Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

Synthesis And Characterization Of Siver Nanoparticles Of Calotropis Gigantea

A. B. Immaculate Angela1, R. Kungumapriya 1, K. Revathi 2*, Anitha A 2, Jasmin vigila 2

1. Pachaiyappa's college, Chennai
2. CRL, Meenakshi Academy of Higher Education and Research
* CORRESPONDING AUTHOR
reva63@rediffmail.com

ABSTRACT

Medicinal plants are a reservoir of biologicallyactive compounds with therapeutic properties thatover time have been discovered and used by diversegroups of people for treatment of various ailments. Phytochemical and biological activities of stem, leaf, flower, root and latex of different solvent extracts of *Calotropis gigantea* was studied *in vitro*. Various secondary metabolites were present in stem, leaf, flower, root and latex of different solvent extracts of *Calotropis gigantea*. Methanolic extract *Calotropis gigantea* showed the maximum level of phytoconstiuents. GC – MS analysis of methanol extract revealed the various compounds might be exhibits various biological activities.

1.INTRODUCTION

C. gigantea is known for various medicinal properties in traditional medicinal system and use to cure a variety of diseases. In last few decades, *C. gigantea* is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. Hence in the present investigation phytochemical and biological activities and synthesis and characterization of silver nano particles from *C. gigantea* was studied.

2. MATERIALS AND METHODS

Extractionof variouspartsof*Calotropisgigantea*

The stem, leaf, flower and root was cleaned shade dried and coarsely powdered, latex wascollected in sterile condition. Successive solvent extraction was done by cold percolation method(Harborne, 1998) by soaking in hexane, chloroform, ethyl acetate, ethanol and methanol successivelyin an aspirator bottle for 48 h. Aqueous extracts of all the parts of *Calotropis gigantea* were alsoprepared. After 48 h, the extracts were filtered by Whatman Filter paper No.1. The solvent wasremoved by distillation using Evator Rotary Evaporator and the extracts were concentrated and driedinLyodelFreezeDryer.

Phytochemicalstudies

Phytochemicalscreeningofstem,leaf,flower,rootandlatexof*Calotropisgigantea*extracts

Qualitative tests were performed to assess the nature of phytochemicals present in various extractsof *Calotropis gigantea* namely hexane, chloroform, ethyl acetate, ethanol, methanol and

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

aqueousextracts.

Liebermann-Burchard Test: Extract is dissolved in minimum of chloroform. Acetic acid wasadded and heated. Few drops of acetic anhydride and concentrated H_2SO_4were added. Greencolourshows thepresenceof Steroid.

a) Noller'sTest: Extractistreatedwithtinandthionylchlorideandwasheatedinawaterbath.

PurplecolourshowsthepresenceofTriterpenoid.

b) Shinoda Test: Extract is dissolved in alcohol. Magnesium bits and concentrated hydrochloricacidwasadded.Itwas heatedinawaterbath. Majentacolourshows thepresenceof Flavonoid.

c) TestforFuran: Extractisdissolvedinalcohol.p-dimethylaminobenzaldehydeandconcentrated hydrochloric acid was added and was heated in a water bath. Pink colour shows thepresenceofFuranoidcompound.

d) Test for Sugar: Extract is treated with anthrone and concentrated H_2SO_4.It was heated in awaterbath. Green colourshows the presenceof Sugar.

e) Test for Coumarin: Extract is shaken with 10% NaOH. Yellow colour shows the presence ofCoumarin.Thesubstanceregenerates when concentrated$H_2SO_4$is added.

f) Test for Quinone: Extract is treated with concentrated H_2SO_4. Red colour shows the presenceofQuinone.

g) TestforSaponin: Extractisshakenwithwater.FrothingshowsthepresenceofSaponin.

h) TestforTannin: Extractisshakenwithwaterandleadacetatesolutionwasadded.Whiteprecipitateshows the presenceof Tannin.

i) TestforAcid: Extractistreatedwithsodiumbicarbonatesolution.Effervescenceshowsthepresenceof Acid.

j) TestforPhenol: Extractisdissolvedinalcohol.Ferricchlorideisadded.Bluishcolourshowsthepresenceof Phenol.

k) TestforAlkaloid: ExtractistakeninaceticacidandfewdropsoffreshlypreparedDragendorff's reagentareadded.Abrickredororangeprecipitateshowsthepresenceof Alkaloids.

Analysisofthedifferentextractsofstem,leaf,flower,rootandlatexof*Calotropisgigantea*by Gas Chromatography-Mass Spectrometry(GC-MS)

GC-MStechniquewasusedinthisstudytoidentifythephytocomponents.GC-MSanalysisofthefractionswasperformedusingGC-MS-QP2010(Shimadzu)andgaschromatographinterfaced to a mass spectrometer (GC-MS) equipped with Elite -1 fused silica capillary column(Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 μm composed of 100 % Dimethylpoly siloxane). For GC-MS detection, an electron

ionization energy system with ionization energyof70Ev was used. Heliumgas(99.999%) was used asthecarriergasataconstantflowrateof 1.51 ml/min and an injection volume of 1 µl was employed (split ratio: 10), Injector temperature240 °C; Ion-source temperature 200 °C. The oven temperature was programmed from 70 °C(isothermal for 3 min), with an increase of 300 °C for 10 min. Mass spectra were taken at 70 eV; ascan interval of 0.5 sec with scan range of 40 – 1000 m/z. Total GC running time was 35 min. Therelative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handlemassspectraandchromatogramswasaGC-MSsolution ver. 2.53.

Identificationofphytocomponents

Interpretationonmass–spectrumGC–MSwasconducted usingthe database of NationalInstituteStandardand Technology(NIST08)andWILEY8.Thespectrumoftheunknowncomponentswascomparedwiththe spectrumofknowncomponentsstoredinthelibrary.Thename,molecularweightandstructureof thecomponentsof thetestmaterials wereascertained.

Quantitativeestimation

Estimationof totalphenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compoundswith slight modifications (Zinshen. J et.,al., 1999). One hundred µL of water dissolved methanolextract of latex (1mg/mL) was mixed with 900 µL of methanol and 1mL of FolinCiocalteaureagent (1:10 diluted with distilled water). After 5min, 1mL of 20% (w/v) of Na_2CO_3solutionwas added. The mixture was then allowed to stand for 30 min incubation in dark at roomtemperature. The absorbance was measured at 765nm in UV-Vis spectrophotometer. The totalphenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is acommonreferencecompound.

Estimationof totalflavonoids

The total flavonoid content was determined using aluminium chloride reagent methodwith slight modification (Ahmed. D et.,al., 2014). Five hundred µL of water dissolved methanolextract of latex (1mg/mL) was mixed with 500 µL of methanol and 500µL of 5% (w/v) sodiumnitrite solution followed by 500µL of 10% (w/v) aluminium chloride solution was added andincubated for 5min at room temperature. Then 1 mL of 1 M NaOH solution was added and thetotal volume was made up to 5mL with distilled water. Absorbance was measured at 510nm inUV-Visspectrophotometer.Theresultwasexpressedas (µg/mgofextract)quercetinequivalent.

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis of different solvent extract of Methanolic extract of stem, leaf, flower, root and latex of *Calotropis gigantea* exhibited flavonoid, steroid, alkaloid, saponin, triterpenoid, quinone, tannins, coumarin, and phenol. Likewise ethanol extract of stem, leaf, flower, root and latex of *Calotropis gigantea* exhibited flavonoid, steroid, alkaloid, saponin, triterpenoid, quinone, tannins and coumarin respectively. Maximum level of phytoconstituents was observed at methanol extract of latex of *Calotropis gigantea;* Whereas in hexane, chloroform, ethylaceate and aqueous showed the moderate level of phytoconstituents (Table 1-5). Methanol extract of latex showed the total phenols and flavonoids was found to be 0.351 and 0.142 GAE/100g.

GC – MS analysis of methanol extract of root, stem, leaf, flower and latex of *Calotropis gigantea* revealed the presence of various bio active compounds such as 2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene,9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-,acetate,(3.beta.,4.alpha.,5.alpha.),2r-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol,lup-20(29)-en-3-ol,acetate,(3.beta.),cholest-22-ene-21-ol, **3,5-dehydro-6-methoxy-,pivalate**,**4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2,urs-12-en-24-oicacid,**3-oxo-,methylester,(+)-,2h-pyran,2-(7-heptadecynyloxy)tetrahydro, (-)-isolongifolol,acetate,3-o-acetyl-6-methoxy-cycloarteno,2r-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol,12-oleanen-3-ylacetate,(3.alpha.),9,19-cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3.beta.,4.alpha.,5.alpha.)-a'-neogammacer-22(29)-ene,4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2,hop-22(29)-en-3.beta.-ol,**3H-1,5-Benzodiazepine**, 2,4-dimethyl,Isobutyricacid,2-isopropoxyphenyl,Propenamide, 2-acetamido-3-phenyl-N-(3-hydroxypropyl**),2-(1H-Benzoimidazol-2**-methylsulfanyl)-4,6-dimethyl-nicotinonitrile,(Table 6-10 & Fig 1-5).

4. CONCLUSION

Calotropisesjuiterpenol, calotropisesterterpenol, calotrobenzofuranone, calotronaphthalene, were the chiefphytochemicalcompoundsfromtherootextractofthe*Calotropisgigantean.*Pharmacologi calactivityincludesNeuropharmacologicalactivity,cytotoxic activity, antitumor activity, antibacterial activity, antidiarrheal activity, anticonvulsantand central nervous system activity, antiinflammatory activity, procoagulantactivity, woundhealing activity, hepatoprotective activity etc. Herbs provide many unique qualities that are verylimited in conventional medicine, such as anticancer, antiviral, and immunoregulation properties.*Calotropisgigantea* is aconstant herbwith a long past of usein traditionalmedicine.This proves to be a posh bequest with such dynamic phytochemical compounds.

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

Table 1 Phytochemical screening of stem extract of C*alotropis gigantea*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann-Burchad test(Steroid)	-	-	-	-	+	-
2	Noller's test(Triterpenoid)	-	+	-	+	+	-
3	Shinoda test(Flavonoid)	-	+	-	+	+	-
4	Furan test	-	-	-	-	+	-
5	Coumarin test	-	-	-	-	+	-
6	Sugar test	-	-	-	-	-	+
7	Quinone test	-	-	+	-	-	-
8	Saponin test	-	-	-	-	+	-
9	Acid test	-	-	-	-	-	-
10	Tannin test	-	+	-	-	+	-
11	Phenol test	-	-	+	+	+	-
12	Alkaloid test	-	+	-	+	-	-

Table 2 Phytochemical screening of leaf extract of C*alotropis gigantea*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann-Burchad test(Steroid)	-	-	-	-	+	-
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	+	+	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	+	+	-
6	Sugar test	-	-	-	-	+	+
7	Quinone test	-	-	-	-	-	-
8	Saponin test	-	-	-	+	+	-
9	Acid test	-	-	-	-	+	-
10	Tannin test	-	-	-	-	+	-
11	Phenol test	-	-	+	-	+	+
12	Alkaloid test	-	-	-	-	-	-

Table 3 Phytochemical screening of flower extract of C*alotropis gigantea*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann-Burchad test(Steroid)	-	-	-	-	+	-
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	-	+	+	+
4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	-	-	-
6	Sugar test	-	-	-	+	+	+
7	Quinone test	-	-	-	-	-	-
8	Saponin test	-	+	+	-	+	-
9	Acid test	-	-	+	+	+	+
10	Tannin test	-	+	-	-	+	-
11	Phenol test	-	-	-	+	+	-
12	Alkaloid test	-	-	-	-	-	-

Table 4 Phytochemical screening of root extract of C*alotropis gigantea*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann-Burchad	-	-	-	+	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

	test(Steroid)						
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	-	+	+	-
4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	-	-	-
6	Sugar test	-	-	-	-	-	-
7	Quinone test	-	+	-	-	+	-
8	Saponin test	-	-	+	-	+	-
9	Acid test	-	-	-	+	+	+
10	Tannin test	-	+	-	+	+	-
11	Phenol test	-	-	-	+	+	+
12	Alkaloid test	-	-	-	-	-	-

Table 5 Phytochemical screening of latex extract of C*alotropis gigantea*

S.no	Phytoche-mical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann-Burchad test(Steroid)	-	-	+	+	+	+
2	Noller's test(Triterpenoid)	-	-	-	+	+	+
3	Shinoda test(Flavonoid)	-	+	-	+	+	+
4	Furan test	-	-	+	-	+	+
5	Coumarin test	-	-	+	+	+	+
6	Sugar test	-	+	+	+	+	+
7	Quinone test	-	-	+	+	+	+
8	Saponin test	-	-	-	-	+	+
9	Acid test	-	-	-	+	+	+
10	Tannin test	-	-	+	+	+	+
11	Phenol test	-	+	+	+	+	+
12	Alkaloid test	-	-	-	-	+	+

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

Table 6 GC – MS analysis of methanolic extract of *Calotropis gigantea stem*

S. No	Compound Name	Molecular Weight	Molecular Formula
1	urs-12-en-28-ol	222	C15H260
2	2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	424	C30H480
3	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	468	C32H52O2
4	2r-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	282	C17H30O3
5	lup-20(29)-en-3-ol, acetate, (3.beta.)-	264	C32H52O2

Fig.1GC – MS analysis of methanolic extract of stem of *Calotropis gigantea*

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

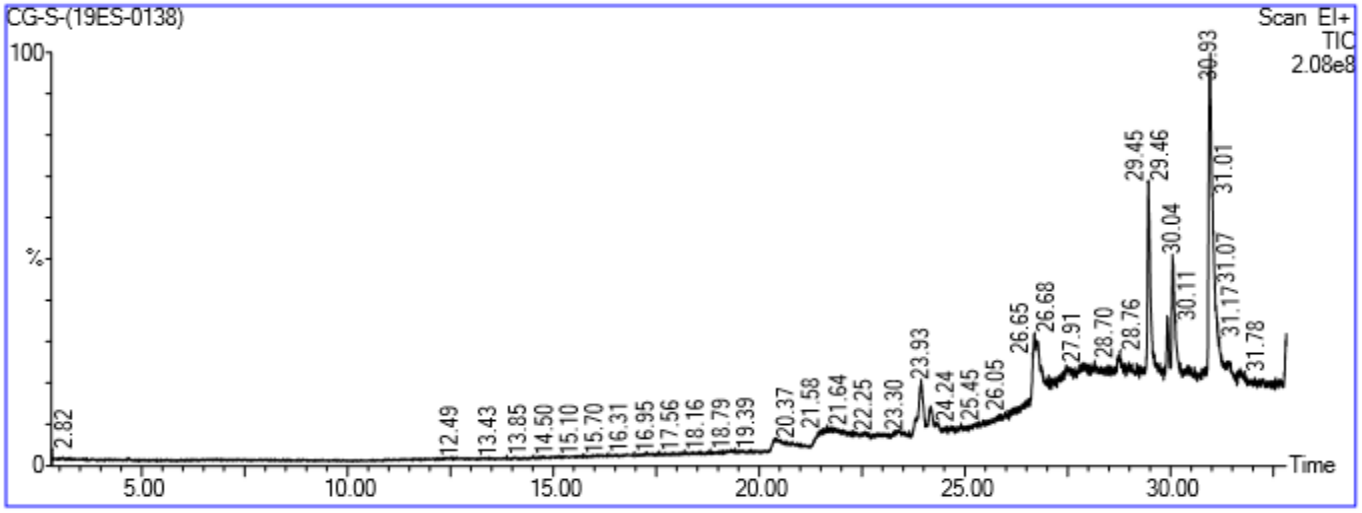


Table 7 GC – MS analysis of methanolic extract leaf of *Calotropis gigantea*

S. No	Compound Name	Molecular Weight	Molecular Formula
1	cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	498	C33H54O3
2	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2	424	C30H480
3	urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	468	C31H48O3
4	2h-pyran, 2-(7-heptadecynyloxy)tetrahydro-	336	C22H40O2
5	(-)-isolongifolol, acetate	264	C17H28O2

Fig. 2GC – MS analysis of methanolic extract of leaf of *Calotropis gigantea*

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

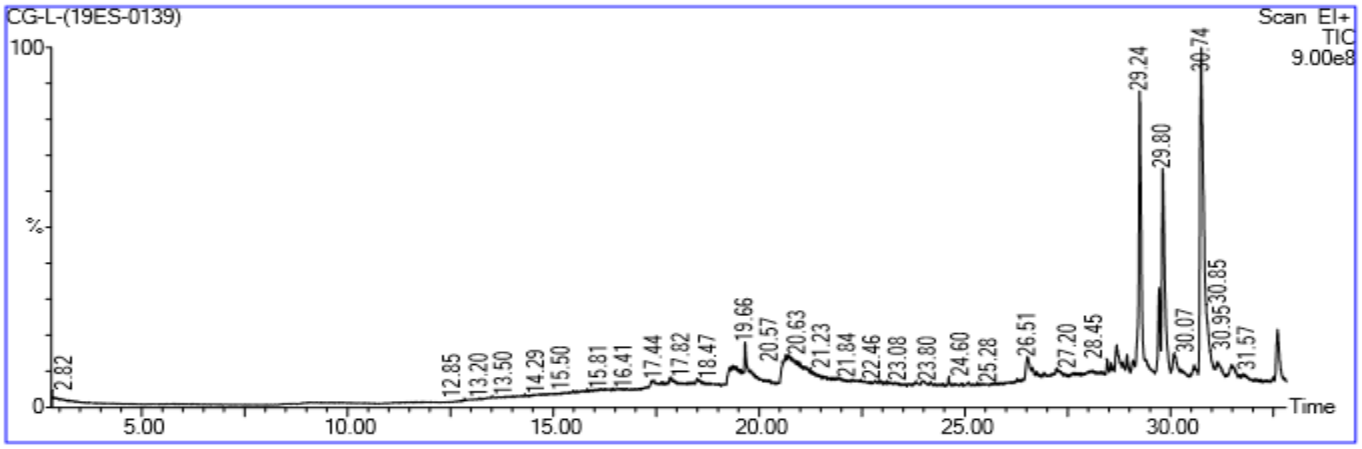


Table 8 GC – MS analysis of methanolic extract of flower of *Calotropis gigantea*

S. No	Compound Name	Molecular Weight	Molecular Formula
1	3-o-acetyl-6-methoxy-cycloarteno	498	C33H54O3
2	2r-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	282	C17H30O3
3	12-oleanen-3-yl acetate, (3.alpha.)-	468	C32H52O2
4	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	468	C32H52O2
5	a'-neogammacer-22(29)-ene	410	C30H50

Fig.3GC – MS analysis of methanolic extract of flower of *Calotropis gigantea*

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

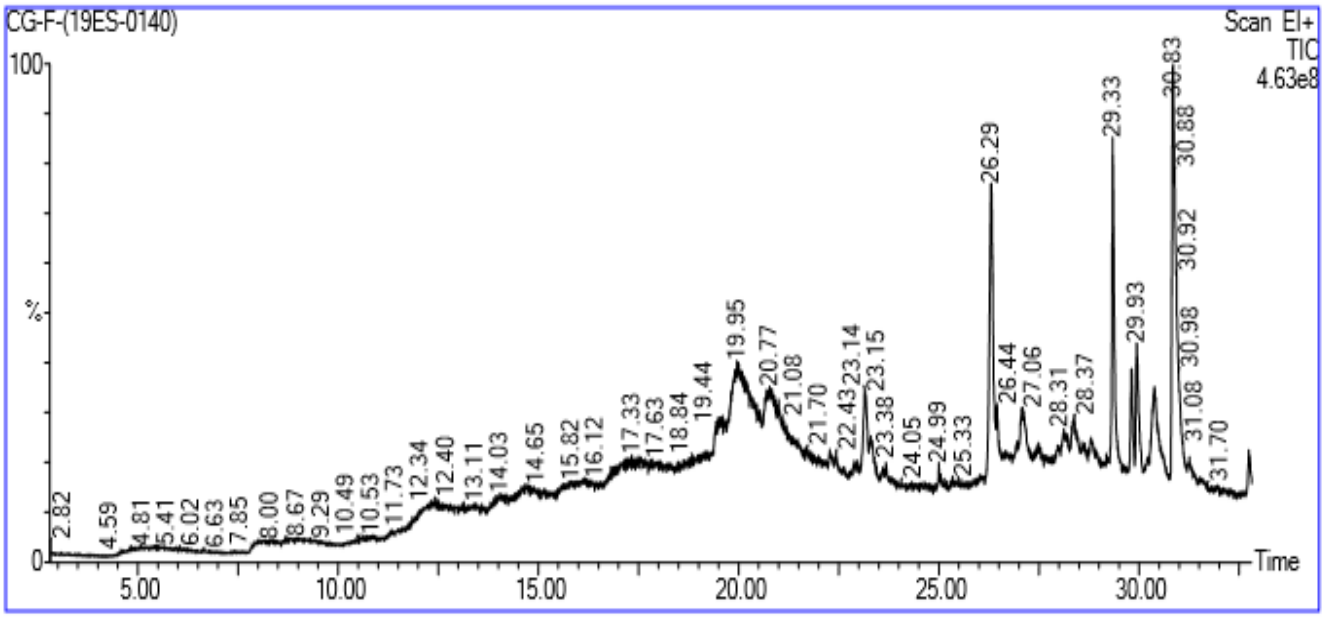


Table 9 GC – MS analysis of methanolic extract of root of *Calotropis gigantea*

S.no	Compound name	Molecular weight	Molecular formula
1	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2	424	C30H480
2	3-o-acetyl-6-methoxy-cycloartenol	498	C33H54O3
3	hop-22(29)-en-3.beta.-ol	426	C30H500

Fig.4GC – MS analysis of methanolic extract of root of *Calotropis gigantea*

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 1023 - 1035
Received 18April2020; accepted 23June2020

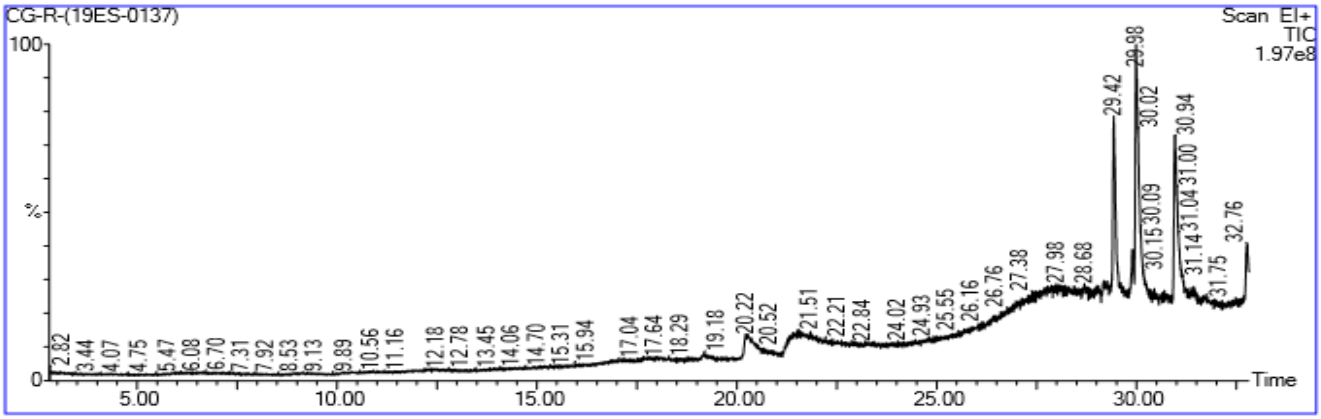
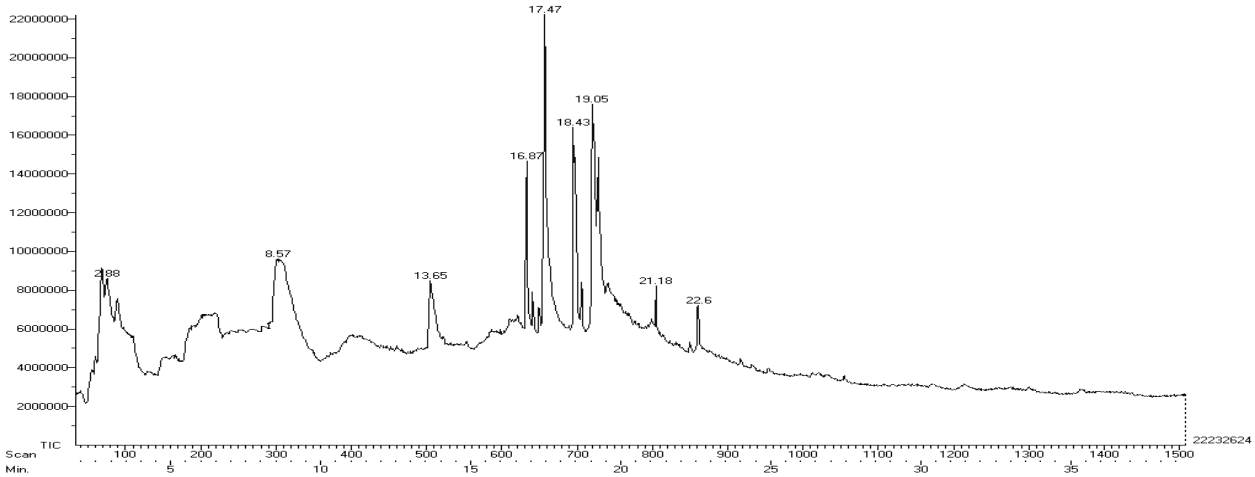


Table 10 GC – MS analysis of methanolic extract of latex of *Calotropis gigantea*

S. No	Name of the Compound	Molecular weight
1	3H-1,5-Benzodiazepine, 2,4-dimethyl-	172
2	Isobutyric acid, 2-isopropoxyphenyl	222
3	Propenamide, 2-acetamido-3-phenyl-N-(3-hydroxypropyl)-	262
4	2-(1H-Benzoimidazol-2-methylsulfanyl)-4,6-dimethyl-nicotinonitrile	294

Fig.5GC – MS analysis of methanolic extract of latex of *Calotropis gigantea*



REFERENCES

1. Ahmed D, Fatima K, Saeed R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. Antioxidants 2014; 3: 671–683.
2. Harborne. J. B., 1998. Phytochemical Methods A guide to modern techniques of plant analysis. 3rd Edition, published by Chapman & Hall, London., pp. 302.
3. National Institute Standard and Technologies NIST08 and Wiley8 Mass Spectral Libraries database https://chemdata.nist.gov.
4. Shimadzu corporation Gas Chromatography Mass Spectrometry GCMS-QP 2010 manual.
5. Zinshen. J, Megcheng. T, Jianming. W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry 64 (1999) 555-559.