academicJournals

Vol. 7(40), pp. 2986 – 2991, 25 October, 2013
DOI: 10.5897/JMPR12.803
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Analysis of phytochemical constituents of the ethanolic and chloroform extracts of *Calotropis procera* using gas chromatography-mass spectroscopy (GC-MS) technique

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Accepted 7 June, 2012

The phyto-components of *Calotropis procera* leaves were screened by qualitative and gas chromatography-mass spectroscopy (GC-MS) analysis. *C. procera* R.Br. is a perennial plant abundantly found in all parts of the country (India) and wild in nature. The leaves of the plant were found to contain various primary and secondary metabolites. Qualitative analysis showed the presence of alkaloids, terpenoids, saponins, tannins and cardiac glycosides and absence of flavonoids in ethanolic extract of *C. procera*, while the chloroform leaf extract showed absence of flavonoids and cardiac glycosides. This work deals with the phytochemical screening and GC-MS studies of the ethanolic and chloroform leaf extracts of *C. procera*. In ethanol leaf extract, highest peak area (%) of 21.36 was obtained by 9-octadecenoic acid (Z), methyl ester ($C_{19}H_{36}O_2$) at retention time of 14.843 and the lowest peak area (%) of 0.31 was obtained by cyclohexanol-3-methyl ($C_7H_{14}O$) at retention time of 18.599, whereas in chloroform leaf extract, the highest peak area (%) of 33.14 was obtained by 2, 6, 10-trimethyl, 14-ethylene-14-pentadecne ($C_{20}H_{38}$) at retention time of 13.136 and the lowest peak area (%) of 0.78 was obtained by 2-tert-butyl-4-(1,1,3,3-tetramethylbutyl) $C_{18}H_{30}O$ at retention time of 10.731. The study summarizes the information concerning the phytochemical constituents present in ethanolic and chloroform leaf extracts. These constituents may be responsible for pharmacological activities.

Key words: *Calotropis procera*, phytochemical analysis, GC-MS, metabolites, leaves.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been used for centuries as remedies for human and animal diseases as they contain phyto-chemicals of therapeutic value.

Different parts (root, stem, leaves, flowers and seeds) of *Calotropis procera* are traditionally used to cure a number of diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea. It is a traditional medicinal plant (Rastogi and Mehrotra, 1991) with unique properties (Oudhia and Tripathi, 1998). The aim of the present work was to phytochemically screen the plant metabolites present in the plant material in ethanol and chloroform extracts qualitatively by applying phytochemical tests and quantitatively by gas chromatography-mass spectroscopy (GC-MS) analysis. In GC-MS analysis the percent area represents the percentage wise amount of the respective compound.

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Table 1. Phytochemical screening of *C. procera*.

Chemical constituents	Test	Leaf extract	
		CHL	ETOH
Alkaloids	Hanger's test	+++	+++
	Mayer's test	++	+++
	Wagner's test	++	+++
	Tannic acid teat	++	+++
Terpenoids	Salkowaski test	++	+++
Saponins	Frothing test	++	+++
Tannins	$Fecl_3$ test	++	++
Flavonoids	$Fecl_3$ Solution test	-	+
Cardiac glycosides	Keller- Kiliani test	-	++
Steroids	Libermann-Burchard's test	+	++

CHL= chloroform; ETOH= Ethyl alcohol; +++ = copiously present; ++ = moderately present; + = slightly present; - = absent.

EXPERIMENTAL

Plant collection, identification and authentification

The plant leaves were collected along the road sides of Dayalbagh Educational Institute, Dayalbagh, Agra. They were identified in Taxonomy Division, Botanical Survey of India (BSI), Allahabad as *C. procera* (R. Br.) and the assigned accession number is 79385 (BSA). A herbarium sample of the material is stored in the Herbarium, Taxonomy Division, Botanical Survey of India (BSI), Allahabad (India).

Extraction and processing

The shade dried plant material (200 g) was crushed and soxhlet extracted using petroleum ether, chloroform, ethyl acetate and ethyl alcohol successively (Green, 2004). The extracts were filtered using Whatman 40 filter paper and were concentrated. The concentrated extracts (chloroform and ethanol) were subjected to qualitative phytochemical analysis and GC-MS analysis.

Chemical reagents

Chemicals and reagents like chloroform, ethanol, sulphuric acid, Mayer's, Hagner's, and Wagner's reagents, tannic acid solution, acetic anhydride, lead acetate, magnesium and benzene were used to analyze phytochemicals present in the aerial parts of *C. procera* (Which grade & company).

Screening of phytochemical constituents

Phytochemical screening of the extracts was carried out as per standard methods prescribed by Harborne (1973), Trease and Evans (1989) and Sofwara (1993) to decipher the presence of various constituents in chloroform and ethanol leaf extracts only. It is due to the presence of good activity of both extracts against some species of human pathogenic fungi as compared to other two leaf extracts.

GC-MS analysis

The GCMS analysis of both extracts was performed using GC-MS SHIMADZU MS 2010 instrument equipped with AB innowax column (60 × 0.25 mm id, film thickness 0.25 µm). Initially, oven temperature was maintained at 50×C for 3 min and temperature was gradually increased up to 280°C at 30 min and 0.2 µl of sample was injected for analysis. Helium was the carrier gas. The flow rate of helium gas was 1.2 ml/min. The sample injector and mass transfer line temperature were set at 270 and 280°C and split ratio is 20 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 40 to 1000 m/z for the duration of 35 min. Identification of components was based on comparison of their mass spectra with those of Wiley and NIST libraries and those described by Adams (1995) as well as on comparison of their retention indices with literature (Vanden and Kratz, 1963).

RESULTS AND DISCUSSION

The phytochemical active compounds present in *C. procera* leaves were qualitatively analyzed using standard methods prescribed by Harborne (1973), Trease and Evans (1989) and Sofwara (1993) and the results are presented in Table 1. The phytochemical screening of the crude ethanolic extract of leaves of *C. procera* showed that both the extracts contain alkaloids, saponins, tannins, terpenoids, glycosides and steroids. This confirms previous reports (Hassan et al., 2006; Oladimeji et al., 2006). However, flavonoids were found to be absent in the ethanolic extract.

GC-MS analysis of the extracts

In the GC-MS analyses of *C. procera*, 26 compounds were identified in the ethanolic extract and 17 compounds in chloroform extract. The identification of phytochemical compounds is based on the peak area (which represents the percentage of that compound), molecular weight and molecular formula. The chromatogram (Figure 1) of ethanol leaf extract shows 6 prominent peaks as 9-octadecenoic acid (Z)-methyl ester ($C_{19}H_{36}O_2$) with

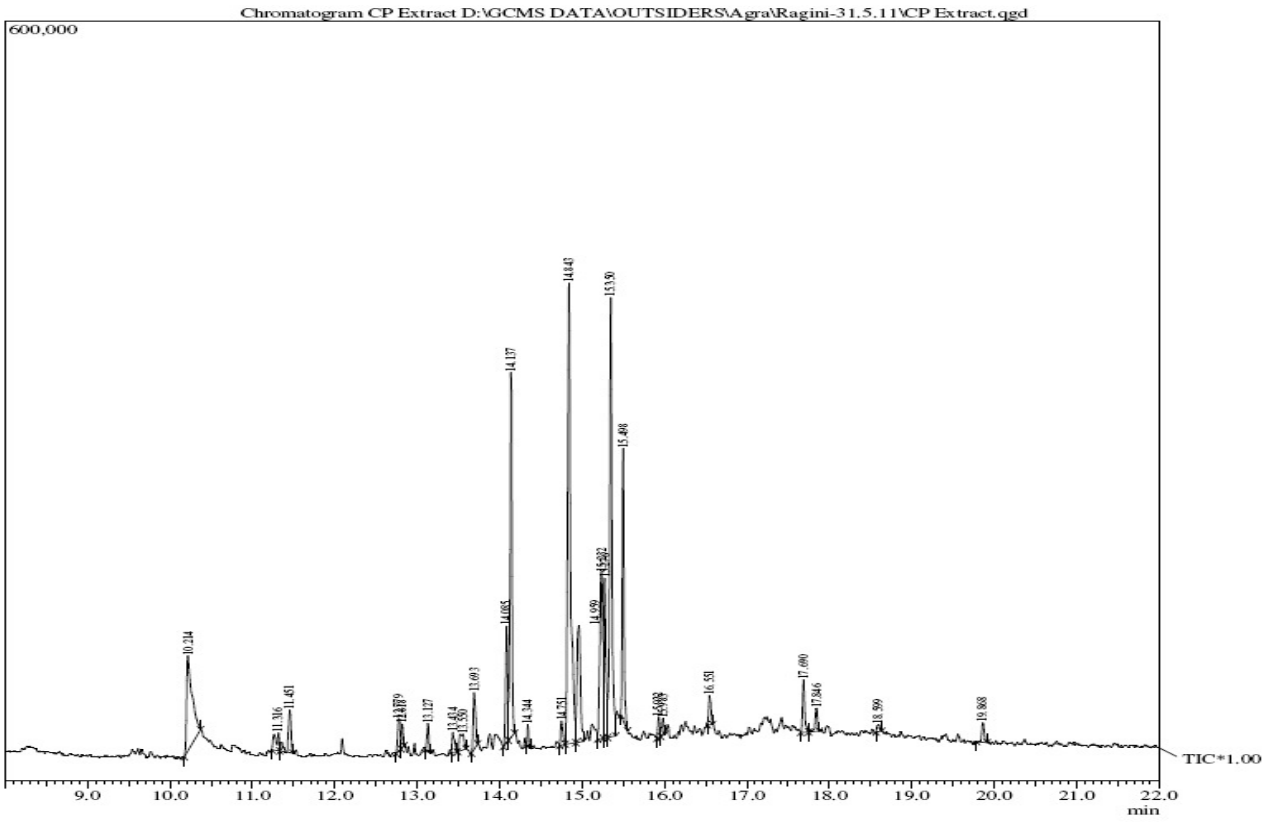


Figure 1. Chromatogram (GC/MS) of the ethanolic extract of *C. procera*.

retention time of 14.843 and peak area of 21.36, alpha-D-glucopyranoside ($C_{32}H_{68}O_7Si_3$) with retention time of 15.350 has the peak area of 15.43. Hexadecanoic acid, ethyl ester ($C_{17}H_{34}O_2$) with retention time of 14.137 has the peak area of 10.24, L-Glutamic acid ($C_5H_9NO_4$) with retention time of 10.214 has the peak area of 8.10, 1,2,3,4-Tetrakis-O-(trimethylsilyl)pentopyranose ($C_{17}H_{42}O_5Si_4$) with retention time of 15.498 has the peak area 7.44, 9,12-Octadecadienoic acid (Z,Z) ($C_{20}H_{36}O_2$) with 15.232 retention time has the peak area of 7.43.The other less prominent peaks at other retention times are shown in Table 2. The total ion chromatograph (TIC) showing the peak identities of the various compounds identified are as shown in Figure. The structures of prevailing compounds of ethanol leaf extract are presented in Table 3.

The chromatogram (Figure 1) of chloroform extract shows 5 prominent peaks as 2, 6, 10-trimethyl, 14-ethylene-14-pentadecne ($C_{20}H_{38}$) with retention time of 13.136 and peak area of 33.14, methyl 9-octadecenoate ($C_{19}H_{36}O_2$) with retention time of 14.855 has the peak area of 17.98, beta-L-galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl) ($C_{18}H_{44}O_5Si_4$) with retention time of 15.351 has the peak area 9.93, 6-octen-1-ol, 3,7-dimethyl ($C_{10}H_{20}O$) with retention time of 14.960 has the peak area of 7.84, D-xylopyranose, 1,2,3,4-tetrakis-o-(trimethylsilyl ($C_{17}H_{42}O_5Si_4$) with 15.498 retention time has the peak area of 5.80. The other less prominent peaks at other retention times are shown in the Table 4. TIC showing the peak identities of the compounds identified are as shown in Figure 2. The structures of prevailing compounds of chloroform leaf extract are presented in Table 5.

This study highlights the presence of many secondary metabolites in the aerial parts of *C. procera,* provide an overview of the different classes of molecules present that have led to their pharmacological activities. This study confirmed that the plant extract could be used for the treatment of various diseases. The GC-MS analysis of extracts showed the presence of various types of compounds in *C. procera* leaves like 9-octadecenoic acid (Z) - methyl ester ($C_{19}H_{36}O_2$) having anti-carcinogenic activity (Yeong et al., 1989), L-glutamic acid ($C_5H_9NO_4$) is biologically significant amino acid and is used as plant growth regulator and fungicide and 9, 12-octadecadienoic acid (Z,Z) ($C_{20}H_{36}O_2$) has been reported to have insecticidal and anti-feedant activities.

Conclusion

The results reveal that the extracts have a quite number of chemical constituents, which may be responsible for

Table 2. Chemical constituents present in the ethanolic extract using GC-MS analysis.

Peak number	RT	Area (%)	Name of the compound	Molecular weight	Molecular formula
1	10.214	8.10	L-glutamic acid	$C_5H_9NO_4$	147
2	11.316	1.07	Butane, 2,2-dimethyl	C_6H_{14}	86
3	11.451	1.74	1,2-Benzenedicarboxylic acid, dimethyl ester	$C_{12}H_{14}O_4$	222
4	12.779	1.07	1-Dodecene	$C_{12}H_{24}$	168
5	12.818	0.57	Heptane, 3,3-dimethyl	C_9H_{20}	128
6	13.127	0.70	6-Octen-1-Ol, 3,7-dimethyl acetate	$C_{12}H_{22}O_2$	198
7	13.434	0.63	(+)-(1s,2's)-3-(2'-isopropylcyclopropyl)-1-propanol	$C_9H_{18}O$	142
8	13.550	1.05	Per(trimethylsilyl)-D-fructose	$C_{21}H_{52}O_6Si_5$	540
9	13.693	1.84	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
10	14.085	3.95	1,2,3,4-Tetrakis-O-(trimethylsilyl)pentopyranose	$C_{17}H_{42}O_5Si_4$	438
11	14.137	10.24	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
12	14.344	0.44	1-[(T-Butyl)dimethylsilylthio)butane	$C_{10}H_{24}SSi$	204
13	14.751	0.84	1-Octanol	$C_8H_{18}O$	130
14	14.843	21.36	9-Octadecenoic acid (Z)- methyl ester	$C_{19}H_{36}O_2$	296
15	14.959	5.51	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	328
16	15.232	7.43	9,12-Octadecadienoic acid (Z,Z)-	$C_{20}H_{36}O_2$	280
17	15.276	4.93	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	308
18	15.350	15.43	Alpha-D-glucopyranoside, methyl 2,3,4-Tris-O-(trimethylsilyl)-, hexadecanoate	$C_{32}H_{68}O_7Si_3$	648
19	15.498	7.44	1,2,3,4-Tetrakis-O-(trimethylsilyl)pentopyranose	$C_{17}H_{42}O_5Si_4$	438
20	15.932	0.64	Ethanamine, 2,2'-oxybis[N,N-dimethyl	$C_8H_{20}N_{2O}$	160
21	15.983	0.48	1,3-Hexanediol, 2-ethyl	$C_8H_{18}O_2$	146
22	16.551	0.82	2-Dodecanol, 1,1-dichloro	$C_{12}H_{24}Cl_2O$	254
23	17.690	1.77	1,2-Benzenedicarboxylic acid, diisooctyl	$C_{24}H_{34}O_4$	390
24	17.846	0.86	1-Octanol, 3,7-dimethyl	$C_{10}H_{22}O$	158
25	18.599	0.31	Cyclohexanol, 3-methyl	$C_7H_{14}O$	114
26	19.868	0.72	(E,E)-4,8,12-Trimethyl-3,7,11-tridecatriene-1-Ol	$C_{16}H_{28}O$	236
-	-	100.00	-	-	-

RT: Retention time.

Table 3. Chemical structures of the most prevailing compounds of ethanol leaf extract of *C. procera.*

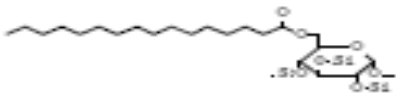
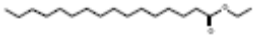
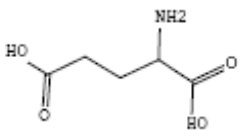
Name of the compound	Chemical structure of the compound
9-Octadecenoic Acid (Z) - Methyl Ester	
Alpha-D-Glucopyranoside	
Hexadecanoic Acid, Ethyl Ester	
L-Glutamic Acid	

Table 3. Contd.

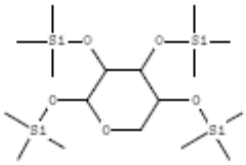
1,2,3,4-Tetrakis-O-(Trimethylsilyl)Pentopyranose	
9,12-Octadecadienoic Acid (Z,Z)	

Table 4. Chemical constituents present in the chloroform extract using GC-MS analysis.

Peak number	RT	Area (%)	Name of the compound	Molecular formula	Molecular weight
1	10.731	0.78	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl) phenol	$C_{18}H_{30}O$	262
2	12.800	1.69	2-Methylene-1,5-pentanediol	$C_6H_{12}O_2$	116
3	13.136	33.14	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
4	13.310	3.15	Bicyclo[4.1.0]heptane, 7-butyl	$C_{11}H_{20}$	152
5	13.439	6.54	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
6	13.728	2.02	Nonanoic acid, 7-methyl methyl ester	$C_{11}H_{22}O_2$	186
7	14.142	2.97	Bis-(3,5,5-trimethylhexyl) ether	$C_{18}H_{38}O$	270
8	14.855	17.98	Methyl 9-octadecenoate	$C_{19}H_{36}O_2$	296
9	14.960	7.84	6-Octen-1-ol, 3,7-dimethyl	$C_{10}H_{20}O$	156
10	15.247	0.92	(+)-(1r,2r)-2,7,7-Trimethyl-3-oxabicyclo[4.1.1.]Octan-4-one	$C_{10}H_{16}O_2$	168
11	15.282	0.99	6(e),9(z),13(e)-Pendectriene	$C_{15}H_{26}$	206
12	15.351	9.93	Beta-L-galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)	$C_{18}H_{44}O_5Si_4$	452
13	15.498	5.80	D-xylopyranose, 1,2,3,4-tetrakis-o-(trimethylsilyl)	$C_{17}H_{42}O_5Si_4$	438
14	16.551	1.38	Bis-(3,5,5-trimethylhexyl) ether	$C_{18}H_{38}O$	270
15	17.696	1.82	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390
16	17.844	1.15	1-(2-Hydroxyethoxy)-pentadecane	$C_{17}H_{36}O_2$	272
17	19.868	1.91	2,6,10-Dodecatrienoic acid, 7,11-dimethyl-3-(trifluorpmethyl)-, methyl ester, (Z,Z)	$C_{16}H_{23}F_3O_2$	304
-	-	100.00	-	-	-

RT: Retention time.

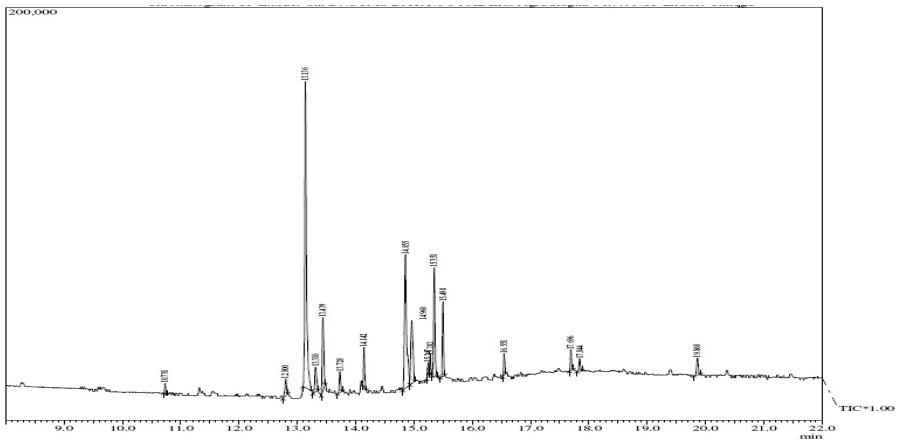
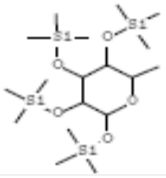
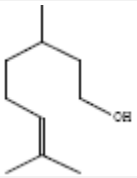
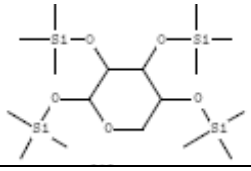


Figure 2. Chromatogram (GC/MS) of the chloroform extract of *C. procera*.

Table 5. Chemical structures of most prevailing compounds of chloroform leaf extract of *C. procera*.

Name of the compound	Chemical structure of the compound
2, 6, 10-Trimethyl, 14-ethylene-14-pentadecne	
Methyl 9-octadecenoate	
beta-L-Galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)	
6-octen-1-ol, 3,7-dimethyl	
D-xylopyranose, 1,2,3,4-tetrakis-o-(trimethylsilyl)	

many pharmacological activities. Further studies are needed on these extracts in order to isolate, identify, characterize and elucidate the structure of these compounds.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged Professor V.G Das, Director, Dayalbagh Educational Institute, Dayalbagh, Agra, for providing necessary research facilities. Financial support from University Grant Commission (UGC) New Delhi in the form of research fellowship for meritorious students in sciences is gratefully acknowledged. Dr. Ajay Kumar, Technician, AIRF, JNU, New Delhi for spectral analysis of the sample is also acknowledged.

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