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GC-MS Analysis of Chemical Constituents from Chloroform Extracts of *Calotropis procera (Ait.) R. Br* (Asclepiadaceae) Roots Collected in Sudan

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Abstract

The present study aim to investigate the chemical constituents of the Chloroform-soluble extracts from the roots of *Calotropis procera* using GC-MS (Gas Chromatography - Mass Spectrometry). The characterization and identification of compounds were confirmed by interpretation of their mass spectra fragmentations data and comparing their data with those published in literature. The Study has revealed to the identification of 25 components which classified in to three categories; 8 fatty acid ester derivatives, ethyl hexadecanoate 1, ethyl heptadecanoate 2, ethyl 3-trans-octadecenoate 3, ethyl octadecanoate 4, ethyl eicosanoate 5, Ethyl do-cosanoate 6, ethyl tricosanoate 7, and ehyl tetracosanoate 8; one phthalate derivative, Di-butyl phthalate 9 and sixteen Pentacyclic triterpenes derivatives, Ursa-9(11):12-diene-3yl acetate 10, Oleana-9(11):12-dien-3-yl acetate 11, Olean-12-en-3β-yl octanoate 12, Ursa-12-en-3β-yl acetate 13, Olean-9(11), 12-dien-3-yl decanoate 14, Olean-12-en-3β-yl acetate 15, Ursa-12-en-3β-yl nanoate 16, Ursa-9(11), 12-dien-3-yl petanoate 17, 11-oxo-β-amyrin acetate 18, Ursa-12-en-3β-yl hexanoate 19, 11-oxo- α -amyrin acetate 20, Ursa-12-en-3β-yl pentanoate 21, ψ-Taraxasteryl pentanoate 22, Taraxasteryl pentanoate 23, 11-oxo-β-amyrin pentanoate 24, and 11-oxo- α -amyrin pentanoate 25. These compounds might be related to the folk utilization of *Calotropis procera* and responsible for its strong antibiotic.

Keywords

Calotropis, procera, Asclepiadaceae, GC-MS, Chemical Constituents

1. Introduction

Genus Calotropis procera belonging to family (Asclepiadaceae), widely distributed in tropical and subtropical Africa and Asia [1]. Two common species of Calotropis have been mentioned in literature by ancient writers, C. procera and C. gigantean. Both of these species contain similar phytochemical constituents and could possibly be used as substitutes for one another [2, 3]. In Sudan, this plant is locally known as Usher (in Arabic) and it is grown in Darfur, Kordofan and Northern regions. It is important Sudanese medicinal plant used in traditional medicinal systems for curing wide range of diseases [4]. Fruits, leafs, roots and latex were used as anti-asthmatic or as anti-rheumatic, diphtheria, eye infection, jaundice, ringworm, skin disease, gonorrhea [5]. Previous phytochemicals studies on the latex roots, leaves, flower and fruits of *C. procera* reveals to presence of different compounds such as pentacyclic triterpenes [6–8], Acyclic diterpenic [9, 10], flavonoids [11–13], sterols cysteine protease procerain, alkaloids and numerous cardenolides [14–16] made this plant of scientific attraction for centuries. *C. procera* has been reported to possess many pharmacological effects such as anthelmintic, antibacterial, antimicrobial, anti-inflammatory, anticancer, cardiovascular, antioxidant and anticonvulsant [17–21]. To date, there are no studies on the chemical composition of *C. procera* grown in Darfur. Therefore, this study aim to investigate the chemical constituents in the chloroform extracts of *C. procera* roots.

2. Materials and Methods

2.1. Plant Material

C. procera roots were collected from Zalingei, Darfur region, Sudan. The plant was authenticated and a voucher specimen (No. 20161016) has been deposited in the herbarium of author's laboratory

2.2. Sample Extraction

A 100 g of dried roots powder were extracted in 500 ml of chloroform in an orbital shaker for three days. Repeated extraction was done with the same solvent until a clear colorless solvent was obtained. The extracts were evaporated to dryness and stored at 4°C in an airtight container for further use.

3. Instrumentation

The analyses were performed using GC-MS instrument (Shimadzu Japan equipped with an HP-5 MS capillary column (BPX5-5% phenyl (equivalent)/95% methyl polysilphenylene/siloxane phase, 30 m × 0.25 µm x 0.25 µm, Shimadzu). The injector port temperature was set at 250°C, and column temperature program was set at 50°C (2 min) with an increasing rate of 5°C/min and maintenance at 300CC for 5 min. Helium (99.999%) was used as the carrier gas with its flow-rate of 0.8 mL/min. The split ratio used was 1:10. The MS conditions included an ion source and ionization voltage used was electron ionization (EI) and 70eV respectively. The ion source temperature was set at 230°C, ionization energy of 70 eV, and the interface temperature was 250°C with its solvent cut-off time of 2.0 min. The start time was set at 19 min and the end time was set at 82 min. and mass scan range of 40–550 amu.

4. Results and Discussion

The chloroform extract of the roots of *C. procera* was investigated by GC-MS and it corresponding chromatogram was presented in Figure 1. The chromatogram revealed the presence of twenty five components which were presented in Table 1. The identification of the compounds was confirmed by interpretation of their mass spectra fragmentations data and comparing their data with those published in literature.

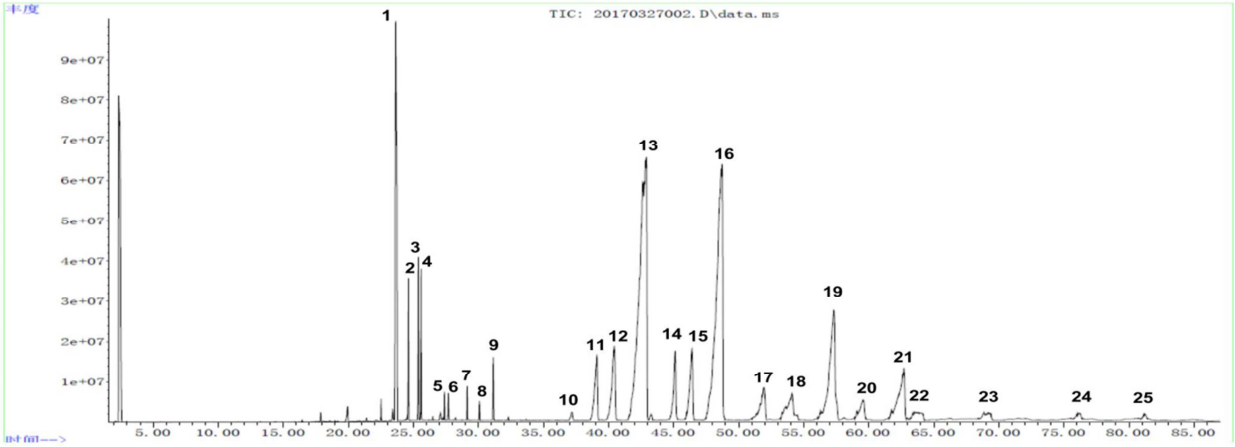

Figure 1. *Chromatogram of the chloroform extract of the roots of C. procera.*

The peaks appeared at 23.62, 24.61, 25.41, 27.30, 29.13, 30.12 and 31.12 min were identified as, ethyl hexadecanoate 1, ethyl heptadecanoate 2, ethyl 3-trans-octadecenoate 3, ethyl octadecanoate 4, ethyl eicosanoate 5, Ethyl do-cosanoate 6, ethyl tricosanoate 7, and ehyl tetracosanoate 8 respectively Figure 2, by comparison their mass spectra with those reported in the lipid website [22] and the literature [23–32].

Table 1. *Chemical constituents of the chloroform extract of the roots of C. procera.*

Com	RT	M^+	BP	Key fragment ions	Name of compound
1	23.62	284	101	255, 241, 227, 213, 199, 185, 157	Ethyl hexadecanoate
2	24.61	298	88	255, 213, 185, 157, 115, 101	Ethyl heptadecanoate
3	25.41	310	55	264, 222, 199, 180, 123, 101, 88	Ethyl 3-trans- otadecanoate
4	25.61	312	88	269, 213, 199, 157, 101, 43	Ethyl octadecanoate
5	27.39	340	88	297, 269, 241, 199, 157, 143, 101	Ethyl ei-cosanoate
6	27.65	278	55	258, 211, 183, 167, 149	Di-(2-ehylhexayl) phthalate
7	29.13	368	88	325, 297, 269, 199, 157, 101	Ethyl do-cosanoate
8	30.12	382	88	339, 283, 199, 157, 115, 101	Ethyl tri-cosanoate
9	31.12	396	88	353, 297, 255, 207, 157, 101	Ethyl tetra-cosanoate
10	37.26	466	255	406, 313, 281, 218, 119, 69, 43	Ursa-9(11):12-diene-3yl acetate
11	39.09	466	255	407, 391, 295, 218, 119, 69, 43	Oleana-9(11):12-dien-3-yl acetate
12	40.46	552	218	468, 393, 257, 218, 203, 189, 119	Olean-12-en-3β-yl octanoate
13	42.87	468	218	393, 257, 203, 189, 119, 43	Ursa-12-en-3β-yl acetate

Com	RT	M^+	BP	Key fragment ions	Name of compound
14	45.12	578	255	563, 407, 391, 295, 218, 69, 43	Olean -9(11), 12-dien-3-yl decanoate
15	46.44	468	218	426, 393, 257, 203, 189, 119, 43	Olean-12-en-3β-yl acetate
16	48.64	566	218	468, 393, 257, 203, 189, 119, 43	Ursa-12-en-3β-yl nanoate
17	51.94	508	255	406, 391, 295, 218, 119, 85	Ursa-9(11), 12-dien-3-yl petanoate
18	54.19	482	273	407, 355, 218, 175, 135, 43	11-oxo- β -amyrin acetate
19	57.24	524	218	482, 408, 270, 255, 203, 189	Ursa-12-en-3β-yl hexanoate
20	59.55	482	273	407, 355, 299, 232, 218, 135, 43	11-oxo- α -amyrin acetate
21	62.65	510	218	409, 291, 257, 203, 189, 135	Ursa-12-en-3β-yl pentanoate
22	63.96	510	189	409, 393, 291, 257, 218, 161, 135	Ψ-Taraxasteryl pentanoate
23	69.15	510	189	409, 393, 365, 218, 203, 191, 175	Taraxasteryl pentanoate
24	76.17	524	373	423, 407, 339, 299, 232, 175, 135	11-oxo-β -amyrin pentanoate
25	81.26	524	273	422, 407, 339, 299, 232, 175, 135	11-oxo- α -amyrin pentanoate

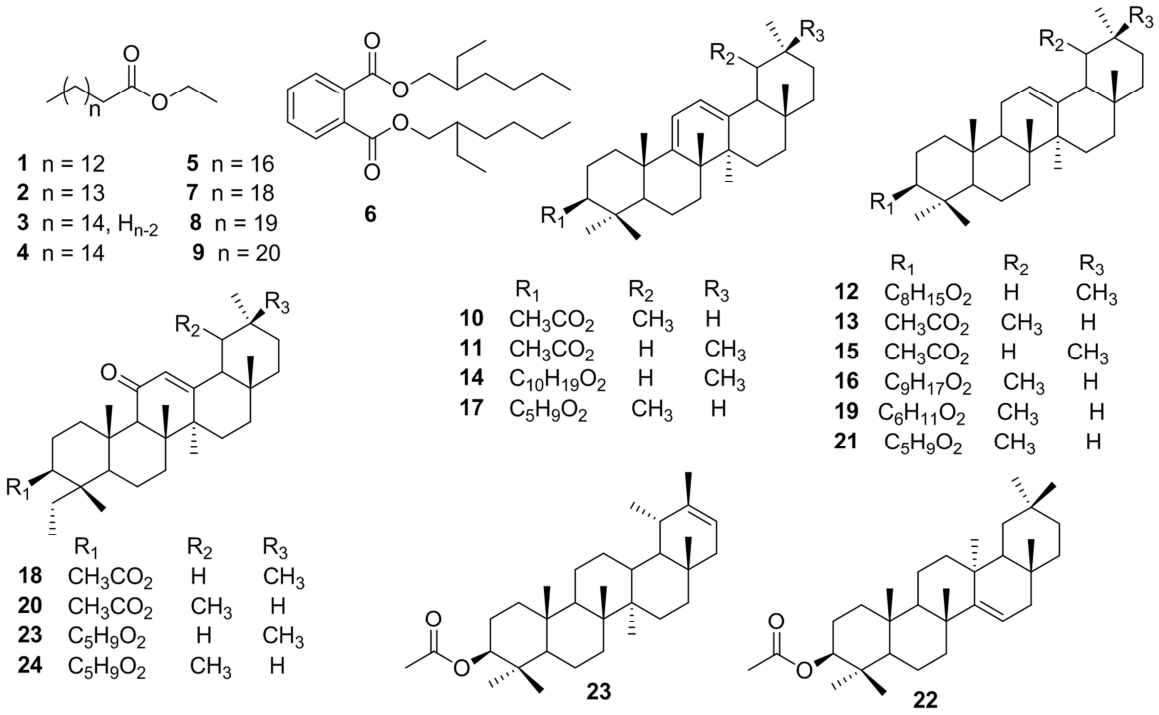

Figure 2. *Structure of Chemical constituents identified from the chloroform extract of the roots of C. procera. Numbers correspond to compounds listed in Table 1.*

Compound 9, appeared at 27.65 min was produced molecular ion m/z at 390.5 $[M]^+$ in it mass spectra corresponded to an elemental composition $C_{24}H_{38}O_4$ and fragment ions at m/z 279.2 $C_{16}H_{22}O_4$, 167.4 $[C_8H_7O_4]$ and 149.6$[C_8H_5O_3]$. This compound was identified as di (2-ehylhexayl) phthalate, by comparing it MS data with references [1, 33–36] which were in full agreement.

Compound 10, 11, 14 and 17 appeared at 37.26, 39.09, 45.12 and 51.94 min respectively in GC chromatogram, their mass spectra characterized by two intense peaks which were observed at *m/z* 255 and 313, indicating the presence of the cisoid diene at C-9 (11): 12 of a pentacyclic triterpene carbon skeleton [37]. Compound 10 showed molecular ion m/z at 466.6 $[M]^+$ corresponded to molecular formula $C_{32}H_{50}O_2$ and 406 [M-HOAc], also showed arising peaks at *m/z* 255$[M\text{-}C_{13}H_{24}O_2]^+$ and 313$[M - C_{11}H_{22}]$ respectively, by direct comparison with ursa-9(11):12-dien-3-yl acetate reported in [37, 38] was full agreement. The mass spectrum of 11 has characteristic fragments of oleana- 9(11):12-dien with significant M^+ at *m/z* 466, and M-HOAc-CH_3 to *m/z* 391 and a minor loss of acetic acid to *m/z* 407[37], the fragment ions at m/z 313, 295 and 255 are also evident due to the loss from the molecular ion of $C_{21}H_{32}O_2$, $C_{11}H_{22}$ and $C_{12}H_{25}$ from ring B and D ring respectively. So Compound 11 identified as oleana-9(11):12-dien-3-yl acetate [39]. Compound 14 and 17 have mass spectra fragment ions similar to that characterize the cisoid diene at C-9 (11): 12 of a pentacyclic triterpene carbon skeleton [40]. Also their El-MS spectra showed intense peaks [M+] ions at *m/z* 578 and 508 which corresponded to molecular formula $C_{40}H_{66}O_2$, $C_{35}H_{56}O_2$ respectively, these compounds was identified as Olean -9(11), 12-dien-3-yl decanoate 14 and Ursa-9(11), 12-dien-3-yl petanoate 17.

In the mass spectra of compound 12, 13, 15, 16, 19 and 21, characterized by a base peaks at *m/z* 189, 203 and 218 can be related to the typical fragmentation pathway of oleanane (β-Amyrine) and ursine (α-Amyrine) -type molecules with a double bond in position 12 [41]. In particular, the ion at m/z 218 is due to a classical retro-Diels-Alder fission of ring C and ions at m/z 189 and 203 arise from fragmentation rearrangements of the radical ion at m/z 218 [42]. The

differentiation between oleanane- and ursane could be seen by examination of the relative intensities of the peaks at *m/z* 189 and 203: β-amyrin has a *m/z* 203 peak around twice the intensity of the *m/z* 189 peak, while α-amyrin spectra shows both peaks with similar intensities [38]. The mass spectrum of compound 12 and 15 have a base peaks at *m/z* 218 and intense ions at *m/z* 203 and 189, which, with the M^+ at *m/z* 552 and 468 respectively, compound 12 characterized by typical fragments at *m/z* 468[M-C_6H_{12}], 425 [M-$C_{10}H_{16}O_2$], and 393[M-$C_{10}H_{16}O_2$-H_2O], indicate to an olean-12-en-3β-yl octanoate and compound 15 characterized by typical fragments at M^+ $[C_{32}H_{52}O_2]^+$ at *m/z* 468, 425 [M-C_2H_5OH], and 393[M-C_2H_5OH] which identified oleanane acetate. Their mass spectra are good fits with those reported in [37, 39].

The mass spectrum of compound 13, 16, 19 and 21 are seem to be similar, with the M^+ at *m/z* 468, 566, 524, and 510, corresponded to molecular formula $[C_{32}H_{52}O_2]^+$, $[C_{39}H_{66}O_2]^+$, $[C_{36}H_{60}O_2]^+$ and $[C_{35}H_{58}O_2]^+$ respectively, they have typical intense ions fragments ions at *m/z* 218, 189(31) and 203(26), can be related to the typical fragmentation pathway of ursane-type (α-Amyrine) molecule with a double bond in position 12, as reported in [38, 40], their mass spectra are also good fits with those reported in [38, 42]. According to the above justification these compounds identified as Ursa-12-en-3β-yl acetate 13, Ursa-12-en-3β-yl nanoate 16, Ursa-12-en-3β-yl hexanoate 19 and Ursa-12-en-3β-yl pentanoate 21.

The mass spectrometric fragmentation Compound 18, 20, 24 and 25 were showed intense fragment ions at *m/z* 232, *m/z* 273 and *m/z* 135, similar to that characteristic of the triterpenes of the 11-oxo-α-amyrin and 11-oxo-β-amyrin [38, 43]. El-MS of 18 and 20 showed an [M+] ions at *m/z* 482, corresponded to molecular formula $C_{32}H_{50}O_3$ for each, unequivocal differentiation between 18 and 20 could be seen by examination of the relative intensities of the peaks at *m/z* 135 and 218: Compound 18 has a *m/z* 135 (100) and 218 (89), this compound identified as 11-oxo-β-amyrin acetate and compound 20 has a *m/z* 135 (89) and 218 (100), this one identified as 11-oxo- α -amyrin acetate [38].

El-MS of 24 and 25 showed an fragment ions [M^+] ions at *m/z* 524, corresponded to molecular formula $C_{35}H_{56}O_3$ for each. While EI-MS of compound 24 showed fragment ions at *m/z* 273 (100) and 232 (79), EI-MS of compound 25 showed fragment ions at *m/z* 273 (100) and 232 (89), by direct comparison with those reported in [38]. Compound 24 and 25 identified as 11-oxo-β -amyrin pentanoate and 11-oxo- α -amyrin pentanoate respectively. Compound 22 and 23 appeared at 69.15 and 69.15 min their El-MS showed an [M^+] ion at *m/z* 510 for each, corresponded to molecular formula $C_{35}H_{58}O_2$, the differentiation between these isomer, the MS spectra of compound 22 showed fragment ions at m/z 409 ($C_{20}H_{49}$, 8), 393 ($C_{29}H_{45}$, 8), 291 ($C_{19}H_{31}O_2$, 5), 191 ($C_{14}H_{23}$, 18), 189 ($C_{14}H_{21}$, 100), while spectra of compound 23 has characteristic fragment ions at m/z 409 ($C_{20}H_{49}$, 8), 393 ($C_{29}H_{45}$, 10), 291 ($C_{19}H_{31}O_2$, 8), 191 ($C_{14}H_{23}$, 33), 189 ($C_{14}H_{21}$, 100), by direct comparison with ψ - Taraxasteryl pentanoate and Taraxasteryl pentanoate reported in [39], compound 22 and 23 respectively were good agreement.

5. Conclusions

GC-MS has proved to be a very powerful tool affording both the separation and characterization of saturated, unsaturated fatty acid ester derivatives and pentacyclic triterpene. MS data furnish a fast differentiation among important skeleton of pentacyclic triterpenes types, some of them with potential biological interest shown in literature. These compounds may be related to the folk utilization of *Calotropis procera* as strong antibiotics.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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