BIOCHEMICAL PROFILE OF *Calotropis procera* FLOWERS

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Abstract

Calotropis procera (Aiton) W.T. Aiton is a medicinal weed of family Asclepiadaceae. This study was carried out to explore the biochemical profile of *C. procera* flowers collected from Southern Punjab region of Pakistan. Methanolic flower extract of *C. procera* was subjected to GC-MS analysis. There were 30 compounds identified in this extract. The predominant compound was γ-sitosterol with 15.39% peak area. Other abundantly occurring compounds included stigmasterol (9.22%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (9.01%), campesterol (8.63%), α-amyrin acetate (8.25%), β-amyrin (8.09%), hexadecanoic acid, methyl ester (7.91%), 11-octadecenoic acid, methyl ester (6.15%), and 2-methoxy-4-vinylphenol (5.66%). Moderately abundant compounds included nonacos-1-ene (2.83%), methyl stearate (1.57%), pentacosane (1.44%)**,** phytol (1.33%), heptacos-1-ene (1.20%), heneicosane (1.19%), and 1-hexacosene (1.09%). The remaining less abundant compounds were present with peak areas less than 1%. Literature survey showed that the major compounds identified in the flower extract of *C. procera* possess various bioactivities including ant-diabetic, anticancer, antihyperglycemic, antioxidant, antimicrobial and anti-inflammatory.

Keywords: *Calotropis procera,* Flowers, GC-MS analysis, Phytochemicals.

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INTRODUCTION

Calotropis procera is a perennial medicinal shrub of family Asclepiadaceae that grows mainly in arid to semi-arid regions (Radhaboy *et al.,* 2019). The plant is native to Middle East Asia, Latin America, Africa and Southwestern Asia (Kaur *et al.,* 2021). It reproduces from seeds and commonly has a height of 1–2 m. It is an erect spreading shrub that comprises of a single or few primary stems giving rise to several secondary and tertiary stems (Fig. 1). Being a drought and salt tolerant plant, it profusely grows and can survive under harsh environmental conditions (Coêlho *et al.,* 2019). It is commonly found along watercourses, roadsides, coastal dunes, overgrazed pastures and river flats. The successful invasion of *C. procera* can be attributed to its high reproductive potential (Kaur *et al.,* 2021).

Since ancient times, *C. procera* is utilized in traditional medicine systems to cure human diseases (Kumari and Sood, 2020). It contains flavonoids, tannins, cardiac glycosides, alkaloids, triterpenes and/or sterols possessing antioxidant, pharmacological, and cytostatic properties (Radwan *et al.,* 2019; Ghramh *et al.,* 2021). The plant also has antidiarrheal, analgesic, anti-inflammatory, antiulcer, antimicrobial and insecticidal activities (Waheed *et al.,* 2016; Falana and Nurudeen, 2020; Kumari and Chaudhary, 2021). In Pakistan, mudarin and asclepsin compounds have been isolated from this plant with bactericidal, emeto-cathartic, vermicidal and digitalic properties (Taylor, 2004). Western and Central African countries utilize aerial parts of this plant to cure skin diseases, sores, sinus, diarrhea, fistula and wounds (Yaniv and Koltai, 2018). Moreover, root bark is used for treatment of fever, leprosy, snake bite, malaria, dysentery, dermatitis and elephantiasis. Plant leaves serve as an effective cure for burn injuries, rheumatism and mumps, whereas flowers are used as a tonic to treat catarrh and asthma (Mali *et al.,* 2019). Traditionally, dry *C. procera* powder is given to the patients for the treatment of asthma, bronchitis, hepatic and spleen enlargement (Paul and Kumar, 2018). In addition, processed plant latex is commercially available to treat eyes, tooth aches, hair fall, paralysis, rheumatoid and intermittent fevers (Meena *et al.,* 2011). Keeping in view ethnophormacological importance of *C. procera,* this study was carried out to identify various phytoconstituents present in flowers of this plant through GC-MS analysis.

Fig. 1: *Calotropis procera* growing on a deserted land of Bahawalpur.

MATERIALS AND METHODS

Collection of flowers

The fresh and healthy full bloomed flowers of *C. procera* were plucked from Bahawalpur district (Southern Punjab). These plants were growing along the roadside; nationally known as N5 under the jurisdiction of National Highways Authority. The flowers were kept in a paper bag and shifted to the laboratory. Before proceeding to further procedures, the plant specimen was identified and confirmed by a botanist Dr. Arshad Javaid (also a co-author of this article).

Preparation of methanolic extract

The flowers were dried under room temperature to evaporate the moisture. Thereafter, the flowers were also kept in the oven at a temperature of 35 ºC for one day for complete moisture evaporation. The dried flowers were then ground into a fine powder form by using a pastel and mortar. This finely grounded powder (10 g) of the *C. procera* flowers was completely soaked in 50 mL of analytical grade methanol in a graduated flask for 15 days. After that, the flower extract was filtered and subjected to GC-MS examining for biochemical profiling (Ferdosi *et al.,* 2021a).

GC-MS analysis

The gas chromatographic (GC) machine model 7890B (Agilent, USA) and mass spectroscopic machine model 5977A (Agilent, USA) were used for phytochemical profiling of methanolic flower extract of *C. procera* following the procedure described by Ferdosi *et al.* (2020). The column used was DB 5MS (30 m × 0.25 μm × 0.25 μm); injection volume was 1 μL; helium was used in a split less mode as a carrier gas. Oven ramping temperature at the start was 80 ºC and then raised 10 ºC per min up till 300 ºC. Inlet temperature was 280 ºC with run time 50 min. MS conditions were: scan range 50–500 m/z; solvent delay time 5 min; and source temperature was 230 ºC. Run time was 50 minutes. The spectra were compared with NIST library of 2017 version for the identification of chemical constituents and arranged in the ascending order of their retention times, respectively. The relative abundance of compounds was reported by using their peak areas. The ChemDraw software was used for drawing the structures of major compounds.

RESULTS AND DISCUSSION

There were 30 compounds identified in flower extract of *C. procera* as presented in Fig. 2. Details of these compounds are given in Table 1. Structures of the abundantly occurring compounds are presented in Fig. 3. The predominant and the most abundant compound in the extract was γ-sitosterol (15.39%). Other abundantly occurring compounds included stigmasterol (9.22%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (9.01%), campesterol (8.63%), α-amyrin acetate (8.25%), β-amyrin (8.09%), hexadecanoic acid, methyl ester (7.91%), 11-octadecenoic acid, methyl ester (6.15%), and 2-methoxy-4-vinylphenol (5.66%) with peak areas above 5%. Seven compounds including nonacos-1-ene (2.83%), methyl stearate (1.57%), pentacosane (1.44%), phytol (1.33%), heptacos-1-ene (1.20%), heneicosane (1.19%), and 1-hexacosene (1.09%) were categorized as moderately abundant ones. The remaining compounds with peak areas less than 1% were ranked as the less abundant ones. These consist of docosanoic acid, methyl ester (0.85%), stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-

(0.77%), eicosanoic acid, methyl ester (0.73%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (0.69%), tetradecanal (0.69%), 1-tetracosene (0.68%), 9,19-cyclolanostan-3-ol, 24-methylene-, (3.beta.)- (0.67%), phenol, 2-methoxy- (0.61%), benzofuran, 2,3-dihydro- (0.54%), and pentacos-1-ene (0.50%).

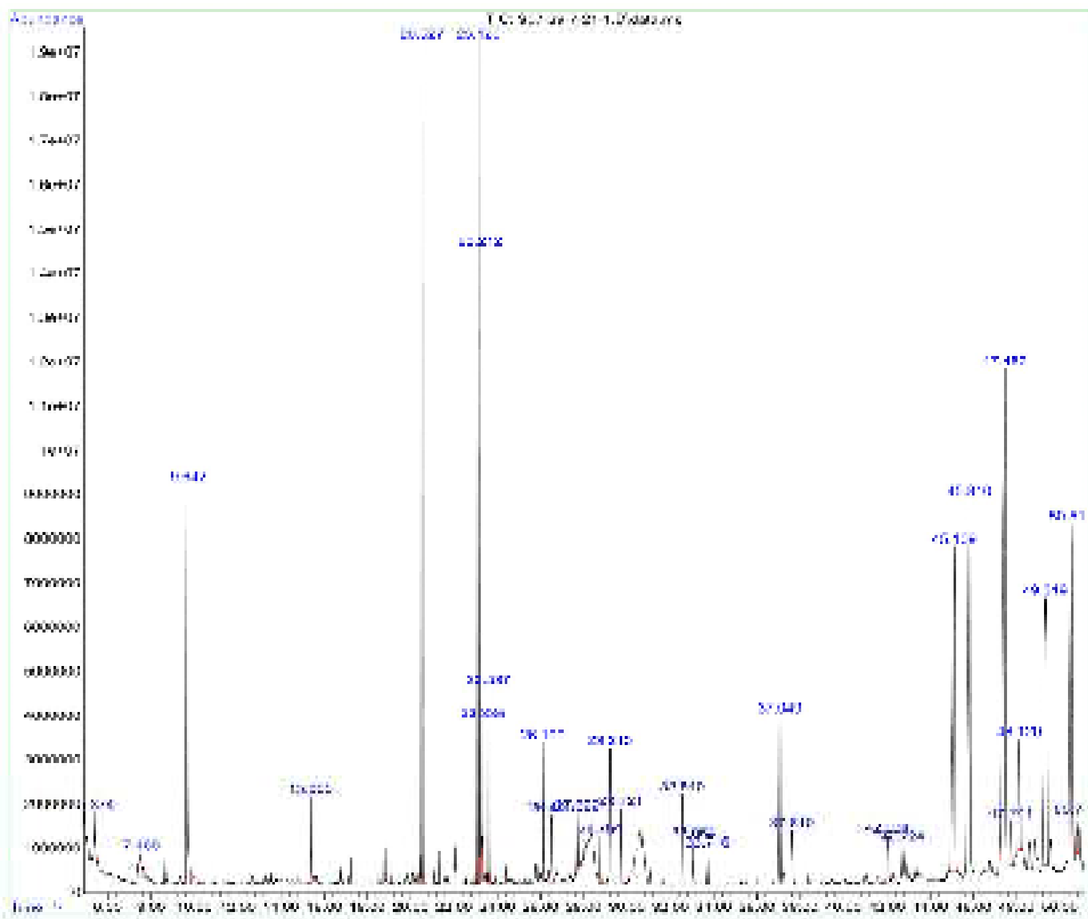

Fig. 2: Chromatogram of flower extract of *Calotropis procera*.

The most abundant compound γ-sitosterol (15.39%) has been identified in many plant species including *Acacia nilotica, Lippia nodiflora* and *Chenopodium quinoa* with antidiabetic and anticancer activities (Balamurugan *et al.,* 2011; Sundarraj *et al.,* 2012; Khan and Javaid, 2020a). It was also found as the major compound in different species of genus *Lagerstroemia* with peak areas from 14.70–34.44% showing antihyperglycemic activity (Sirikhansaeng *et al.,* 2017). Stigmasterol (9.22%), an unsaturated sterol, was previously identified in a number of plant species such as *Cirsium arvense* flowers (Ferdosi *et al.,* 2021b), *C. quinoa* leaves (Khan and Javaid, 2020), and *Chenopodium murale* stem (Naqvi *et al.,* 2020). It has been shown to possess anti-inflammatory and anti-diabetic (Wang *et al.,* 2017; Zeb *et al.,* 2017). Different derivatives of this compound namely stigmasterol glucoside, cyasterone, fucosterol epoxide, and spinasterol, fucosterol are known for their pharmacological properties. In addition, this important compound also has its involvement in the formation of a variety of hormones such as estrogens, androgens corticoids and progesterone (Kaur *et al.,* 2011). 9,12-octadecadienoic acid (Z,Z)-, methyl ester (9.01%), hexadecanoic acid, methyl ester (7.91%), 11-octadecenoic acid, methyl ester (6.15%), docosanoic acid, methyl ester (0.85%), eicosanoic acid, methyl ester (0.73%) and tetracosanoic acid, methyl ester (0.52%) belong to fatty acid methyl esters group. Such compounds are known for their antimicrobial and antioxidant activities (Ali *et al.,* 2017; Pinto *et al.,* 2017). β-Amyrin (8.09%) has been found in various plant species including *Monotheca buxifolia, Myrcianthes pungens* and *Melia azedarach,* showing antifungal, anti-inflammatory and antioxidant activities (Jabeen *et al.,* 2011; Okoye *et al.,* 2014; Cardoso *et al.,* 2020; Javed *et al.,* 2021). α-Amyrin acetate (8.25%) showed antihyperglycaemic activity in rats (Singh *et al.,* 2009). Campesterol (8.63%), an anticancer compounds having a structure similar to that of cholesterol (Choi *et al.,* 2007), reduces cholesterol absorption in the intestine by competing with cholesterol (Choudhary and Tran, 2011).

Table 1: Compounds identified in flower extract of *Calotropis procera*.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Phenol, 2-methoxy-	$C_7H_8O_2$	124.13	5.374	0.61
2	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	7.460	0.54
3	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	9.642	5.66
4	Tetradecanal	$C_{14}H_{28}O$	212.37	15.365	0.69
5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	20.527	7.91
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	23.126	9.01
7	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48	23.212	6.15
8	Phytol	$C_{20}H_{40}O$	296.53	23.335	1.33
9	Methyl stearate	$C_{19}H_{38}O_2$	298.50	23.597	1.57
10	Heneicosane	$C_{21}H_{44}$	297.57	26.100	1.19
11	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.55	26.490	0.73
12	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340.49	27.699	0.69
13	Pentacos-1-ene	$C_{25}H_{50}$	350.66	28.764	0.50
14	Pentacosane	$C_{25}H_{52}$	352.68	29.213	1.44
15	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	29.721	0.85
16	Heptacos-1-ene	$C_{27}H_{54}$	378.71	32.540	1.20
17	Tetracosane	$C_{24}H_{50}$	338.65	33.064	0.68
18	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.66	33.716	0.52
19	Nonacos-1-ene	$C_{29}H_{52}$	400.72	37.049	2.83
20	1-Hexacosene	$C_{26}H_{52}$	364.69	37.610	1.09

21	1-Nonadecene	$C_{19}H_{38}$	266.50	42.034	0.85
22	1-Tetracosene	$C_{24}H_{48}$	336.63	42.724	0.68
23	Campesterol	$C_{28}H_{48}O$	400.68	45.109	8.63
24	Stigmasterol	$C_{29}H_{48}O$	412.69	45.810	9.22
25	γ-Sitosterol	$C_{29}H_{50}O$	414.70	47.452	15.39
26	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-	$C_{29}H_{48}O$	412.69	47.741	0.77
27	α-Amyrin	$C_{30}H_{50}O$	426.71	48.120	2.26
28	β-Amyrin	$C_{30}H_{50}O$	426.71	49.319	8.09
29	α-amyrin acetate	$C_{32}H_{52}O_2$	468.8	50.517	8.25
30	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{31}H_{52}O$	440.74	50.752	0.67

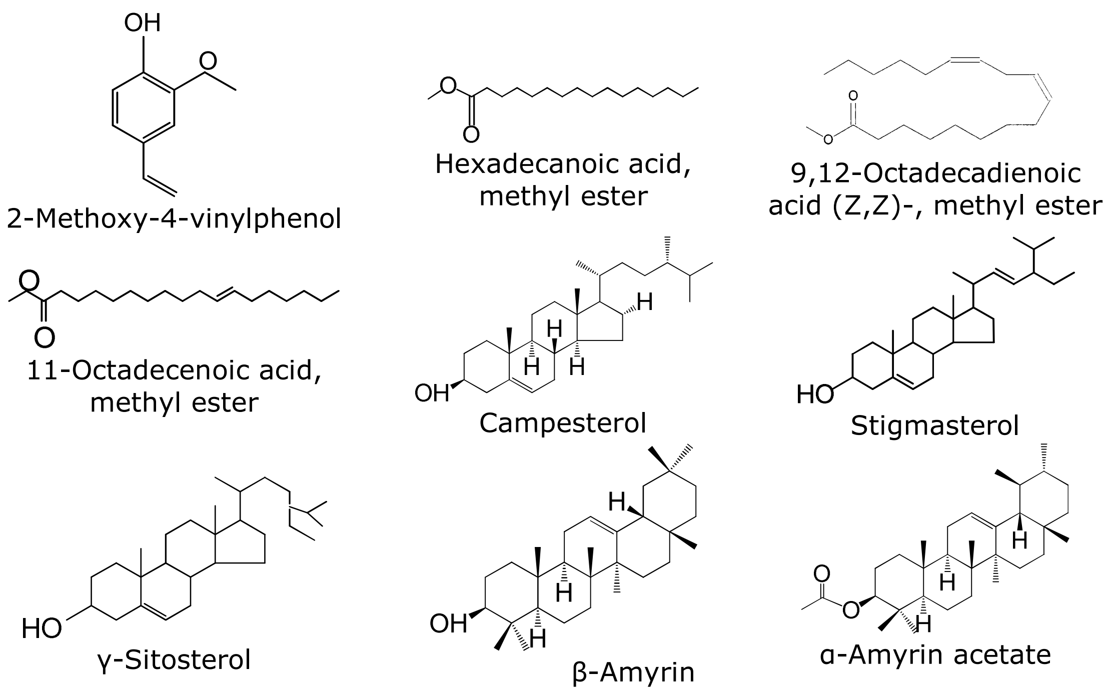

Fig. 3: Structures of major compounds in flower of *Calotropis procera*.

Conclusion

This study concludes that flowers of *C. procera* growing in desert land of Bahawalpur contains a number of important compounds such as γ-sitosterol, stigmasterol, campesterol, β-amyrin, α-amyrin acetate, and different types of fatty acids methyl esters with antidiabetic, antihyperglycemic, anticancer, antimicrobial, antioxidant and/or anti-inflammatory activities.

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