J. Indian Chem. Soc.,
Vol. 97, No. 11a, November 2020, pp. 2311-2320

Correlation between *in vitro* antioxidant activity and GC-MS evaluation of *Calotropis gigantea* (L.) R.Br. root extract

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Manuscript received online 22 October 2020, revised and accepted 10 November 2020

The present experiment was performed to evaluate the antioxidant potential and to identify the different bio-active components present in the methanol root extract of *Calotropis gigantea*. The preliminary phytochemical identification of *Calotropis gigantea* root extract was carried out, as to confirm the presence of primary and secondary metabolites. Antioxidant activity of methanolic root extract was evaluated using 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide radical scavenging assays. Phytochemical analysis revealed the presence of phenolic compounds, tannins, saponin, flavonoids, terpenoids, steroids and carbohydrates in root extract of *Calotropis gigantea*. Methanol root extract of *C. gigantea* possessed significant *in vitro* antioxidant activity when compared with ascorbic acid, as standard. And the GC-MS showed twelve chemical components present in methanol root extract of the plant. The above research concluded that the plant does contain different phytochemical components, especially phenolic compounds and flavonoids as of their pharmaceutical importance and become a crucial part in arresting the progression of several diseases and disorders.

Keywords: Extract, GC-MS, phytochemical, methanol, root.

Introduction

India is considered as a largest producer of herbal medicine, and called as "Botanical garden of the World"[1]. The herbs are used for the formulation of various effective traditional medicine, that contain a wide range of phyto-components used to cure and treat several diseases[2].

Calotropis gigantea (L.) R.Br. is a perennial herb, commonly called as "madar" in hindi; "crown flower" or "giant milk weed" in english, belongs to family *Asclepiadaceae*[3]. The plant is distributed abundantly in tropical and even in warm climatic regions. Traditionally the plant is widely used to treat various diseases including skin diseases, fever, rheumatism, cough, cold, asthma, nausea, vomiting, diarrhea, elephantiasis and indigestion. It is believed that the whole plant is used as an expectorant, depurative, anthelmintic and tonic[4]. Root bark is used as depurative, anthelmintic, expectorant, laxative and febrifuge. The powdered form of root is used in the treatment of bronchitis, dyspepsia and asthma. Plant leaves and flowers are used in treating intermittent fevers, paralysis, inflammations, arthralgia, digestant, astringent, stomachic and tonic[5].

Herbal extraction is the simple method, which is rapid and inexpensive. The GC-MS (gas chromatography-mass chromatography) analysis is an interesting tool can be used to identify the amount of active phyto-components present in the obtained extracts which is utilized in drugs, cosmetic, food and pharmaceutical industry[6]. This technique involves combination of two analytical methods to analyze complex mixtures of chemical components. GC chromatography used to isolate the active principles from mixtures, and then the mass spectroscopy helped in analyzing each separated out phyto-chemcials. Chemical investigation has reported that the roots of the plant contains naphthalene derivative, calotropnaphthalene, two terpene derivatives, calotropise squiterpenol, calotropises terterpenol, an aromatic compound, calotropbenzofuranone along with sucrose[7].

Root bark contained triterpene esters, lupenyl-1-acetate[8]; stigmasterol[9], β-sitosterol, β-sitosterolacetate[10]; resin, β-amyrin, β-amyrin actate[11]; fatty acids, isovaleric acid[12].

With this respect, still now no such antioxidant activity along with the identification and characterization of active ingredients have been performed on *Calotropis gigantea* root. Therefore, the current study was carried out to identify the active principles present in the methanol root extract of *Calotropis gigantea* (L.) R.Br. using GC-MS analytical technique and its antioxidant approach was calculated via *in vitro* free radical scavenging assays. Even a statistical relationship has been measured between different variables including total phenolic content, total flavonoid content and antioxidant potential.

Results and discussion

Percentage extractive yield:

About 18.02 g (12.01% w/w) semi-solid crude methanolic extract of *C. gigantea* was obtained from 150 g of coarsely powdered roots of the plant using continuous hot extraction.

Preliminary phytochemical screening for primary and secondary metabolites:

Preliminary phytochemical screening showed the presence of primary and secondary metabolites, including carbohydrates, phenolic compounds, tannins, flavonoids, saponin, terpenoids, and steroids in the root extract of *Calotropis gigantea* as detailed in Table 1.

Table 1. Preliminary phytochemical screening of methanol root extract of *Calotropis gigantea* (L.) R.Br. root

Sl. No.	Plant metabolites	Identification tests	Observations
1.	Primary	Carbohydrates	+
2.		Protein and amino acids	–
3.		Fats and oils	–
4.	Secondary	Volatile oils	–
5.		Terpenoids and steroids	+
6.		Alkaloids	–
7.		Glycosides	–
8.		Tannins and phenolic compounds	+
9.		Saponin glycosides	+
10.		Flavonoids	+
11.		Resins	–

'+': presence; '–': absent.

Determination of total phenolic content and total flavonoid content:

Total phenolic content of root extract is presented in the Table 2 and expresses as mg/g gallic acid equivalent (GAE). The linear regression equation which is obtained from the standard plot of gallic acid (shown in Fig. 1) is given below as:

$y = 0.004x + 0.0559$; $R^2 = 0.9916$

where y = absorbance and x = the amount of gallic acid (μg).

Table 2. Total phenolic and flavonoid content of methanol root extract of *Calotropis gigantea* (L.) R.Br.

Extract	TPC (mg GAE/g root extract wt)	TFC (mg QE/g root extract wt)
Calotropis gigantea (L.) R.Br. root	70.525±0.50	47.247±0.44

n = 3; TPC: total phenolic content; TFC: total flavonoid content; GAE: gallic acid equivalent; wt: weight; QE: quercetin. Values are expressed as mean ±SD.

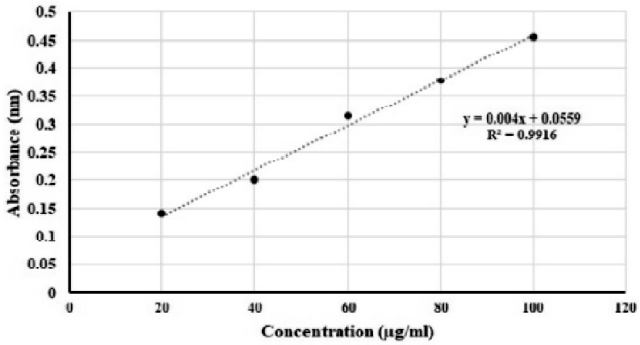

Fig. 1. Calibration of gallic acid.

Methanol root extract was observed to contain the satisfactory amount of phenolic compounds (70.525±0.50 mg of GAE/g of the plant extract). The total flavonoid content of root extract was calculated using linear regression equation from the standard plot of quercetin (as shown in Fig. 2) and are expressed as mg/g of quercetin equivalent (QE).

$y = 0.0035x + 0.0123$; $R^2 = 0.9902$

where y = absorbance and x = the amount of quercetin (μg).

The amount of total flavonoid content found present in the root extract of the plant was 47.247±0.44 mg of QE/g of the plant extract.

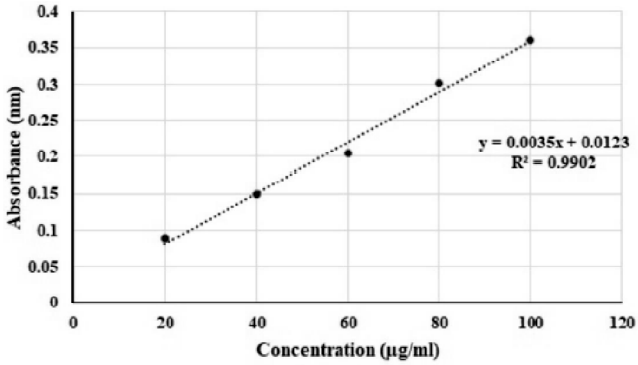

Fig. 2. Calibration of quercetin.

In vitro antioxidant assay:

The antioxidant activity of *C. gigantea* root extract was evaluated by using commonly employed free radical scavenging assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide. Radical scavenging effect of the plant on DPPH and hydrogen peroxide (H_2O_2) radicals was expressed as % inhibition (percentage inhibition) and compared against ascorbic acid, as standard (as shown in Fig. 3 and Fig. 4). The half maximal inhibitory concentration of the plant was observed as 48.91 as compared to the standard, ascorbic acid (IC50 30.51). Maximum antioxidant activity measured by hydrogen peroxide was found 100 µg/mL. The half maximal inhibitory concentration of the methanolic root extract of *Calotropis gigantea* was 73.02 (tabulated in Tables 3 and 4).

Correlation between TPC, TFC and antioxidant activity using Pearson correlation coefficient analysis:

The correlation between TPC, TFC and *in vitro* antioxidant activity of methanol root extract was statistically evaluated and observed using Pearson correlation coefficient as shown in Table 5. Positive correlation was observed among

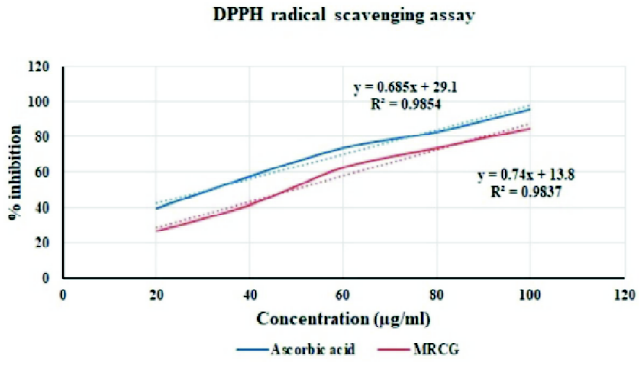

Fig. 3. *In vitro* antioxidant activity: DPPH free radical scavenging assay of ascorbic acid and *Calotropis gigantea*. MRCG: methanolic root extract of *Calotropis gigantea;* % inhibition: percentage inhibition.

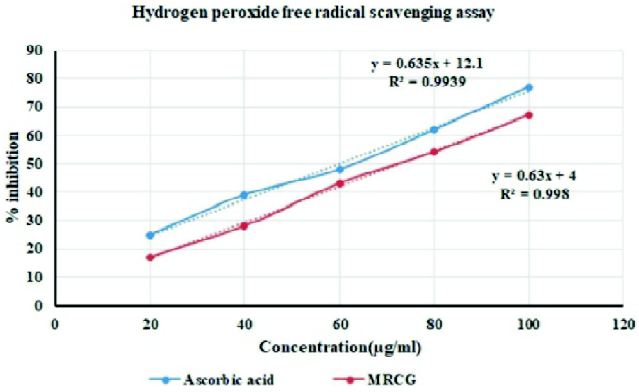

Fig. 4. *In vitro* antioxidant activity: Hydrogen peroxide free radical scavenging assay of ascorbic acid and *Calotropis gigantea*. MRCG: methanolic root extract of *Calotropis gigantea;* % inhibition: percentage inhibition.

Table 3. Percentage inhibition of *Calotropis gigantea* root and ascorbic acid using DPPH assay

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	Methanol root extract of *C. gigantea*
20	40.02±0.23	27.13±0.47
40	58.12±0.11	42.02±0.15
60	74.10±0.12	63.38±0.02
80	83.40±0.18	74.41±0.46
100	96.32±0.08	85.39±0.92
IC_{50}	30.51	48.91

n = 3; IC_{50} = half maximal inhibitory concentration. Values are expressed as mean ±SD.

Table 4. Percentage inhibition of *Calotropis gigantea* root and ascorbic acid using hydrogen peroxide assay

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	Methanol root extract of *C. gigantea*
20	25.09±0.008	14.021±0.09
40	39.11±0.12	23.12±0.12
60	48.23±0.32	34.01±0.14
80	62.03±0.21	53.16±0.32
100	77.26±0.01	64.09±0.67
IC_{50}	59.69	73.02

n = 3; IC50 = half maximal inhibitory concentration. Values are expressed as mean ±SD.

Table 5. Pearson correlation coefficient of TPC, TFC and *in vitro* antioxidant parameters observed in *Calotropis gigantea*

	TPC	TFC	DPPH	H_2O_2
TPC	1			
TFC	0.540285	1		
DPPH	0.656153	0.987875	1	
H_2O_2	0.842822	0.756016	0.800122	1

TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay; H_2O_2: hydrogen peroxide radical scavenging assay.

all the parameters employed in the experimental work. TPC revealed to contain maximum correlation (r = 0.84) to H_2O_2 radical scavenging assay and; on other side it possesses satisfactory correlation towards TFC (r = 0.54) and DPPH free radical scavenging assay (r = 0.60). TFC showed perfect positive correlation (r = 0.99) with DPPH assay and marked correlation (r = 0.76) with H_2O_2 assay. However, both the free radical scavenging assay contained significant level of correlation (r = 0.80) with each other.

GC-MS analysis of bioactive components:

The different phyto-components present in the root extract of the plant were identified using GC-MS as summarized in Table 6. The methanolic root extract of *Calotropis gigantea* contained *trans*-tetrahydrofuran-3,4-diol; glycerin; α-L-galactopyranoside, methyl-6-deoxy; 2-*p*-nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose; 1,3-butadiene-1-carboxylic acid; ethanedioic acid, bis(trimethylsilyl)ester; cyclopentane undecanoic acid, methyl ester; 3-nitropropanoic acid; butoxyacetic acid; 7-nonenoic acid, methyl ester; 3-decen-1-ol, (*E*); 3,5-dimethyl-5-hexen-3-ol.

However, the technique confirmed various phytochemical components along with their retention times were detailed in Fig. 5. The biological activity of twelve identified compo-

Table 6. Bio-active components identified in the methanol root extract of *Calotropis gigantea* by GC-MS analysis

Sl. no.	RT (min)	Name of compound	Molecular formula	Molecular weight	Area%	Nature of compound
1.	4.01	*trans*-Tetrahydrofuran-3,4-diol,	$C_4H_8O_3$	104.1	0.12	Alcohol
2.	4.07	Glycerin	$C_3H_8O_3$	92.09	0.35	Alcohol
3.	4.11	α-L-Galactopyranoside, methyl-6-deoxy	$C_7H_{14}O_5$	178.183	0.28	Alcohol
4.	9.30	2-*p*-Nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose	$C_{33}H_{31}NO_8$	569.6	0.57	Carbohydrate
5.	9.41	1,3-Butadiene-1-carboxylic acid	$C_5H_6O_2$	98.09	4.04	Acid
6.	10.92	Ethanedioic acid, bis(trimethylsilyl)ester	$C_8H_{18}O_4Si_2$	234.4	7.01	Ester
7.	14.18	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268.4	3.05	Ester
8.	14.45	3-Nitropropanoic acid	$C_3H_5NO_4$	119.08	2.36	Acid
9.	14.56	Butoxyacetic acid	$C_6H_{12}O_2$	132.16	44.62	Acid
10.	15.33	7-Nonenoic acid, methyl ester	$C_{10}H_{18}O_2$	170.25	5.64	Ester
11.	15.71	3-Decen-1-ol, (*E*)	$C_{10}H_{20}O$	156.26	21.21	Alcohol
12.	16.24	3,5-Dimethyl-5-hexen-3-ol	$C_8H_{16}O$	128.21	0.99	Alcohol

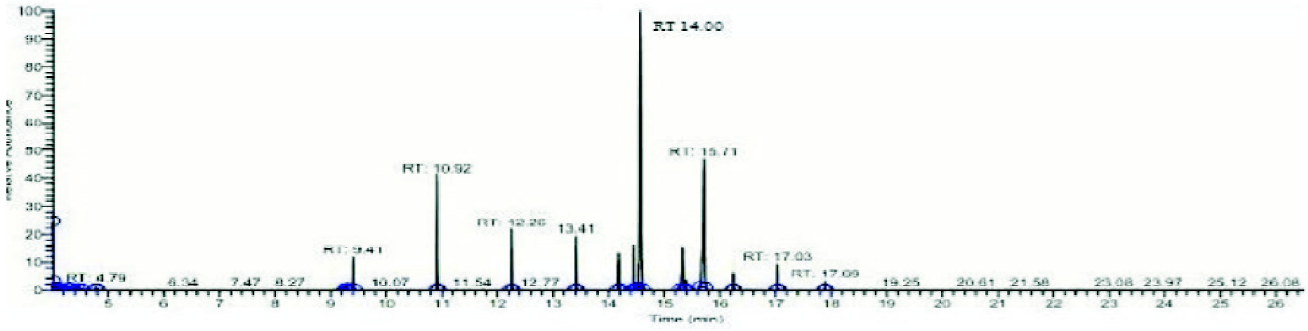

Fig. 5. GC-MS chromatogram of methanol extract of *Calotropis gigantea* (L.) R.Br. root.

nents from *Calotropis gigantea* were analyzed by Duke's[13] and discussed in Table 7. The fragmentation of large components into small compounds may lead to the formation of different peaks at *m/z* ratios. The obtained spectra were considered as a fingerprint of such compounds which can be identified from the data library. Even the spectra helped in predicting the mass formula and structure of twelve phyto-components as summarized in Figs. 6, 7, 8. The future findings of this detailed work is to screened out the pharmacological activity of isolated bioactive compounds, which will be considered as an important tool for novel drug formulation.

Table 7. Biological activity of identified phyto-components of *Calotropis gigantea*

Sr. No.	Name of compound	Activity
1.	*trans*-Tetrahydrofuran-3,4-diol	Transdermal
2.	Glycerin	Moisturizer
3.	α-L-Galactopyranoside, methyl-6-deoxy	Anticancer, antioxidant, Antidote (Lead)
4.	2-*p*-Nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose	DNA protective, anticancer, antileukotriene, CNS Depressant, coronary dilator
5.	1,3-Butadiene-1-carboxylic acid	Acidifier, urinary acidulant
6.	Ethanedioic acid, bis(trimethylsilyl)ester	Acidifier, acidulant
7.	Cyclopentaneundecanoic acid, methyl ester	Acidifier, acidulant
8.	3-Nitropropanoic acid	Urine acidifier, urinary acidulant
9.	Butoxyacetic acid	Acidifier, acidulant
10.	7-Nonenoic acid, methyl ester	Methyl donor, urine acidifier, urinary acidulant
11.	3-Decen-1-ol, (*E*)	Oligosaccharide provider, anticancer, antitennis-elbow, anti-tumour(esophagous), ecbolic, edemagenic
12.	3,5-Dimethyl-5-hexen-3-ol	Oligosaccharide provider

Discussion

Diversity of herbs contain number of bioactive components, known for exhibiting pharmacological activity[14]. Most of the secondary metabolites from herbs are of significant nature as an antioxidant property preferably over synthetic drugs because of fewer or negligible amount of side effects[15]. Such secondary metabolites have the capability to decline the progression and severity of life threatening diseases including diabetes, cancer, hepato-injury, through free radical scavenger mechanism[16].

The experimental results of phytochemical screening revealed the presence of bioactive components in methanolic root extract of *C. gigantea* namely. Furthermore, many reports which are available on polyphenolic components exerted potential therapeutic activities like antidiabetic, antioxidant, anti-inflammatory , antibacterial etc.[17,18]. Mainly, the antioxidant property of phenolic components is due to their free radical scavenging activity in which neutralize the reactive oxygen species[19]. According to the literature survey, it has found that tannins and flavonoids are the most propitious polyphenolic component among secondary metabolites[20]. On the basis of preliminary phytochemical screening results, both the total phenolic content and flavonoid content of the plant extract were determined and thereby the plant's antioxidant activity was evaluated using DPPH and hydrogen peroxide free radical scavenging assay.

Methanol extract of *C. gigantea* root contained significant total phenol (70.525±0.50 mg GAE/g root extract wt) and total flavonoid content (47.247±0.44 mg QE/g root extract wt), respectively.

Free radical scavenging activity of the plant *C. gigantea* root was determined using *in vitro* assays, including DPPH and hydrogen peroxide free radical scavenging methods. The scavenging activity of the extract was compared with the ascorbic acid used as standard. In the DPPH assay, the intensity of discoloration of DPPH solution represents the scavenger property of the test sample. With all the experiment results, the plant extract contains various primary and secondary metabolites like carbohydrates, phenolic compounds, tannins, flavonoids, saponin, terpenoids, and steroids etc. Therefore, these bioactive ingredients have the capability to discolor the DPPH solution[21]. In case of hydrogen peroxide assay, hydroxyl radical would get generate and may leads to cytotoxicity. Thus, any component can scavenge the hydrogen peroxide, may have the possibility to protect the living being[22]. Such activities are reliable on the concentration of the extracts. It is concluded that the antioxidant property of the plant may be due to the polyphenolic components.

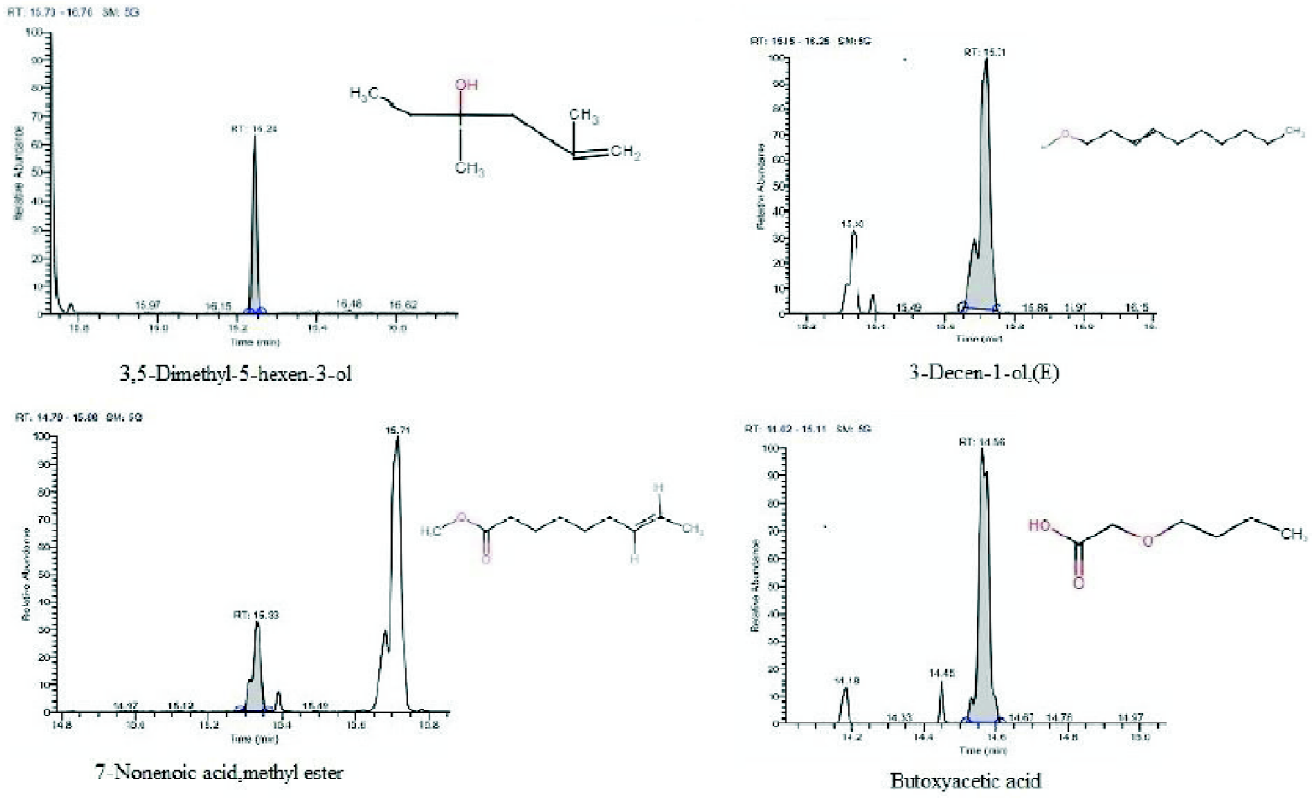

Fig. 6. GC-MS spectra of identified compound from *Calotropis gigantea* (L.) R.Br. root extract: 3,5-Dimethyl-5-hexen-3-ol-3 decen-1-ol, (*E*); 7-nonenoic acid, methyl ester; butoxyacetic acid; 3-nitropropanoic acid.

A total number of twelve bioactive chemical components were identified and their structural elucidation were characterized in the methanol root extract using GC-MS analysis. Results of preliminary phytochemical screening and GC-MS analysis revealed that *C. gigantea* roots contained number of bioactive primary and secondary ingredients, represented various group of chemical compounds such as carbohydrates, phenolic compounds, tannins, flavonoids, saponin, terpenoids, and steroids.

On the quantification analysis, it is concluded that the root extract exhibited satisfactory antioxidant activity against DPPH and hydrogen peroxide radical scavenging models as they are rich in polyphenolic components. Based on the above results, it can be summarized that *C. gigantea* root may be used as a source of antioxidant ingredient in many novel formulations that ultimately provide good health to human being by providing protection against oxidative stress. For further future findings, both *in vitro* as well as *in vivo* studies along with the isolated bioactive components are required to enhance more novel diseases preventing and curing strategies.

Materials and methods

Chemicals used during the experiment:

DPPH, gallic acid and quercetin, all these chemicals were procured from Sigma-Aldrich. And other chemicals and reagents utilized during the study were of pure analytical grade.

Plant material collection and authentication:

Roots of *Calotropis gigantea* were collected from the local areas of Kolkata, West Bengal and authenticated with the help of Dr. V. Sampath Kumar, Botanist, Botanical Survey of India, Government of India, Howrah, West Bengal. The voucher specimen was preserved for further findings and references.

Plant material extraction method:

Around 150 g of dried coarse powdered of *C. gigantea* root was subjected to methanol extraction using Soxhlet

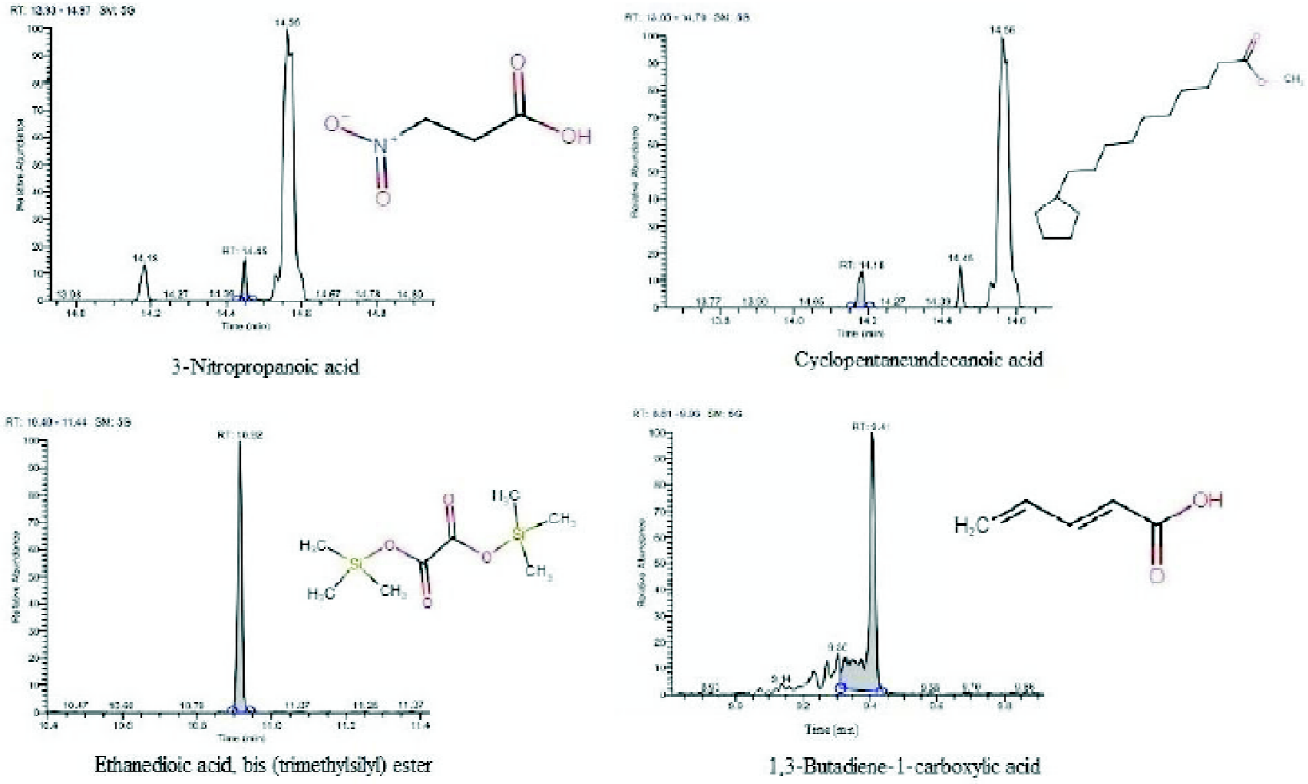

Fig. 7. GC-MS spectra of identified compound from *Calotropis gigantea* (L.) R.Br. root extract: Cyclopentaneundecanoic acid, methyl ester; ethanedioic acid, bis(trimethylsilyl)ester; 1,3-butadiene-1-carboxylic acid.

extractor for 48 h. The resultant extract was concentrated to dryness under vacuum using rotary evaporator and stored in an airtight container for future use[23,24].

Preliminary phytochemical screening:

Methanolic root extract of the plant was evaluated for preliminary phytochemical screening of primary and secondary metabolites as described by Kokate[24–26].

Determination of total phenolic content:

Evaluation of total phenolic content of methanolic root extract of the plant was carried out using Folin-Ciocalteau assay. Gallic acid at a concentration range of 20–100 µg/ml was used as a standard for plotting standard calibration curve. 1 ml of the plant extract or the standard, gallic acid with the concentration range from 20–100 µg/ml were allowed to mix with 1.5 ml of Folin-Ciocalteau reagent, followed by the incorporation of 2.5 ml of 7% w/v sodium carbonate solution. After the addition, the mixtures were kept in a dark place for around 30 min with random shaking. After 30 min of incubation period, the absorbance of resulting color was evaluated at 765 nm using UV-Vis spectrophotometer[27]. Total phenolic content of sample was calculated using linear regression equation while plotting the standard graph of gallic acid. The results were calculated as mean ±SD in triplicate form and were expressed as mg/g gallic acid equivalent of plant extract.

Determination of total flavonoid content:

The total flavonoid content of plant extract was estimated using aluminium chloride method. In this, quercetin as flavonoid was used as a standard for the construction of calibration curve. With the concentration range from 20–100 µg/ml of quercetin or 1 mg/ml of plant extract in 2 ml of methanol were mixed with 1 ml of aluminium chloride (10%), 0.1 ml of 1 *M* of potassium acetate and 3 ml of distilled water, respectively. The mixtures were then incubated at room temperature for 30 min and then the absorbance was measures using UV-Vis spectrophotometer at 415 nm against blank. A blank solution contained all the reagents but devoid of alu-

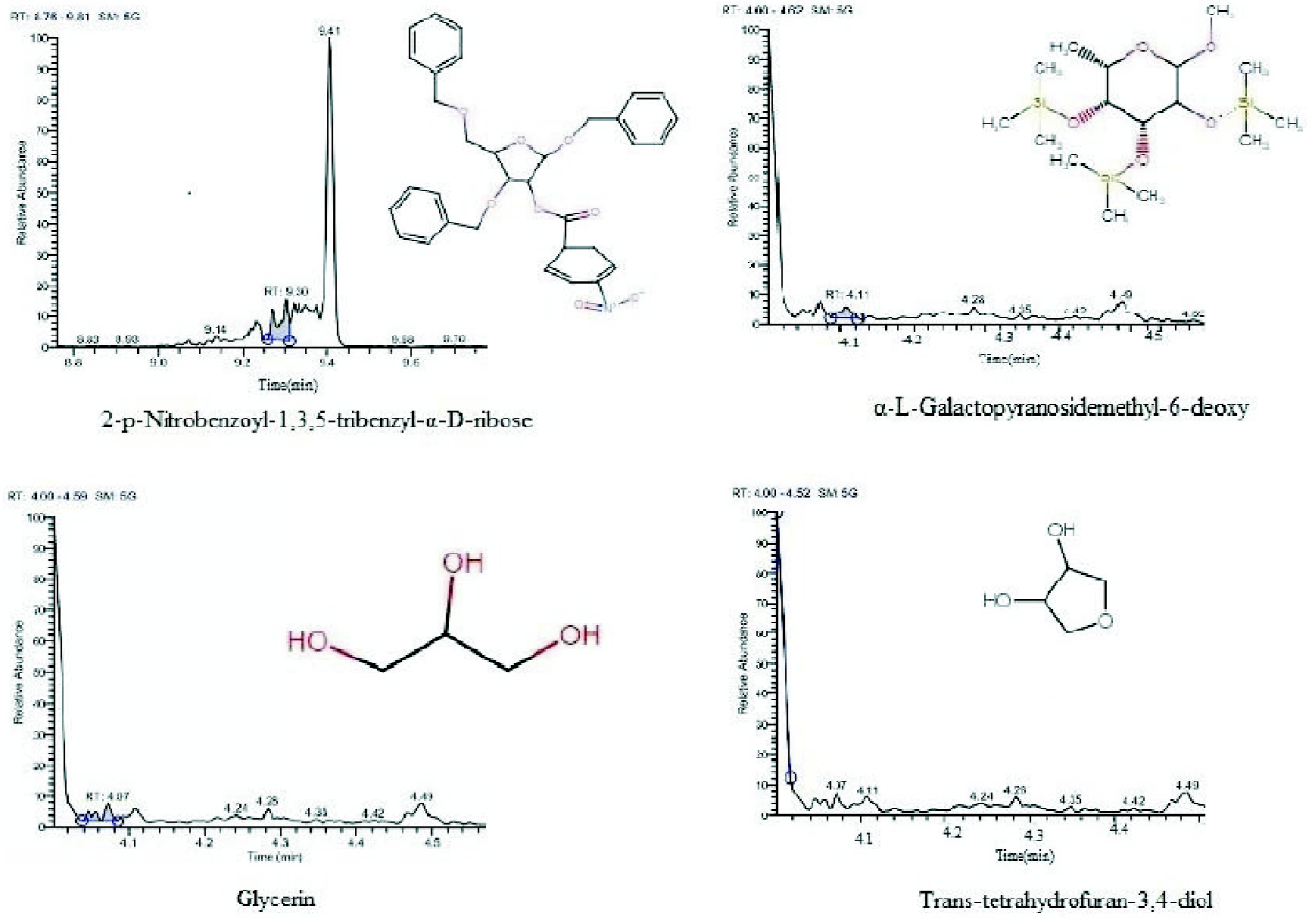

Fig. 8. GC-MS spectra of identified compound from *Calotropis gigantea* (L.) R.Br. root extract: 2-*p*-Nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose; α-L-galactopyranosidemethyl-6-deoxy; glycerin; *trans*-tetrahydrofuran-3,4-diol.

minium chloride. The flavonoid content was determined from linear regression equation and calculated in triplicated value as mean ±SD. All the values were expressed as mg/g of quercetin equivalent of plant extract[28].

In vitro antioxidant activity

DPPH free radical scavenging assay:

Antioxidant activity of crude extract of *C. gigantea* was determined using DPPH free radical scavenger method as per reported in Singh *et al.*[29]. Different concentration of crude extract as test sample and ascorbic acid as standard were taken in test tubes. About 2 mL of DPPH (1 mmol/L) solution in methanol was added into each test tubes. After this, the mixtures were mixed rapidly and kept at 37°C for thirty minute in dark places. The blank solution contained all other chemicals except the incorporation of test sample and standard. Absorbance was measured at 517 nm using UV-Vis spectrophotometer. The percentage of free radical scavenger property was computed using the given formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 = the absorbance of the control and A_1 = the absorbance of crude extract/known standard.

Hydrogen peroxide scavenging assay:

Hydrogen peroxide scavenging property of methanol root extract of the plant was evaluated as per the method reported by Nabavi *et al.*[30]. Hydrogen peroxide (40 mmol/L) solution was prepared in phosphate buffer (50 mmol/L) at pH 7.4. Various concentrations of 20, 40, 60, 80 and 100 μg/mL of test sample (1 mL) and the standard, ascorbic acid were prepared with 2.5 mL, hydrogen peroxide solution and after 10 min, the absorbance was measured using UV-Vis

spectrophotometer at 230 nm against blank solution. The blank solution was containing only phosphate buffer; and it was devoid of hydrogen peroxide and the crude extract or ascorbic acid. The percentage of hydrogen peroxide scavenger activity was calculated by using following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 = the absorbance of the control and A_1 = the absorbance of crude extract/known standard.

Gas chromatography-mass spectrometry analysis:

The GC-MS analysis of plant root was evaluated by using Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. The MS part of an instrument consisted of Triple Quadrupole which is paired with the TRACE 1300 GC and Auto-sampler. The purpose of Auto-sampler is meant for automated sample handling. The gas chromatograph consisted of split/splitless injectors and multi-mode (including on-column) programmed temperature vaporizing, column temperature 400°C, polar as well as nonpolar columns are available. The detectors used flame ionization detector and electron capture detector. The components were identified and compared along with their retention indices available in the computer library (NIST library) attached to the GC-MS instrument and then the obtained results are tabulated.

Statistical analysis:

Experimental work was carried out in triplicates and the results were calculated and expressed as mean ±SD (standard deviation). Correlation was studied between TPC, TFC and *in vitro* antioxidant activity of root extract using Pearson correlation coefficient.

Conclusion

Herbal plants form the backbone of herbal formulations, as it has been extensively studied for their excellent pharmacological properties. Even they are considered to be the main source for the development of many new components of novel therapeutic qualities. Therefore, the characterization of bioactive components presents in *Calotropis gigantea* was performed by using GC-MS technique. This analysis shows the presence of twelve chemical components including, *trans*-tetrahydrofuran-3,4-diol; glycerin; α-L-galactopyranoside, methyl-6-deoxy; 2-*p*-nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose; 1,3-butadiene-1-carboxylic acid; ethanedioic acid, bis(trimethylsilyl)ester; cyclopentane undecanoic acid, methyl ester; 3-nitropropanoic acid; butoxyacetic acid; 7-nonenoic acid, methyl ester; 3-decen-1-ol, (*E*); 3,5-dimethyl-5-hexen-3-ol. In addition to this, *in vitro* antioxidant activity was also performed using DPPH and H_2O_2 radical scavenging assay. With all these reports, it has been concluded that the plant root contain significant proportion of polyphenolic and flavonoid components, which have been further supported by the identification of lead molecule with the application of GC-MS analysis. However, the determination of pharmacological property of such compounds are still going on with more proper evidence. It has been concluded from above results that the herb *Calotropis gigantea* is of pharmaceutical and therapeutically importance.

Acknowledgement

The authors are thankful to the Sophisticated Analytical Instrumentation Facility, CIL Panjab University, Chandigarh, India for evaluating the GC-MS analysis of the given sample.

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