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Research Article

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GC-MS Analysis and Antimicrobial Activity of Ethanolic Extract of *Calotropis procera* (Ait.) R. Br. Leaves

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ABSTRACT

Calotropis procera (Ait) R. Br. is evergreen small woody shrub growing wildly in many parts of the world especially warm places. Its leaves have significant bilirubin lowering, antioxidant, antiplasmodial, anti-inflammatory, wound healing properties and are used to treat body pain, burns, injuries, rheumatism, sinus fistula and snake bite. GC-MS analysis of ethanolic extract of the leaves showed the presence of triterpenes (16.62%) including norolean-12-ene (2.11%), methyl commate A (4.23%), 9,19-cyclolanost-24-en-3β-ol (1.31%), lup-20(29)-en-3-olβ acetate (2.43%) and lupeol (1.64%) and steroids (14.07%) viz., cholesta-4,6-dien-3β-ol benzoate (1.14%), (24R)-ergost-5-en-3β-ol (3.75%), stigmast-5-en-3β-ol (3.56%) and stigmasta-5,24(28)-dien-3β-ol (1.31%).The other compounds detected in the leaf extract were alkanes (4.53%), aliphatic amides (2.59%), fatty esters (1.84%), oxo-alkane (1.06%), diterpene alcohol, (1.07%) and acyclic diterpene (1%) were also present. The leaf ethanolic extract showed significant antimicrobial activity against Escherichia coli and Staphylococcus aureus in all concentrations. Mild antibacterial activity was observed with Bacillus subtilis and Streptococcus pyogenes in high concentrations. Futher the isolation, chemical characterization and clinical activities of the major chemical constituents of the leaves can be carried out.

Keywords: *Calotropis procera*; Leaves; Ethanolic extract; Chemical composition; Antimicrobial activity

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INTRODUCTION

Calotropis procera (Ait.) R. Br. (Asclepidaceae) is a wild plant which spreads in regions suffering from environmental stress such as drought and salinity [1]. Different parts of plant are used as a remedy for leprosy, eczema, inflammation, skin infections, syphilis, malarial, low hectic fevers, and as an abortifacient [2]. The powdered leaves are used for the fast healing of wounds, purgative, or used as treatment for indigestion, jaundice, joint pain, skin disorders, swelling, hepatic problems and to promote sexual health including penile dysfunction [3]. An aqueous extract of the leaves showed significant bilirubin lowering and wound healing properties in rats [4]. Leaves were responsible to cause tachycardia and transitory cardiac arrhythmias in sheep may be due to cardiotoxicity and hepatotoxicity by stimulation of inflammation, increased oxidative stress and suppression of antioxidant defense system [5]. A root methanol extract of *C. procera* has shown ameliorative effect in diabetic neuropathy which may be attributed by its multiple actions including potent hypoglycemic and antioxidant effects [6]. The leaves are used as a homeopathic medicine and to alleviate ringworms [7]. It showed good antimicrobial activity against both Gram positive and Gram negative bacteria [8]. The leaf extracts also possessed remarkable larvicidal, adult emergence inhibitor, repellent and oviposition deterrent effects against both *Anopheles arabiensis* and *Culex quinquefasciatus* and might be used as natural biocides for mosquito control and for motorcycle helmet disinfection [9]. The roots contained two steroidal derivatives, *n*-triaconthexene-1-ol, urs-12,20(30)-dienyl laurate,

1′-β-sitosteryl-2′-caproyl glycerol, and two fatty acids [10], saponins, tannins, cardiac glycosides, alkaloids and flavonoids [11]. The leaves possessed quercetagetin-6-methyl ether 3-O-glycosides and lupeol [12]. The flavonoids were present in highest quantity in leaves of *C. procera* [13]. In Jazan region of KSA major compounds present in stem bark were triterpenes, fatty esters and aliphatic amides which vary from the compounds grown in other parts of the world [14]. Alkaloids, saponins, tannins and flavanoids were the main constituents present in leaf extract of plant and exhibited significant antimicrobial activity [15]. This manuscript describes the GC-MS analysis and antimicrobial activity of an ethanolic extract of the leaves of the *C. procera* collected from Jazan, Saudi Arabia.

MATERIALS AND METHODS

Plant Material

The leaves of *C. procera* were collected from Jazan, Saudi Arabia and identified by Dr. Yahiya Masruhi, Department of Botany, Faculty of Science, Jazan University. A voucher specimen of the sample is preserved in the herbarium of the Department, College of Pharmacy (KSA).

Preparation of the Leaves Extract

Coarsely powdered 500 g of the air-dried leaves were exhaustively extracted with 450 ml of ethanol in a Soxhlet apparatus for three days (48 hrs). The extract was then concentrated on a steam-bath. 50 g of the dark brown mass was achieved by drying the concentrated extract under reduced pressure. The residue was then stored in the dark at 4ºC for subsequent experiments.

Analysis of Extract by GC-MS

GC-MS analysis were carried out on a Shimadzu Gas Chromatograph instrument fitted with a capillary column TR-5MS (30 m × 0.25 mm), film thickness 0.25 µm. The carrier gas was He, flow rate 1.2 ml/min. The initial temperature was 70ºC and then heated at a rate of 15ºC per minute to 290ºC and held for 16 minutes. The chromatograph was coupled to Shimadzu QP2010 Ultra MS detector 70 eV.

Identification of Constituents

The most constituents were identified by GC by comparing their retention indices with those of authentic standard available in the laboratory or were in close agreement with the references. Further identification was achieved by MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer data base using the NIST08 and Wiley 9 built libraries.

Antimicrobial Activity

Microbial strains:

Escherichia coli, Staphylococcus aureus, Bacillus subtilis and *Streptococcus pyogenes* bacterial pure cultures of were obtained from Microbiology Department, College of Pharmacy, Jazan University, Jazan, KSA. All the four bacterial cultures were maintained on nutrient agar medium at 4ºC.

Standard for antimicrobial activity:

Tetracycline (50 µg/ml) antibiotic was used as standard which was prepared in dimethyl sulphoxide.

Media preparation and sterilization:

Deionized water was used to prepared nutrient agar media. 28 g of dehydrated nutrient agar medium [composed of sodium chloride (5.0 g), peptone (5.1 g), beef extract (1.5 g), yeast extract (1.5 g) and agar (1.5 g)] was accurately weighed and suspended in 1 L of distilled water in a conical flask and dissolved. Autoclave was used to sterilize the dissolved medium at 121ºC for 15 min at lbs/in^2 pressure [16].

Bacterial culture:

Previously maintained slants of bacterial culture on nutrient agar medium at 4ºC were then transferred to fresh slants after one month. The fresh slants were then incubated at 37ºC for 24 hours. After that slants were washed using 15 ml of sterilized normal saline solution. Then a dilution was selected such that the optical density of 1.5 at 600 nm was obtained and subsequently the culture was done. The test organisms were then stored under refrigeration for further experiments.

Anti-microbial assay:

Agar well diffusion was carried out for *in vitro* antimicrobial assay [17]. The plates were prepared by transferring the liquefied and sterilized nutrient agar into petri-plates of 100 mm size and kept undisturbed for solidification. The specific test organisms were streaked over the solidified media in aseptic conditions. 6 mm ID stainless steel borer was taken to made holes in each plate. Different dilutions (95 µl) of the extract of calotropis leaves were made having concentrations of 4 µL/ml, 7 µL/ml, 10 µL/ml and 13 µL/ml of solution. Tetracycline solution was used as a standard. The plates were labeled as Co (control), S (standard), A (*Escherichia coli),* B *(Staphylococcus aureus),* C *(Bacillus subtilis)* and D *(Streptococcus pyogenes)* with four different holes, labeled as 4, 7, 10 and 13 for different concentrations. All dilutions were made in DMSO solvents. The plates were then left untouched for 3 hours at 4ºC for proper diffusion of the drug/test dilutions. After complete diffusion process all the petri plates were incubated for 24 h at 37ºC. After 24 h plates were examined and the diameters of zones of inhibition were accurately measured [18].

RESULTS AND DISCUSSION

The chemical composition of the ethanolic extract of the leaves of *C. procera* is tabulated in Table 1. The ethanolic extract consisted mainly ten sterols (14.07%), five alkanes (4.53%), two aliphatic amide (2.59%) and oxo-alkane (1.06%), three fatty esters (1.84%), one of each aromatic acid ester (0.52%), cyclic alkane (0.56%), halo-alkane (0.68%), halo-aromatic derivative (0.56%), heterocyclic compound (0.5%), sulphur compound derivative (0.51%), vitamin E (0.6%), chalcone derivative (0.43%) and aliphatic ketone (0.5%). The predominant compounds were derivatives of triterpens like six triterpenes (6.28%), three triterpenic esters (3%), two acyclic triterpenes (1.49%), one of each diterpene alcohol (1.07%), acyclic diterpene (unsaturated) (1%), monoterpene derivative (0.54%), pentacyclic triterpene (1.53%), sesquiterpene (0.71%) and triterpenic glycoside (4.23%). Thirty one compounds occurred in trace amounts. Maximum percentage of the compounds present in the leaf extract were different derivatives of triterpenes (15.84%) includes squalene, lanosterol, norolean-12-ene, urs-12-ene-3β,11β-diol diacetate, methyl commate A, 13, 27-cyclours-11-en-3-ol acetate, 12-oleanen-3β-yl acetate, lupeol, taraxasterol acetate and lup-20(29)-en-3β-ol acetate. The ethanolic leaf extract also contained sterols (14.07%) including cholesta-4,6-dien-3β-ol, stigmasta-4,7,22-trien-3α-ol, cholesta-4,6-dien-3β-ol benzoate, ergost-5-en-3β-ol, ergosta-8,24(28)-dien-3-ol, 4,14-dimethylSilane, trimethyl- [stigmast-5β-en-3-yl oxy], stigmast-5-en-3β-ol, stigmasta-5,24(28)-dien-3β-ol, 24-methylene-lophenol and 9,19-cyclolanost-24-en-3β-ol. The alkanes (4.53%) present in the leaf ethanolic extract were *n*-dotriacontane, *n*-tetracosane, *n*-docosane, *n-h*exacontane and *n*-tetrapentacontane. Methyl myristate, *n*-butyl 2,4,6,8-tetramethylundecanoate and methyl nonahexacontanoate were three fatty esters (1.84%). *n*-Hexadecanamide and 9-*n*-octadecenamide were the aliphatic amides (2.59%). Oxo-alkane (1.06%) was characterized as 1,1′-oxybis-*n*-octadecane. The diterpene alcohol (1.07) was identified as 3,7,11,15-tetramethyl-2-hexadecen-1-ol. The acyclic diterpene was formulated as (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene (1%).

The alcoholic extract was examined for antibacterial activity against *E.coli, S.aureus, B.subtilis* and *S. pyogenes.* The alcoholic extract showed good antimicrobial activity against clinically isolated pathogenic microbial strains in comparison to standard, tetracycline. The observations were recorded in Table 2.

The leaves of *C. procera* could be useful for treatment of skin disorders like eczema, psoriasis, rashes and allergies. The antimicrobial activity was reported due to the presence of phenols, alkaloids, tannins and quinines [19]. On the bases of GC-MS analysis of *C. procera* growing in Jazan region of Saudi Arabia was devoid of these compounds. The phytochemical analysis revealed that different derivatives of sterols (14.07%) and triterpenes (15.84%) were present in maximum quantity which could be the reason for antimicrobial effect. These compounds could show good inhibitory activity against three human cell lines including lung cancer, glioblastoma and prostate cancer. Natural sterols present in herbal components attenuates all necessities of a human body's immune response which is important for general disease prevention, in degenerative diseases also sterols enhance immune system and delay various aging processes [20]. These phytosterols are nontoxic chemicals. They lower cholesterol in human blood, by inhibiting the absorption of cholesterol from the gut. Sitosterols are effective for Tuberculosis, Rheumatoid Arthritis, Benign Prostatic Hyperplasia, HIV/AIDS, Anti-inflammatory and antipyretic activities [21,22]. Due to the presence of large percentage of steroids and triterpenes this plant could have great value in the field of cardiology and immunology.

Table 1: Chemical constituents of the ethanolic extract of the leaves of *C. procera*

S No	Retention time	Component	% Area
1	11.9	3,7,11,15-tetramethyl-2-hexadecen-1-ol	1.07
2	12.4	*n*-Hexadecanamide	1.32
3	13.4	9-*n*-Octadecenamide	1.27
4	14.2	Methyl myristate	0.57
5	14.4	Mono(2-ethylhexyl) phthalate	0.52
6	14.6	*n*-Dotriacontane	1.51
7	14.7	*n*-Butyl 2,4,6,8-tetramethylundecanoate	0.4
8	14.9	*n*-Tetracosane	0.57
9	15.1	*n*-Octadecyl-cyclohexane	0.56
10	15.2	Methyl nonahexacontanoate	0.87
11	15.4	*n*-Docosane	0.64
12	15.5	1-Iodo-octadecane	0.68
13	15.7	*n*-Hexacontane	1.13
14	15.9	Squalene	0.93
15	16.1	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	1
16	16.2	1,1′-Oxybis-*n*-octadecane	0.42
17	16.3	*n*-Tetrapentacontane	0.68
18	16.7	Neryl linalool isomer	0.54
19	16.8	2-[4-Cyclohexylbutanoylamino]-3-chloro-1,4-naphthoquinone	0.56
20	16.9	1,1′-Oxybis-n-octadecane	0.64
21	17.2	7,8-Dimethoxy-3,5-dimethyl-4-mesitylimino-3,4-dihydro-5H-indeno[1,2-d]pyrimidine	0.5
22	17.3	1,8-Dioxa-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl	0.51
23	17.3	Isochiapin B	0.71
24	17.4	2,6,10,15,19,23-Hexamethyl (2,6,10,14,18,22)-tetracosahexene	0.56
25	17.5	Cholesta-4,6-dien-3β-ol	0.48
26	17.7	Stigmasta-4,7,22-trien-3α-ol	0.65
27	17.8	γ-Tocopherol	0.6
28	18	5,8-Tridecadione	0.5
29	18.3	Cholesta-4,6-dien-3β-ol benzoate	1.14
30	20.1	(24R)-Ergost-5-en-3β-ol	3.75
31	21.1	4,14-Dimethyl ergosta-8,24(28)-dien-3-ol	0.68
32	21.2	Trimethyl[[-stigmast-5β-en-3-yl]oxy] silane	0.49
33	21.4	Stigmast-5-en-3β-ol	3.56
34	21.7	Stigmasta-5,24(28)-dien-3β-ol	1.31
35	21.8	24-Methylene-lophenol	0.7
36	21.9	Trans-4-ethoxy-4′-methoxychalcone	0.43
37	22	Lanosterol	0.56
38	22.2	Norolean-12-ene	2.11
39	22.3	Urs-12-ene-3β,11β-diol diacetate	0.74
40	22.7	9,19-Cyclolanost-24-en-3β-ol	1.31
41	22.9	Methyl commate A	4.23
42	23.2	13,27-Cyclours-11-en-3-ol acetate	0.45
43	23.9	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-2H-picen-3-one	1.53
44	24.8	12-Oleanen-3β-yl acetate	0.7
45	25	Lupeol	1.64
46	26.8	Taraxasterol acetate	0.65
47	27.1	Lup-20(29)-en-3β-ol acetate	2.43

Table 2: Antimicrobial activity of the leaf ethanolic extract of *C. procera* tested on pathogenic microbes

Sample conc. (µl/ml)	Zone of inhibition (mm) *E. coli*	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *B. subtilis*	Zone of inhibition (mm) *S. pyogenes*
4(A)	2	7	0	0
7(B)	10	13	0	0
10(C)	11	21	8	0
13(D)	15	21	11	5
50 (Co)	---	---	---	---
50 (S)	20	16	34	24

CONCLUSION

The leaves of *Calotropis procera* from Jazan region of Saudi Arabia contained triterpenes (15.84%), steroids (14.07%), alkanes (4.53%), aliphatic amides (2.59%), fatty esters (1.84%), oxo-alkane (1.06%), diterpene alcohol, (1.07%) and acyclic diterpene (1%). The leaf ethanolic extract showed significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* at all concentrations and mild antibacterial activity against *Bacillus subtilis* and *Streptococcus pyogenes* at high concentrations. This work has enhanced understanding about the phytoconstituents of the plant. On the basis of the major chemical compounds present in the leaves further study on the isolation, chemical characterization and clinical activities of the plant can be carried out.

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