anti-

oxidant activity. Antioxidants repair the damages caused by free radicals thus protecting proteins, enzymes, lipids and nucleic acids. Antioxidants are also lower the risk of degenerative disorders [22]. During oxidative stress, flavonoids are induced in plants indicating their role in ROS homeostasis [23]. Plants exhibit antioxidant activities through their bioactive compounds and many plants were studied for their potent antioxidant activities. With the advancement of technology, elucidation of phytochemicals and biological activities are being explored for treatment of various ailments. Antioxidant capacity is a function of drought tolerance in plants and to acclimate to drought, *Calotropis* is equipped with antioxidants system. The antioxidant activity also interferes with recovery from water limitation and resurrection from dehydration [24]. Drought-induced deregulation of metabolism enhances generation of reactive oxygen species (ROS) which in turn affect the redox regulatory state of the cell. There are many pharmacological reports for *Calotropis procera* and the antioxidant properties of various extract of *C. procera* is reported elsewhere [25-28] however, the chemometric profile of various parts of *Calotropis gigantea* and their antioxidant properties is not compared earlier. In this study, antioxidant effect was compared among latex, leaf and flower extract of *Calotropis gigantea* and is first report on comparing the gas chromatography-mass spectrometry (GC-MS) chemometric profiling of various parts of *Calotropis gigantea*. The findings give hope of searching new and more efficient antioxidant compounds from *Calotropis gigantea*.

EXPERIMENTAL

Plant sample collection: *Calotropis gigantea* collected from Pollachi (10.669823ºN, 76.980639ºE), Tamil Nadu state, India was authenticated and a specimen voucher was deposited at the Herbarium, Department of Botany, Bangalore University, Bangalore, India.

Reagents and standards of analytical grade such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,22-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) [$ABTS^+$] were purchased from Sigma-Aldrich (India).

Extract preparation: Fresh leaves and flowers were collected from *Calotropis gigantea*, washed thoroughly with water, air dried and subjected to chloroform extraction using Soxhlet′s apparatus. Latex was collected from internodes of the plant and immediately subjected to extraction with ethanol. The latex mixture was filtered, air dried and pulverized. Extracts were concentrated under vacuum in a rotary evaporator, dried and stored in vacuum desiccators for further analysis.

Sample preparation for GC-MS analysis: Concentrated chloroform and ethanol extracts (25 mg) were redissolved in the respective solvents, vortexed properly and filtered through 0.22 µm syringe filter. Aliquot sample solution (1 µL) was injected into the GC-MS system for the requisite analysis.

GC-MS analysis: GC-MS analysis of extracts of *Calotropis gigantea* was carried out on DSQ system (Perklin-Elmer Clarus SQ8C) and gas chromatograph interfaced to a mass spectrometer equipped with a DB 5-ms capillary standard non-polar column of 30 m length, 0.25 µm thickness. Helium was used as a carrier gas at a constant flow of 1 mL/min and the temperature was set with initial oven temperature at 60 ºC and held for 2 min and the temperature of the oven was raised to 270 ºC for 10 min (leaf and flower extract) and 350 °C for 20 min (latex extract). The sample of 100 mL was dissolved in 1mL of acetone and injected with split less mode.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: Free radical scavenging activity of the flower extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. The DPPH solution (0.1 mM) in ethanol was prepared and 1mL of this solution was added to 3 mL of extracts solution (or standard) in water at different concentrations. After 30 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH of radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The antioxidant activity was expressed as IC_{50} (concentration of samples necessary to inhibit by 50% the formation of DPPH radicals, in µg/mL)

2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) [$ABTS^+$] radical scavenging assay: $ABTS^+$ radical scavenging activity was determined according to Re *et al.* [29]. $ABTS^+$ radical was freshly prepared by adding 5 mL of 4.9 mM ammonium persulfate solution to 5 mL of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with ethanol (99.5%) to yield an absorbance of 0.70 ± 0.02 at 734 nm and the same was used for assay. To a ABTS radical solution (950 µL), added 50 µL of extract solution (25-500 µg/mL) and the reaction mixture was vortexed. Absorbance was recorded after 6 min at 734 nm and compared with the control ABTS solution. Percentage inhibition was calculated using the following eqn.:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100$$

RESULTS AND DISCUSSION

Investigation of *Calotropis gigantea* was based on traditional use, ecological region and biological reports in the available literature. Plant material was chosen that meet the specific criteria (antioxidant activity) of the study. Solvent was considered based on the type of plant material and appropriate for the compound isolation relevant to the selected criteria. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Hence, ethanol (latex) and chloroform (leaves and flower) were used to give appropriate secondary metabolite yields. GC-MS analysis of solvent extracts of *C. gigantea* revealed the presence of 28 different chemotypes in latex, 27 in leaves and 32 in flowers which were characterized and identified by comparison of their mass fragmentation patterns with the similar in NIST database library. This is the first report on comparing the GC-MS chemometric profiling of the latex, leaf and flower extract of *C. gigantea*. The major

phytochemical groups identified in this study were fatty acid ethyl esters, aromatic carbons and triterpenes. All these compounds identified by GC-MS analysis (Figs. 1-3) were further investigated for their biological activities and most of them were found to possess a diverse range of positive pharmacological actions.

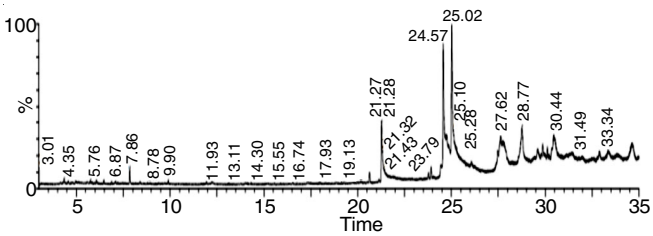

Fig. 1. Chromatogram of *Calotropis gigantea* latex extract

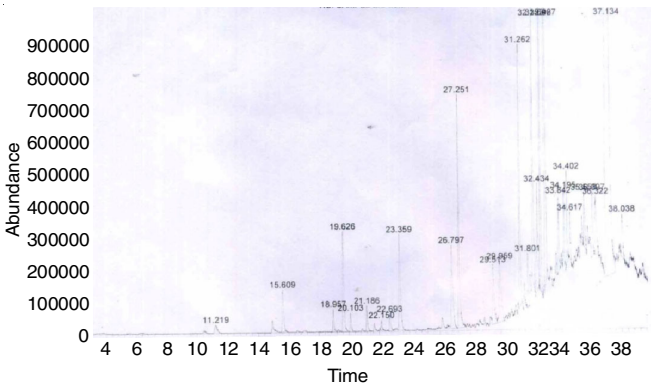

Fig. 2. Chromatogram of *Calotropis gigantea* leaves extract

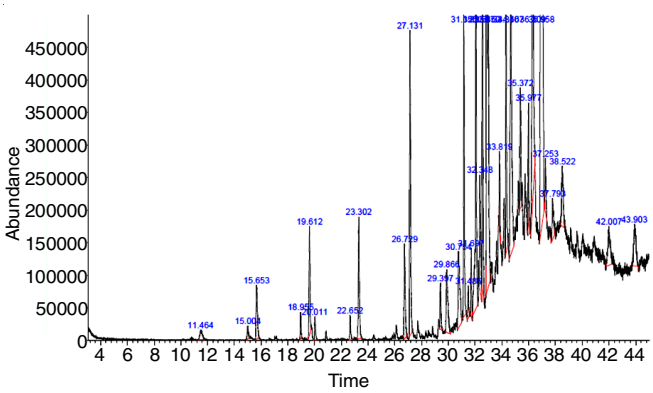

Fig. 3. Chromatogram of *Calotropis gigantea* flower extract

The ethanol extract of *C. gigantea* latex revealed the presence of 36 different chemotypes which were characterized and identified by comparison of mass fragmentation patterns with compounds similar in NIST library. Of the identified compounds, oleic acid (12.88%), octadecanoic acid (10.3%), cholesterol (9.47%), *n*-hexadecanoic acid (8.01%), megastigma-4,6(*E*),8(*Z*)-triene (6.18%), 1*H*-inden-1-one, 2,3-dihydro-3,3,4,6-tetramethyl (5.04%), butyl-6,9,12,15- octadecatetraenoate (3.49%), benzene, 1,2-*bis*(9-borabicyclo[3.3.1]non-9-yloxymethyl) (1.59%) were found to be the major constituents. Among the compunds identified in the ethanol extract of *C. gigantea* latex, fatty acid ethyl esters, terpenes (botulin), coumarins (ethoxy-4-methylcoumarin) and carbocycles (indene) were observed (Table-1). Similarly, fatty acid esters, volatile compound, terpenes were observed by Pattnaik *et al*. [30] from leaf extracts of *Calotropis gigantea.* Specific inhibition of acyl-CoA oxidase-1 and decreased reactive oxygen species by 10,12-tricosadiynoic acid is studied by Zeng *et al*. [31]. Betulin, a triterpene is shown to possesses anticancer properties [32]. In a study by Celikezen *et al*. [33], 6-ethoxy-4-methylcoumarin exhibited a cytotoxic effect and antioxidant activity. Inhibitory activity of protein kinase enzymes by pyrazolo[3,4-*d*]pyrimidines and their role as anticancer agents have been explored [34,35]. Indanes and indenes are carbocycles present in several natural products of biological relevance due to their pharmacological activities [36,37]. Presence of a chemical diversity and bioactivity of the identified compounds are in good agreement with the results of many other researchers.

Chloroform extract of *Calotropis gigantea* leaves revealed the presence of 27 different chemotypes which were characterized and identified (Table-2). Of the identified compounds, *bis*(2-ethylhexyl) phthalate (55.86%), dibutyl phthalate (12.29%), pthalic acid (9.82%), phenol,2,4-*bis*(1,1-dimethyethyl) (3.88%), eiosanal (2.01%), 1-octadecanol (2.52%), behenic alcohol (1.96%), *N*-tetracosanol-1 (1.82%), hexadecanoic acid (1.31%), hexa-butyl selenatrisitane (1.28%), hexa-*t*-butyl selenatrisiletane (1.02%) were found to be the major constituents. Other minor compounds present in the extract were 17-pentatriacontene (0.81%), 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)cyclohexanol (0.78%) and linolenic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.78%).

The chloroform extract of *Calotropis gigantea* flowers was subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The components were identified by comparison of the retention time of GC peaks with those obtained using NIST library and confirmation was done with the reported literature. From the GC-MS analysis, nearly 32 phytochemicals were identified and the results are given in Table-3. Major phytochemicals present in the chloroform extract were *bis*(2-ethylhexyl)phthalate (38.83%), phthalic acid, butyl hex-3-yl ester (11.47%) and nonacosane (8.06%). The minor phytochemicals present in the chloroform extract were hexadecane (0.220%), tricyclo[5.3.1.1(2,6)]dodeca-3,8-diene,11-acetoxy-12-hydroxy-4,5,9-trichloro (0.265%) and benzyl alcohol (0.268%).

Tetradecanol is a fatty alcohol reduced specifically the growth of T cells such as EL-4 T cell and isolated murine CD4+ T cells [38]. Behenic alcohol have therapeutic applications [39]. Tetracosanol had induced antiproliferative effects on CHO-K1 cells and exhibited significantly greater effects on the oncogenic cell lines [40]. DPPH free radical reduction by tetracosanol is investigated by Makhafola *et al*. [41]. In the present study, 32 compounds were identified in the chloroform extract of *C. gigantea* flowers.

DPPH has been widely used to measure the antioxidant property of plants. DPPH free radical scavenging of *C. gigantea* latex, leaves and flower extracts were determined using ascorbic acid as standard (Figs 4-6). Latex extract at the concentration of 10, 20, 30, 40, 50 and 60 µg/mL were used in the study and the highest DPPH scavenging activity of 96% was exhibited

TABLE-1
CHEMICAL CONSTITUENTS PRESENT IN THE ETHANOL EXTRACT OF *Calotropis gigantea* LATEX

Name of the compound	m.f.	Retention time	Percentage of total
Benzeneaceticacid, ethyl ester	$C_{10}H_{12}O_2$	7.855	0.512
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	20.64	0.473
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.28	5.606
à-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3.3.1]non-9-yl]-	$C_{30}H_{51}B_3O_5$	21.54	0.569
Naphthalen-2-yl-acetic acid, 6-hydroxy-6-methyl-cyclo decyl ester	$C_{23}H_{30}O_3$	21.65	0.406
Mannopyranose, 1-O-(trimethylsilyl)-, 2,3:4,6-Dibutaneboronate	$C_{17}H_{34}B_2O_6Si$	21.72	0.491
18-Norcholest-17(20),24-dien-21-oic acid, 16-acetoxy-4,8,14-trimethyl-3,11-dioxo; methyl ester	$C_{32}H_{46}O_6$	21.89	0.378
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	21.95	0.378
10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	23.92	0.571
Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	24.43	0.596
Oleic Acid	$C_{18}H_{34}O_2$	24.57	12.88
Octadecanoic acid	$C_{18}H_{36}O_2$	25.02	9.734
6-Ethoxy-4-methylcoumarin	$C_{12}H_{12}O_3$	26.06	0.510
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	27.47	1.578
Cholesterol	$C_{27}H_{46}O$	27.62	9.474
Megastigma-4,6(E),8(Z)-triene	$C_{13}H_{20}$	28.77	6.180
Benzene, 1,2-*bis*(9-borabicyclo[3.3.1]non-9-yloxymethyl)-	$C_{24}H_{36}B_2O_2$	29.59	1.591
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	29.79	0.375
Glycidyloleate	$C_{21}H_{38}O_3$	29.87	0.914
1H-Indene, 1-hexadecyl-2,3-dihydro-	$C_{25}H_{42}$	30.12	0.446
1H-Inden-1-one, 2,3-dihydro-3,3,4,6-tetramethyl-	$C_{13}H_{16}O$	30.46	5.047
7H-Pyrazolo[4,3-d]pyrimidin-7-one, 1,6-dihydro-3-ribofuranosyl-	$C_{10}H_{12}N_4O_5$	31.3	0.480
1H-Cyclopropa[3,4]benz[1,2-e] azulene-a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9atriacetate	$C_{28}H_{38}O_{10}$	31.35	0.441
Betulin	$C_{30}H_{50}O_2$	31.46	0.922
Olean-12-ene-3,16,21,22,28-pentol	$C_{30}H_{50}O_5$	31.96	0.380
10,12-Tricosadiynoic acid, TMS derivative	$C_{26}H_{46}O_2Si$	32.89	0.860
5,16,20-Pregnatriene-3beta,20-diol diacetate	$C_{25}H_{34}O_4$	33.4	0.530
Butyl 6,9,12,15-octadecatetraenoate	$C_{22}H_{36}O_2$	34.64	3.493

TABLE-2
CHEMICAL CONSTITUENTS PRESENT IN THE CHLOROFORM EXTRACT OF *Calotropis gigantea* LEAVES

Name of the compound	m.f.	Retention time	Percentage of total
Cyclotetra decane	$C_{14}H_{28}$	11.219	0.228
1-octadecanol	$C_{18}H3_8O$	15.609	0.833
Octadecane	$C_{18}H_3$	18.957	0.295
1-octadecanol	$C_{18}H_{38}O$	19.626	1.695
L-Arginine, N [phenyl methoxy carbonyl	$C_{14}H_{20}N_4O_4$	20.103	0.267
3,7,11,15,-tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	21.186	0.392
3,7,11,125,tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	22.150	0.149
Eicosane	$C_{20}H_{42}$	22.693	0.321
Eicosanal	$C_{20}H_{42}O$	23.359	2.006
Behenic alcohol	$C_{22}H_{46}O$	26.797	1.964
Phenol,2,4-*bis*(1,1-dimethyethyl)	$C_{14}H_{22}O$	27.251	3.887
Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	29.513	1.313
N-tetracosanol-1	$C_{24}H_{50}O$	29.959	1.820
Phthalic acid, hept-4-yl-2-isobutyl ester	$C_{19}H_{28}O_4$	31.801	0.581
Phthalic acid, butyl 2-pentyl ester	$C_{17}H_{24}O_4$	31.262	3.319
Di butyl phthalate		32.153	3.386
Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	32.434	2.040
Phthalic acid butyl-2-pentyl ester	$C_{17}H_{24}O_4$	32.642	3.895
Dibutyl phthalate	$C_{16}H_{22}O_4$	32.967	8.913
Linolenic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{36}O_4$	33.038	0.782
Hexa-*t*-butyl selenatrisiletane	$C_{24}H_{54}SeSi_3$	33.842	1.024
17-pentatria contene	$C_{35}H_{70}$	34.195	0.813
Nonacosane	$C_{29}H_{60}$	34.617	0.430
2,2,4-Trimethyl-3-(3,8,12,16,-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	35.558	0.783
Octadecanoic acid, 2-hydroxy-1,3-propanediyl	$C_{39}H_{76}O_5$	36.097	0.749
Hexa-butyl selenatrisitane	$C_{24}H_{54}SeSi$	36.322	1.281
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	37.134	55.862

TABLE-3
CHEMICAL CONSTITUENTS PRESENT IN THE CHLOROFORM EXTRACT OF *Calotropis gigantea* FLOWERS

Name of the compound	m.f.	Retention time	Percentage of total
1-Tetradecanol, methyl ether	$C_{15}H_{32}O$	11.464	0.335
Hexadecane	$C_{16}H_{34}$	15.006	0.220
1-Nonadacene	$C_{19}H_{38}$	15.652	0.885
Octadecane	$C_{18}H_{38}$	18.954	0.244
1-Octadecanol	$C_{18}H_{38}O$	19.612	1.269
Benzyl alcohol	C_7H_8O	20.012	0.268
Eicosane	$C_{20}H_{42}$	22.650	0.300
Behenic alcohol	$C_{22}H_{46}O$	23.303	1.515
n-Tetracosanol-1	$C_{24}H_{50}O$	26.730	1.174
Phenol, 2,4-*bis*(1,1-dimethyethyl)	$C_{14}H_{22}O$	27.131	3.542
Hexadecanoic acid,butyl ester	$C_{20}H_{40}O_2$	29.397	0.497
Tetracosanol	$C_{24}H_{50}O$	29.866	1.175
Heptacosane	$C_{27}H_{56}$	30.753	1.483
Phthalic acid, hept-4-yl iso butyl ester	$C_{19}H_{28}O_4$	31.153	2.912
Tricyclo[5.3.1.1(2,6)] dodeca-3,8-diene,11-acetoxy-12-hydroxy-4,5,9-trichloro	$C_{14}H_{15}O_3Cl_3$	31.485	0.265
Phthalic acid, hex-3-yl isobutyl ester	$C_{18}H_{26}O_4$	31.697	0.521
Dibutyl phthalate	$C_{16}H_{22}O_4$	32.057	4.074
Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	32.349	1.302
Phthalic acid, Butyl 2-pentyl ester	$C_{17}H_{24}O_4$	32.544	3.210
Phthalic acid, Butyl hex-3-yl ester	$C_{18}H_{26}O_4$	32.870	11.472
Nonacosane	$C_{29}H_{60}$	33.820	0.714
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	34.312	2.550
Nonacosane	$C_{29}H_{60}$	34.666	6.085
Nonacosane	$C_{29}H_{60}$	35.370	1.196
Octadecanoic acid	$C_{18}H_{38}O_2$	35.977	1.203
Nonacosane	$C_{29}H_{60}$	36.309	8.063
Bis(2-ethylhexyl) Phthalate	$C_{24}H_{38}O_4$	36.955	38.827
Octadecane, 3-ethyl-5-(2-ethyl butyl)-	$C_{26}H_{54}$	37.253	0.328
5*H*-cyclopropa(3,4)benz(1,2-e)azulen-5-one, 1,1a-α,1b-β,4,4a,7a-α,7b,8,9,9a-decahydro-4a-β,7b-α,9a-α-trihydroxy-3-(hydroxy methyl)-1,1,6,8α-tetramethyl, 9a-isobutyrate	$C_{24}H_{34}O_6$	37.791	0.789
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	38.523	1.066
9,10-secocholesta-5,7,10(19)-triene-1, 3-diol, 25-[(trimethylsilyl)oxy]-, (3β,5Z,7E)-	$C_{30}H_{52}O_3Si$	42.008	1.201
Heptaethylene glycol monododececyl ether	$C_{26}H_{54}O_8$	43.902	1.313

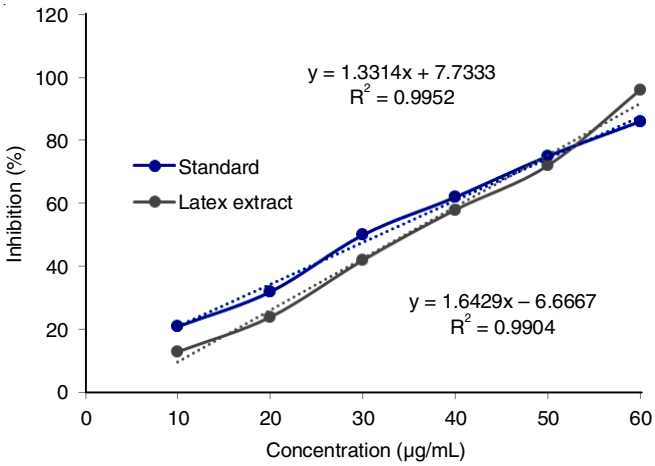

Fig. 4. DPPH radical scavenging activity of *Calotropis gigantea* latex

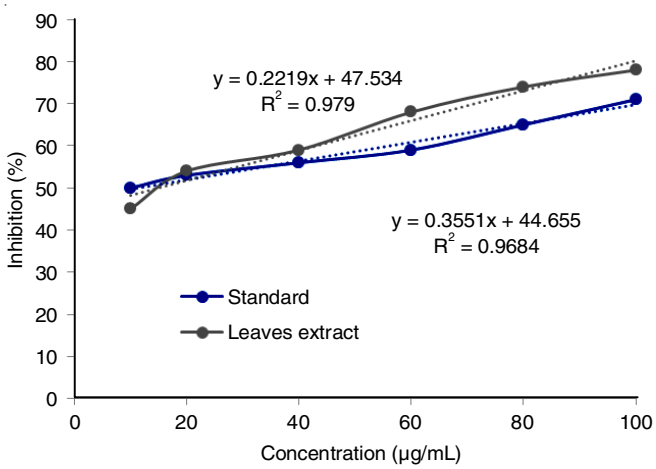

Fig. 5. DPPH radical scavenging activity of *Calotropis gigantea* leaves

at 60 µg/mL concentration. For both leaves and flower extracts, the concentrations tested were 10, 20, 40, 60, 80 and 100 µg/mL as the lower concentrations were not showing higher scavenging activity. In both the cases, scavenging activity was increased at higher concentrations of extract tested. *Calotropis gigantea* leaf extract recorded 96% of scavenging activity at 100 µg/mL concentration where as it was 57% by flower extract. Free radical scavenging activity of *Calotropis gigantea* was studied by other researchers and DPPH free radical scavenging of *C. gigantea* leaves was 85.17% at 400 µg/mL [42] and the IC_{50} value of whole plant was 54.29 µg/mL [43], whereas IC_{50} of 1.7 mg/mL of ethanol extract of *C. gigantea* leaves was

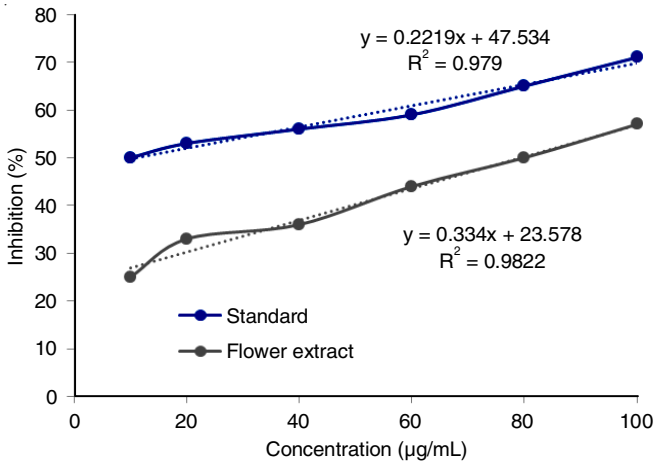

Fig. 6. DPPH radical scavenging activity of *Calotropis gigantea* flowers

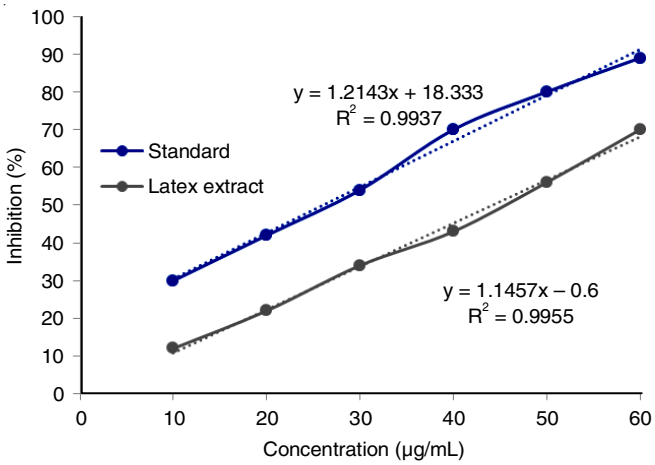

Fig. 8. ABTS⁺ activity of *Calotropis gigantea* latex

reported by Gacche *et al*. [44]. Patel *et al*. [45] observed 68.52 µg/mL free-radical scavenging activity in terms of percent inhibition of DPPH radical in *C. gigantea* leaves. In this study, IC_{50} of 6.74 µg/mL of chloroform extract of *C. gigantea* leaves was observed (Fig. 7). It is well known that low-polarity constituents are not only a major energy source but also containing many biological activities such as antioxidant activity. Highest antioxidant activity of 65.89% at 800 mg/kg from stem extract was reported by Jayakumar *et al*. [46]. Joshi *et al*. [43] reported reducing power of *C. gigantea* leaves as 56.34 mg ascorbic acid equivalent per gram of extract. Chloroform extract of *C. gigantea* on tissue antioxidants was investigated in streptozotocin-induced oxidative damage in rats by Choudhary *et al*. [47]. The results concluded the protection of β cells against reactive oxygen species mediated damage by enhancing cellular antioxidant defense.

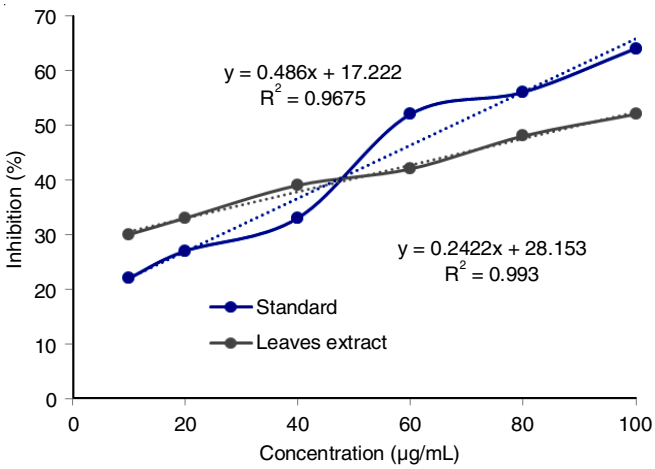

Fig. 9. ABTS⁺ activity of *Calotropis gigantea* latex

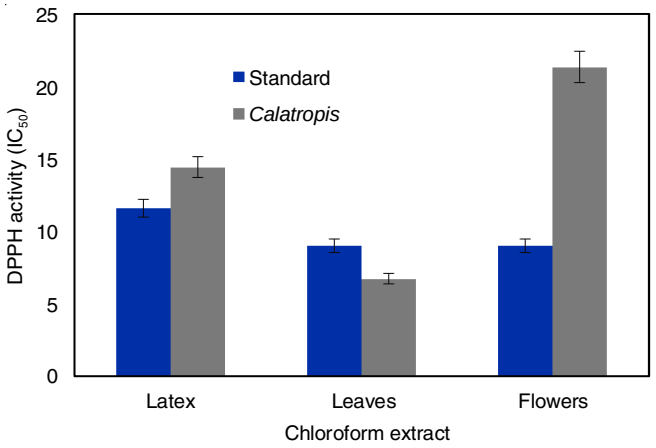

Fig. 7. IC_{50} values for DPPH scavenging activity

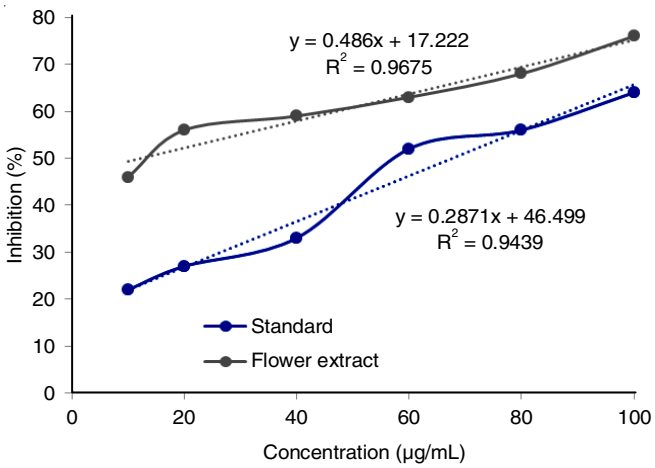

Fig. 10. ABTS⁺ activity of *Calotropis gigantea* flowers

2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) [ABTS⁺] radical scavenging assay are among the most abundant antioxidant capacity assays and is based on interaction between an antioxidant and the pre-generated ABTS⁺ radical cation. Among the extracts tested, *C. gigantea* latex extract exhibited highest ABTS⁺ activity of 76% at 100 µg/mL concentration (Figs. 8-10). The IC_{50} values ranged between 7.91 and 15.07 µg/mL by various components of *C. gigantea* (Fig. 11). The presence of flavonoids and terpenoids present in flower extract is responsible for its antioxidant activity [48].

There is dynamic equilibrium between production and elimination of free radicals in the body under normal conditions. However, if free radical removal function is suppressed, the generated free radicals cause inevitable causes damage to the body. Long term administration of synthetic antioxidants is associated with side effects and there is a need for safe natural

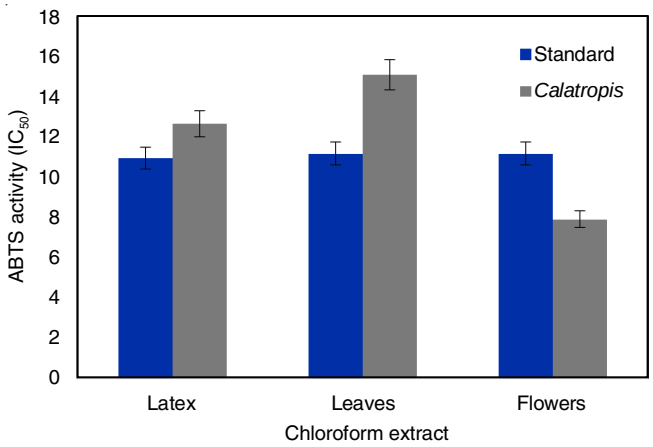

Fig. 11. IC_{50} values for $ABTS^+$ scavenging activity

products with minimal adverse effect to replace synthetic antioxidants. $ABTS^+$ radical assay revealed ethanol extracts of *C. gigantea* had obvious antioxidative and free radical scavenging effect *in vitro* as determined by half inhibitory concentration (IC_{50}) of ABTS. Both DPPH and $ABTS^+$ assay represent free radical-scavenging activities of extracts of several parts of *C. gigantea* and support further studies for estimating their biological effects. Apart from this, there were other bioactive compounds identified from *Calotropis gigantea* in this study which are known to possess pharmacological activities. The findings revealed the potential use of various parts of *Calotropis gigantea* in combating various diseases.

Conclusion

The investigation concludes that the compounds present in *Calotropis gigantea* latex, leaves and flowers have potential to perform antioxidant activity. These findings will definitely use in new directions in pharmacological and therapeutic investigations on *Calotropis gigantea* latex, leaves and flowers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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