Biological activity and investigation of some active constituents with molecular docking from two *Cleome* species

Wael Abdullah[a], Wael M. Elsayed[a], Khaled A. Abdelshafeek[a,b], Naglaa M. Nazif[a], Nasser S.M. Ismail[c], Shams I. Ismail[a], Abdel N.B. Singab[d], Somaia A. Nada[e]

Departments of [a]Chemistry of Medicinal Plants, [e]Pharmacology, National Research Center, [d]Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, [c]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Future University, Nasar City, Egypt, [b]Department of Chemistry, Faculty of Science, Albaha University, Albaha, KSA

Correspondence to Wael M. Elsayed, PhD, Department of Chemistry of Medicinal Plants, National Research Center, Dokki, Cairo, 12311, Egypt. Tel: 0201001490750; fax: 0020233354075; e-mail: waelmatar@hotmail.com

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Objective

Cleome genus belonging to *Cleomeaceae* family is used as an antidiabetic agent in Egyptian folk medicine. The current study aimed to isolated and identify the active constitutes of two species, *Cleome africana* and *Cleome droserifolia*, and to study the biological activities, such as antidiabetic, anti-inflammatory, and hepatoprotective.

Materials and methods

The lipid constituents of two plant species, *C. africana* and *C. droserifolia*, were extracted with petroleum ether and fractionated to acetone-insoluble fraction and acetone-soluble fraction. Molecular docking studies were carried out in isolated compounds. Antidiabetic, anti-inflammatory, and hepatoprotective activities of the promising compounds were investigated.

Results and conclusion

The fatty alcohols and hydrocarbons were identified from the acetone-insoluble fraction. *C. africana* comprises four fatty alcohols, in which tetratetracontanol is the main one, whereas *C. droserifolia* consists of two fatty alcohols, in which dotriacontanol is the main one. The hydrocarbons include seven compounds, with tritriacontane as the main one in both species. The acetone-soluble fraction was saponified to afford the unsaponifiable fraction, which contains in *C. africana* a series of n-alkanes from n-C14 to n-C31, representing 93.66%, with n-C22 as the main one, in addition to a sterol fraction, forming 5.13%, in which β-sitosterol is the main compound, and the fraction of *C. droserifolia* was found to contain a series of n-alkanes from n-C18 to n-C30, representing 60.46%, in which n-C30 is the main compound, besides sterols, representing about 39.54%, with campesterol as a major compound (12.46%), and finally, a triterpene fraction consisting of α-amyrin and β-amyrin. In addition, fatty acids as a mixture of eight acids (saturated and unsaturated) were found in both species; the major two acids are linoleic acid (38.99%) and palmitic acid (33.05%). Extraction of the glucosinolate (GL) constituents from the methanolic extract of *C. africana* led to isolation and identification of one compound (G), identified as 3-ethylsulfonyl-2,3-dimethoxypropyl GLs. Enzymatic hydrolysis of the total GLs allowed us to identify eight isothiocyanate and two thione compounds. Molecular docking of compound G with glucosidase enzyme using C-DOCKER protocol resulted in having higher docking scores relative to the lead molecule and the ligand (Glimepiride). Accordingly, molecular docking studies proved that the molecule of G compound has a promising active hits and can be used as antidiabetic through glucosidase inhibitor and also different extracts exhibited an anti-inflammatory and hepatoprotective activity.

Keywords:
Antidiabetic activity, *Cleome africana*, *Cleome droserifolia*, lipid profile, glucosinolates, molecular docking

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Introduction

Cleome africana and *Cleome droserifolia* belong to *Cleomaceae* family [1]. *Cleome* species exhibit many biological activities such as antidiabetic, antiviral, anticancer, antidiarrhea, analgesic, anti-inflammatory, and hepatoprotective. It contains many chemical constituents like terpenes, flavonoids, glucosinolates (GLs), and alkaloids. Aparadh and Karadge [2] studied the seed's fatty acid composition of *Cleome viscosa*, *Cleome simplicifolia*, *Cleome gynandra*, *Cleome chelidonii*, and *Cleome speciosa* [2] and found that

linoleic and palmitic acid are prominent acids; moreover, glucocapparin, glucocleomin, methyl isocyanate, and 5-ethyl-5-methyl-oxazolidine-2-thione were found [3–5]. This study was carried out to investigate the lipid constituents and the GLs with their hydrolysis products along with their biological activity in addition to molecular modeling studies of some isolated compounds from *C. africana*.

Materials and methods

Plant material

C. africana was collected from Matrouh region, and *C. droserifolia* was collected from the desert of Saint Catherine, Egypt, during spring (May) 2011. Authentication of the plant was kindly confirmed by Prof. Dr Ibrahim, El-garf, Botany Department, Faculty of Science, Cairo University, Cairo, Egypt.

Chemicals and instruments

Silica gel G (Kieselgel G, type 60 F254; Merck, Germany), polyamide (6D; Riedl-deHaen AG.D-3016, Seelze-1, Germany), Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden), Whatmann No. 1MM, and 3MM paper were used. Solvents used are petroleum ether (40–60°C), hexane, ether, chloroform, sulfuric acid, dry HCl, acetone, ethyl acetate, and methanol (El Gomhoreya Company for pharmaceutical and Chemical Industries, Egypt). KOH, Fuller's earth, anhydrous sodium sulfate, distilled water, and acidic aluminum oxide (anionotropic, 1 kg, activity grade-?, type WA-? acidic; Sigma Chemical Co., Germany) were used as well. Gas chromatography–mass spectrometry (GC/MS) analysis was done with Thermo Scientific Trace GC Ultra and ISQ Single Quadruple MS (Thermo Scientific), with a direct capillary interface and fused silica capillary column TG-5MS fused silica (30 m×320 µm×0.25 µm film thickness). Electron impact (EI) mode of ionization, with a mass range of 50–700 m/z, was applied in the GC/MS analysis of the unsaponifiable matter, whereas the GC/MS analysis of the fatty acid methyl esters was with mass range of 50–500 m/z. High-performance liquid chromatography: Perkin-Elmer (USA) series 200 pump system equipped with a Perkin-Elmer series 200 variable ultraviolet (UV) absorbance detector was used. NMR (^{1}H-NMR, 400 MHz) was performed. The NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using Bruker; the chemical shifts values were recorded in δ ppm.

Kits of prostaglandin and interleukin 1 beta (IL-1β) were purchased from Riedel-de Haën, Germany, Biomerieux Diagnostic Kits, France, Orgenium Laboratories, Finland. Kits of glucose, total antioxidant capacity (TAC) and malondialdehyde (MDA), aspartate aminotransferase and alanine aminotransferase (AST and ALT), uric acid, and creatinine were obtained from Biodiagnostic (Cairo, Egypt). Citrate buffer was purchased from Biodiagnostic. Amaryl, streptozotocin (STZ), gum acacia, carbon tetrachloride, liquid paraffin, and sodium chloride were obtained from Sigma Chemical Co.

All experiments in this study were carried out in the central animal house of the National Research Centre, Dokki, Giza, Egypt. Male Sprague Dawley rats (150–170 g) were used throughout the experiments. They were obtained from the central animal house of National Research Centre, Dokki, Giza, Egypt. Animals were housed under standard environmental conditions (23±1°C, 55±5% humidity, and a 12-h light/12-h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues.

Extraction and fractionation of lipid constituents

Approximately 1 kg of fresh *C. africana* and 1 kg of fresh *C. droserifolia* plants were defatted with petroleum ether in a Soxhlet, and the obtained extracts were dried to yield a pale-yellow residue (3.4 g of *C. africana*). Then, 3.0 g *of C. droserifolia* was used for lipid analysis. The lipid fraction was treated with hot acetone to afford 1.8 and 0.9 g of acetone-insoluble fraction and 1.4 and 0.4 g of acetone-soluble fraction as oily material. The acetone-insoluble fraction was crystallized from chloroform/methanol and subjected to GC/MS analysis under the following conditions: helium was carried as carrier gas at ~1.0 ml/min, in pulsed splitless mode. The solvent delay was 3 min, and the injection size was 1.0 µl. The GC temperature program was started at 35°C (3 min), then elevated to 150°C at a rate of 5°C/min and then isothermal at 150°C for 5 min. Wiley7 and Nist05 mass spectral database was used in the identification of the separated peaks. The acetone-soluble fraction was saponified as Johnson and Davenport [6] and El-Said and Amer [7] to yield 700 mg of the yellowish brown semisolid residue of unsaponifiable matter and 500 mg of the semisolid residue of fatty acids. The fatty acids of 0.2 g were converted to their methyl esters as stated in other articles [6,8,9]. The unsaponifiable matter and the fatty

acid methyl esters were subjected to gas-liquid chromatography (GLC) with HP-5 phenyl methyl siloxane column of length 30 m, diameter 320 μm, and film thickness 0.25 μm, and nitrogen as carrier gas with flow rate of 20 ml/min, hydrogen as carrier gas with flow rate of 20 ml/min, and air as a carrier gas, with a flow rate of 200 ml/min. The oven temperature was 70°C, injector temperature was 250°C, detector temperature was 280°C. The used temperature programming started with 110°C for 2 min, increased to 240°C by the rate of 4°C/min, and then isothermally for 25 min.

Investigation of ethyl acetate and butanol fractions of *Cleome africana* and *Cleome* droserifolia by high-performance liquid chromatography

The defatted plant materials, 680 g of *C. africana* and 630 g of *C. droserifolia*, were macerated with 70% methyl alcohol till exhaustion. The alcoholic extracts for both species were evaporated *in vacuo* at about 50°C (60 g). The residue was dissolved in hot distilled water (600 ml), left overnight in the refrigerator, and then filtered. The aqueous filtrate was extracted with successive portions of chloroform (3×500 ml) followed by ethyl acetate (5×500 ml), and finally, with n-butanol (5×500 ml). The solvents were dried, separately, over sodium sulfate anhydrous and evaporated *in vacuo* at 50°C; the chloroform, ethyl acetate, and n-butanol free solvent residues amounted to 0.3, 3.4, and 5 g for *C. africana* and 0.1, 1.0, and 1.2 g for *C. droserifolia*. Approximately 5 μl of the ethyl acetate fraction and 10 μl of butanol fraction were injected in the high-performance liquid chromatography, and the chromatogram was scanned at wavelengths 280, 360, and 320 nm. The obtained signals were compared with different authentics using the following conditions: elution was carried out with mobile phase of 0.1% acetic acid and acetonitrile over 50 min at a flow rate of 1 ml/min using a Phenomenex column RPC18 (Hyper Clone), ODS, 5 μm 250×4.6 mm.

Isolation of the total glucosinolates from *Cleome africana*

One kilogram of fresh plant of *C. africana was defatted* as mentioned before. The defatted plant material of *C. africana* was extracted with methanol (80%) for 3 days (3×2.5 l) and evaporated *in vacuo* (45°C). The residue (60 g) was dissolved in hot distilled water (500 ml), left in refrigerator for 24 h, and then filtered. The aqueous filtrate was introduced to acidic aluminum oxide column chromatography [10]. The column was washed with distilled water, and then eluted with 2% aqueous potassium sulfate solution till the brown zone reached the bottom of the column. The eluate was dried under reduced pressure, dissolved in hot methanol (4×250 ml), filtered, and evaporated *in vacuo* at 40°C till dryness to give a brownish residue [total glucosinolate fraction (TGF); 0.92 g] [11]. Separation of GLs was carried out by preparative paper chromatography to afford compound G, which was further purified by Sephadex LH-20 column eluted with methanol (80%) to afford compound G in pure form (12 mg). The other zones give us small amounts, so we decided to collect all the fractions and subjected them to enzymatic hydrolysis as follows.

Approximately 5 mg of total GLs was dissolved in distilled water (5 ml), and the solution was buffered by addition of citrate buffer solution (pH 5.8) [12]. Approximately 2 mg of myrosinase (Thioglucosidase, Sigma, cod no. T-4528) and few crystals of citric acid were added, and the mixture was incubated at 37°C for 18 h. The hydrolysate solution, after saturation with sodium chloride, was exhaustively extracted with ether. The ether extract was evaporated *in vacuo* at 30°C, and then analyzed by GC/MS to obtain many hydrolysis products, such as isothiocyanates, nitrile, or oxazine.

Evaluation of the biological activities of *Cleome africana* fractions

Study of hypoglycemic effect of Cleome africana fractions

(1) Effect of *C. africana* fractions on glucose loading test

A total of 25 mature rats weighing 150–170 g were divided into five equal groups (five rats each) [13] as follows: the first group rats received saline and were kept as a negative control (negative control); the second group rats received glucose (1 g/kg) and were kept as a positive control (positive control); the third group rats received glucose (1 g/kg) before 45 min from administration of amaryl drug with recommended dose of 0.03 mg/kg and kept as a standard drug; the fourth group rats received glucose (1 g/kg) before 45 min and then were administrated TGF (100 mg/kg); and the fifth group rats received glucose (1 g/kg) before 45 min and then administrated G compound (100 mg/kg). After 60 and 120 min from treatment with amaryl or *C. africana* fractions, blood samples were collected via retro-orbital venous plexus and serum glucose levels were estimated by diagnostic kit method [14].

(2) Antidiabetic effect of C. africana fractions on STZ-induced diabetic rats

Another confirmatory antidiabetic experiment was done using STZ-induced hyperglycemia in rats.

Male mature rats were injected with a single dose of STZ (65 mg/kg) freshly prepared and dissolved in citrate buffer pH 4.5 to induced diabetes. After 48 h of STZ injection, fasting blood glucose was determined using diagnostic kit method [14]. The rats with serum glucose level above 200 mg/dl were selected and used for the present study [15].

A total of 25 mature male rats were divided into equal groups (five rats each). Group 1 rats received saline solution and kept as a negative control (negative control) for 15 days. Group 2 diabetic rats were administrated saline and kept as a diabetic control for 15 days. Group 3 diabetic rats were force fed with recommended dose of amaryl (0.03 mg/kg) for 15 days. Group 4 diabetic rats were force fed TGF (100 mg/kg) for 15 days. Group 5 diabetic rats were force fed with G compound (100 mg/kg) for 15 days. After the end of the experiment period, blood samples were collected via retro-orbital venous plexus, and serum glucose levels were estimated by diagnostic kit method [15]. Inflammation biomarkers of diabetic rats treated with amaryl or *C. africana* fractions, such as serum prostaglandin (PGE2) and serum interleukin 1B, were determined with a rat ELISA (Riedel-de Haën, Germany, Biomerieux Diagnostic Kits, France, Orgenium Laboratories, Finland). Serum TAC activity was determined using Biodiagnostic Kits [16]. Lipid peroxidation biomarker (MDA) was determined using Biodiagnostic kits according to the method of Ohkawa *et al.* [17].

Hepatoprotective effect of *Cleome africana* fractions

A total of 20 adult male rats were divided into four equal groups. Group 1 rats received the vehicle (gum acacia 2.5%) and kept for 9 days as a negative control. Group 2 rats received saline for 8 days and then orally administrated carbon tetrachloride 1.5 ml/kg body weight (50% CCl_4 v/v in liquid paraffin) to induce hepatorenal injury according to the method of Yadav and Dixit [18] and kept as hepatotoxic control. Groups 3 and 4 rats received oral doses of 100 mg/kg of *C. africana* fractions, TGF and G, respectively, for 7 days and then administrated carbon tetrachloride 1.5 ml/kg body weight at the eighth day. After 48 h following CCl_4 administration, blood samples were collected from retro-orbital venus plexus from all animals.

Serum was prepared for biochemical analysis by centrifugation (4000 g and 10 min using Sigma labor zentrifugen). Liver enzyme activities of AST and ALT were estimated according to the method of Reitman and Frankel [19]. Renal function parameters, such as creatinine and uric acid, were determined according to the methods of Thefeld *et al.* [20] and Haisman and Muller [21].

Molecular docking

Computational docking is an automated computer-based algorithm that determines how a compound will bind at the active site of a protein and is designed to estimate two main parameters. The first is to determine the suitable position and the orientation of certain test set molecule's pose inside the binding site in comparison with that of the radiograph crystallographic enzyme–substrate complex. The second term is the calculation of the estimated protein ligand interaction energy, the so-called docking scoring [22]. The C-DOCKER docking protocol in Accelyrs Discovery studio software was used in this study to simulate the interactions of different poses of the test compounds with α-glucosidase enzyme and the calculation of their binding energy. Calculation of the docking score or in other term the binding energy between the target protein and the ligand could be performed by using the following general expression: $E_{binding}=E_{Complex}-(E_{protein}+E_{ligand})$.

This requires the calculation of three energies: the protein ligand complex energy, the protein alone, and the ligand alone. The energy calculation used in C-DOCKER protocol is based on CHARMM Force Field molecular mechanic simulation engine [23].

Results and discussion

The lipid constituents were extracted with petroleum ether and fractionated to fatty alcohols, fatty acids, and unsaponifiable matters, which were identified using GC/MS and/or GLC. The results obtained in Table 1 showed that the GC/MS analysis of the acetone-insoluble fraction of *C. africana* comprises four fatty alcohols and seven hydrocarbons in which tetratetracontanol and tritriacontane are the main ones (16.4 and 24.44%). However, *C. droserifolia* (Table 2) consists of two fatty alcohols and three hydrocarbons, in which dotriacontanol and tritriacontane are the main (27.27 and 53.81%, respectively).

The GLC analysis of the fatty acid methyl esters of both plants *C. africana* and *C. droserifolia* shown in Table 3 revealed the presence of a mixture of nine fatty acids (saturated and unsaturated). The saturated fatty acids for *C. africana* constitute 22.97%, whereas for *C. droserifolia* are 50.62%. The main component is palmitic acid in both plants.

Table 1 Gas chromatography–mass spectrometry data of acetone-insoluble fraction of *Cleome africana*

Peak numbers	Ret. time (min)	Rel. %	Molecular formula	Molecular weight	Compounds
1	53.68	1.99	$C_{26}H_{54}$	366	Hexacosane
2	54.67	0.42	$C_{27}H_{56}$	380	Heptacosane
3	55.90	2.44	$C_{28}H_{58}$	394	Octacosane
4	57.95	6.96	$C_{29}H_{60}$	408	Nonacosane
5	58.39	3.42	$C_{31}H_{64}$	436	Hentriacontane
6	59.86	3.17	$C_{29}H_{58}O$	422	Nonacosanol
7	61.66	24.41	$C_{33}H_{68}$	464	Tritriacontane
8	61.87	14.83	$C_{31}H_{64}O$	452	Hentriacontanol
9	63.39	3.50	$C_{33}H_{66}O$	478	Tritriacontanol
10	65.30	22.44	$C_{44}H_{90}$	618	Tetratetracontane
11	65.43	16.40	$C_{44}H_{88}O$	632	Tetratetracontanol

Table 2 Gas chromatography–mass spectrometry data of acetone-insoluble fraction of Cleome droserifolia

Peak numbers	Ret. time (min)	Rel. %	Molecular formula	Molecular weight	Compounds
1	48.69	12.26	$C_{27}H_{56}$	380	Heptacosane
2	50.08	3.54	$C_{28}H_{58}$	394	Octacosane
3	51.58	53.81	$C_{33}H_{68}$	464	Tritriacontane
4	53.06	3.12	$C_{29}H_{58}O$	422	Nonacosanol
5	55.09	27.27	$C_{32}H_{66}O$	466	Dotriacontanol

Table 3 Gas-liquid chromatography analysis of the fatty acid methyl esters of *Cleome africana* and *Cleome droserifolia*

Peak numbers	R_t (min)	Rel. %		Molecular formula	Compounds
		Cleome africana	*Cleome droserifolia*		
1	12.27	–	2.77	$C_{14}H_{28}O_2$	Myristic acid ($C_{14:0}$)
2	14.809	14.77	33.05	$C_{16}H_{32}O_2$	Palmetic acid ($C_{16:0}$)
3	15.502	2.87	–	$C_{16}H_{30}O_2$	Palmitoleic acid ($C_{16:1}$)
4	18.062	6.74	7.32	$C_{18}H_{36}O_2$	Stearic acid ($C_{18:0}$)
5	18.568	29.40	23.32	$C_{18}H_{34}O_2$	Oleic acid ($C_{18:1}$)
6	19.595	38.99	13.33	$C_{18}H_{32}O_2$	Linoleic acid ($C_{18:2}$)
7	20.818	3.32	8.72	$C_{18}H_{30}O_2$	Linolenic acid ($C_{18:3}$)
8	22.273	1.46	7.48	$C_{20}H_{40}O_2$	Eicosanoic acid ($_{C20:0}$)
9	25.433	2.46	4.01	$C_{20}H_{38}O_2$	Arachidic acid ($C_{20:1}$)

R_t, retention time.

The unsaturated fatty acids comprise mono unsatutated fatty acids are constitute 34.73% of Cleome africana and 27.33% of Cleome droserifolia. The polyunsaturated fatty acids like linoleic and linolenic which constitutes 42.31 and 22.05%, respectively. The main acid in both plants is linoleic acid, which forms 38.99 and 13.33%, respectively. These results agree with that obtained by Aparadh and Karadge [2], where he reported that linoleic and palmitic acid are prominent acids in many *Cleome* species.

The results obtained in Table 4 of the two plants showed that the unsaponifiable fraction of *C. africana* consists mainly of a series of n-alkanes ranging from n-C14 to n-C31, constituting 93.66%, in which n-C22 is the main compound (18.99%), in addition to a sterol fraction, forming 5.13%, in which β-sitosterol is the main compound (1.88%). However, *C. droserifolia* was found to contain a series of n-alkanes from n-C18 to n-C30, representing 60.46%, in which n-C30 is the main compound (12.24%), besides sterols, representing 39.54%, with campesterol as a major compound (12.46%), and finally a triterpene fraction, consisting of α-amyrin and β-amyrin (8.60%), which agree with that obtained by Ranjitha *et al.* [24], where they proved the presence of α-amyrin acetate, α-amyrin, sitosterol, and stigmasterol in the hexane extract of *C. gynandra*.

The obtained data from Table 5 proved that the major flavonoids in the butanol fraction of both species are rutin and naringenin. However, the ethyl acetate fraction of *C. africana* was found to contain hesperidin (462.81 μg/ml) as the main component and *p*-coumaric acid (320.78 μg/ml), whereas that of

Table 4 Gas-liquid chromatography analysis of the unsaponifiable fraction of *Cleome africana* and *Cleome droserifolia*

Peak numbers	R_t (min)	Rel. %		Compounds
		Cleome africana	*Cleome droserifolia*	
1	9.96	0.18	–	C14 n-tetradecane
2	11.97	2.77	–	C15 n-pentadecane
3	14.72	2.48	–	C17 n-heptadecane
4	15.43	1.61	–	Unknown
5	16.00	5.54	3.20	C18 n-octadecane
6	17.53	–	4.72	C19 n-nonadecane
7	18.02	10.89	1.62	C20 n-cosane
8	18.81	6.50	–	Unknown
9	19.19	3.19	2.90	C21 n-hencosane
10	20.15	18.99	4.23	C22 n-dodacosane
11	20.97	4.51	–	Unknown
12	21.96	10.70	–	C23 n-triacosane
13	22.95	–	8.77	C24 n-tetracosane
14	23.57	2.19	5.47	C25 n-pentacosane
15	24.13	3.45	–	Unknown
16	24.81	4.19	5.51	C26 n-hexacosane
17	25.41	3.01	1.73	C27 n-heptacosane
18	25.98	2.76	5.07	C28 n-octacosane
19	26.57	2.19	–	Unknown
20	27.24	4.99	5.00	C29 n-nonacosane
21	28.71	1.75	12.24	C30 n-contane
22	29.69	1.81	–	C31 n-hencontane
23	30.56	0.96	6.35	Cholesterol
24	32.76	0.30	12.46	Campasterol
25	33.42	0.20	–	Unknown
26	34.61	1.46	7.42	Stigmasterol
27	35.35	1.01	–	Unknown
28	36.45	1.88	4.71	β-sitosterol
29	37.36	–	4.68	β-amyrin
30	39.29	0.53	3.92	α-amyrin

R_t, retention time.

C. droserifolia was found to contain naringenin (42.38 μg/ml) followed by *p*-coumaric acid (39.62 μg/ml). From the aforementioned results, we can conclude that *C. africana* has higher concentration of phenolics and flavonoids than *C. droserifolia*.

The GLs were isolated from the methanolic extract of CA by passing through an acidic aluminum oxide column followed by elution with 2% K2SO4 solution to afford the total GLs, which were subjected to preparative paper chromatography to give compound G, which was further purified over Sephadex LH-20 column eluted with methanol (90%).

Compound G, 3-ethyl sulfonyl 2,3-dimethoxypropyl GLs, was isolated as an amorphous white powder; its UV absorption G in methanol showed λ_{max} at 206 nm, and by addition of NaOMe, the bathochromic shifted to 213 nm [25]. The EI-MS of the compound G revealed the presence of a molecular ion peak M^+ at m/z=498 which corresponded to the molecular formula $C_{13}H_{24}O_{13}S_3N$. The presence of peaks at m/z=469 and 439 is owing to loss of CH_3CH_2 and CH_3CH_2+OCH_3. Fragments of group (CH_3CSCH_2, 73), group (CH_3CSH, 60) and isothiocyanate group (NCS, 58). The ^{1}H-NMR displayed different groups of signals at δ=5.07, d, J=7.5 Hz for the anomeric proton of glucose moiety, and 3.66 and 3.63 ppm as two singlets for the two OCH_3 groups at C2 and C3. The other protons of the sugar are located between 3.2 and 3.5 ppm .The CH_2 group protons appear as quartet at 1.58 ppm. The protons at C2 and C3 appears as doublet at 1.21. A triplet at 0.92 ppm represents the protons of CH_3 group. (Table 6). The obtained data from TLC, PC, MS, UV, and ^{1}H-NMR substantiated that the compound G can be tentatively identified as 3-ethylsulfonyl-2,3-dimethoxypropyl GLs. This compound was isolated for the first time from this species (*C. africana*) during the present work.

The GC/MS analysis of the hydrolysis products of total GLs led to identification of the compounds (Table 7).

Table 5 Results of high-performance liquid chromatography ethyl acetate and butanol fractions

Numbers	Compound	EtOAc (μg/ml)		BuOH (μg/mg)	
		Cleome africana	*Cleome droserifolia*	*Cleome africana*	*Cleome droserifolia*
1	Pyrogallol	ND	ND	ND	4.12
2	Gallic	12.78	ND	ND	ND
3	Protochatchuic	60.62	1.03	ND	ND
4	*p*-hydroxybenzoic	ND	ND	ND	ND
5	Gentisic	ND	ND	ND	ND
6	Catachine	ND	ND	ND	ND
7	Chlorogenic	13.65	ND	ND	ND
8	Caffeic	4.46	ND	ND	ND
9	Syrngic	ND	ND	ND	ND
10	Vanillic	ND	ND	ND	ND
11	Scoplatine	ND	ND	ND	ND
12	Ferulic	ND	ND	ND	ND
13	Sinapic	ND	0.90	1.48	7.16
14	Rutin	219.31	26.89	28.64	53.35
15	*p*-coumaric	320.78	39.62	3.11	23.86
16	Naringenin	226.71	42.38	12.60	40.91
17	Hisperdin	462.81	5.09	ND	1.13
18	Apeginin-7-glucoside	ND	2.32	ND	1.03
19	Myrcetin	187.26	2.89	ND	3.93
20	Rosmarinic	149.74	1.21	0.34	0.35
21	Cinnamic	134.81	0.85	ND	ND
22	Quercetin	454.76	5.31	0.78	0.04
23	Apegnin	161.60	1.99	ND	ND
24	Kaempferol	33.70	0.69	ND	ND
25	Chyrsin	115.28	ND	ND	ND

ND, not detected.

Table 6 ^{1}H-NMR analysis of 3-ethylsulfonyl-2,3-dimethoxypropyl glucosinolates

Groups	δ in ppm ^{1}H
Anomeric proton of glucose	5.07, d, J=7.5
OCH_3	3.66, 3H, s
OCH_3	3.63, 3H, s
Rest of sugar protons	3.2–3.5
CH_2	1.58, q
H-2., H-3	1.21, d
CH_3	0.92, t

The biological activities of *Cleome africana* fractions

Antidiabetic effect

The obtained results from the hypoglycemic effect of the tested extracts (Table 8) as illustrated in glucose loading test 1 h after treatment showed significant decrease in glucose level in groups treated with G>TGF>amaryl. However, after 2 h, glucose loading test was significantly decreased than diabetic untreated group as follows: G >TGF>amaryl.

Moreover, glucose level significantly reduced in the fasting diabetic rats treated with G>TGF>amaryl as shown in Table 9. The potential antidiabetic activity of the two fractions (TGF and G) does not vary significantly. These results agree with that obtained by El Naggar *et al* [26].

The data presented in Table 10 showed significant inhibition in serum PGE2 than normal control in group given TGF. However, G and amaryl normalized PGE2 level compared with diabetic nontreated rats. Serum interleukin 1B value significantly reduced by all treatment groups in the following manner: G>TGF>amaryl when compared with diabetic control group. Furthermore, serum TAC significantly reduced in diabetic control than any treated rats. All tested extracts significantly increased TAC level than control group in variable degrees (G>TGF>amaryl). These results agree with that obtained by Gupta *et al.* [27]. The tested antidiabetic agents herein have antioxidant properties as they significantly decreased MDA and increased TAC in serum of treated diabetic groups. Serum MDA value significantly elevated in diabetic nontreated group than control and the other treated animals. G treatment significantly decreased MDA level than normal control. Our results revealed that all treatments inhibited PGE2 and interleukin 1B levels compared with the diabetic control group in different degrees. It was

Table 7 Gas chromatography–mass spectrometry analysis of the hydrolysis products of total glucosinolate

Names	R_f	Moleular ion peak M^+m/z	Molecular formula	Fragmentation pattern m/z	Reference numbers
4-pentenyl ITC	5.14	127	C_6H_9NS	55,59,68,72	[3]
Allyl ITC	12.31	99	C_4H_5NS	57,73,84	
2-hydroxybutyl ITC	12.75	131	C_5H_9ONS	58,72,73,98	
2-methythio ethyl ITC	12.79	133	$C_4H_7NS_2$	58,72,73,85,117	
2-methylbutyl ITC	15.41	129	$C_6H_{11}NS$	43,58,71,72	
4-methylsulfinyl-2-methylbutyl	19.15–19.72	191	$C_7H_{13}ON$	58,72,128,133,158	
3-benzoyloxypropyl	31.15	221	$C_{11}H_{11}O_2$	57,72,77,105,122,205	
3-ethylsulfonyl-2,3-dimethyl-2-propenyl	31.19	251	$C_8H_{13}O_4$	132,176,191,205,220	
5,6-dimethyltetrahydro-1,3-oxazine-2-thione	33.13–37.43	145	$C_6H_{11}ON$	58,72,112,130	
5-ethyl-5-methyl-1,3-oxazoline-2-thion (Cleomin)	33.13–37.43	145	$C_6H_{11}ON$	70,112,116,130	

ITC, isothiocyanate.

Table 8 Effect of *Cleome africana* fractions on glucose loading test

Groups	Negative control	Positive control	Amaryl	TGF	G
After 1 h	70.0±1.40[a]	181.0±1.50[b]	130.0±1.40[c]	127.0±2.10[ce]	118.0±2.50[e]
After 2 h	69.22±1.27[a]	140.10±2.68[b]	110.10±2.62[c]	99.64±3.16[de]	99.14±2.80[e]

Data presented as a mean±SD of five replicates. Analysis of variance one-way at *P* value less than 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism version 5 program. The similar letters are insignificantly different with each other. TGF, total glucosinolate fraction.

Table 9 Effect of *Cleome africana* fractions on fasting serum glucose level in streptozotocin-induced diabetic rats

Groups				
Negative control	Untreated control	Amaryl	TGF	G
69.22±1.45[a]	276.4±1.61[b]	138.2±1.83[c]	113.0±1.46[de]	95.00±1.61[e]

Data presented as a mean±SD of five replicates. Analysis of variance one-way at *P* value less than 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism, version 5 program. The similar letters are insignificantly different with each other. TGF, total glucosinolate fraction.

noticed that diabetes increases inflammatory cytokines as discussed by El-Abhar and Schaalan [12] where they concluded that cyclooxygenase and lipoxygenase play an important role as inflammatory mediators.

Hepatorenal protective effects

CCl_4-intoxicated rats showed significant elevation in ALT, AST, creatinine, and uric acid when compared with any group in the experiment (Table 11). G treatment significantly decreased ALT, AST, uric acid, and the creatinine value. The other treatment TGF significantly decreased ALT, AST, uric acid, and creatinine compared with CCl_4-treatment group.

Molecular docking

Docking of the lead compound

The study was started by determining the binding mode of bioactive conformation of the selected lead compound cocrystallized with alpha glucosidase enzyme having the code; 3l4z[3] obtained from the Brook haven protein data bank (http://www.rcsb.org) without change in its conformation. The binding pocket includes such residues as Arg526, Asp443, Asp542, His600, Tyr299, and Asp327 (Fig. 1). Another validation for the ideal pose was also performed by alignment of the radiograph bioactive conformer with the best fitted pose of the same compound. The alignment showed good coincidence between them (RMSD=0.526 A°), indicating the validity of the selected pose. Accordingly, the aforementioned studies validated that the C-DOCKER study is an ideal one. Thus, the binding mode and the simulated interaction energy of this pose with its binding site are valid and could be used as reference for other interactions between the same binding site and the test set molecules.

Docking of compound G with α-glucosidase binding site

The molecular docking study was carried out between the obtained test set molecule G and the binding site of the alpha glucosidase enzyme using C-DOCKER protocol.

Table 10 Effect of *Cleome africana* fractions on serum inflammation biomarkers prostaglandin and interleukin 1B), total antioxidant capacity, and lipid peroxidation biomarker (malondialdehyde) level in streptozotocin (-induced diabetic rats

Groups	Negative control	Untreated control	Amaryl	TGF	G
Prostaglandin E2 (ng/l)	67.40±1.2[a]	90.23±1.04[b]	63.80±1.95[af]	58.44±1.93[cef]	60.20±1.62[ac]
Interleukin 1B (pg/ml)	2.500±0.130[a]	19.77±1.210[b]	13.21±1.120[ce]	9.32±0.76[de]	8.54±0.59[de]
Total antioxidant capacity (mmol/l)	1.26±0.04[a]	0.88±0.01[b]	1.54±0.05[c]	3.36±0.04[h]	3.89±0.05[j]
Malondialdehyde (nmol/ml)	3.27±0.02[a]	6.80±0.02[b]	5.39±0.09[c]	3.52±0.08[a]	3.19±0.04[a]

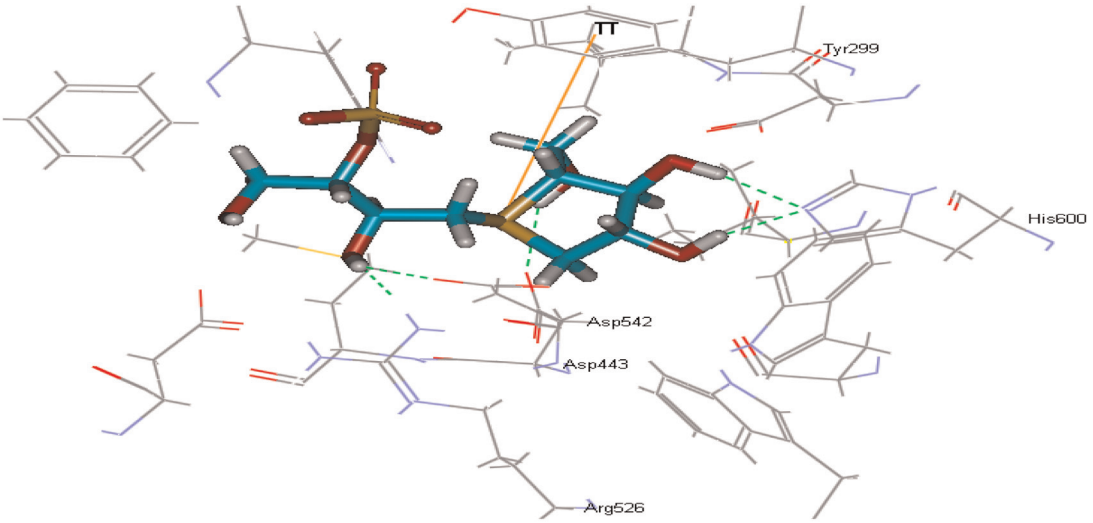
Data presented as a mean±SD of five replicates. Analysis of variance one-way at *P* value less than 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism, version 5 program. The similar letters are insignificantly different with each other. TGF, total glucosinolate fraction.

Table 11 Effect of *Cleome africana* fractions on serum liver and renal functions of CCl_4-induced hepatotoxicity rats

Groups	Negative control	CCl_4	TGF	G
ALT (U/l)	25.61±1.01[a]	45.98±1.00[b]	25.00±1.30[af]	23.20±1.16[a]
AST (U/l)	41.14±0.92[ae]	96.40±1.36[b]	45.40±1.21[e]	41.00±1.00[de]
Uric acid (mg/dl)	1.66±0.15[ae]	5.56±0.15[b]	1.44±0.05[e]	0.95±0.06[d]
Creatinine (mg/dl)	0.35±0.01[ac]	4.72±0.10[b]	0.33±0.01[ad]	0.31±0.01[ad]

Data presented as a mean±SD of five replicates. Analysis of variance one-way at *P* value less than 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism, version 5 program. The similar letters are insignificantly different with each other. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TGF, total glucosinolate fraction.

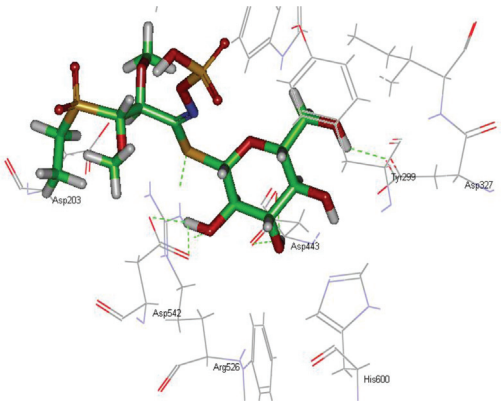
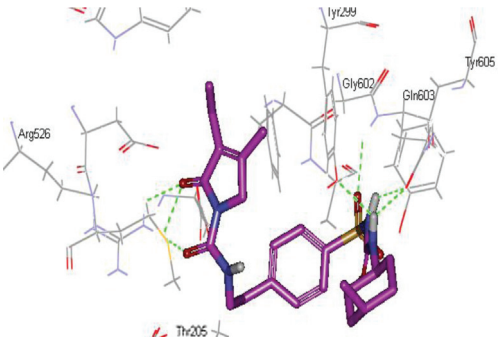
Figure 1



Interaction diagram of lead compound with the binding site of α-glucosidase enzyme.

Each tested molecule gave 10 possible docked poses. The ideal pose of each molecule was selected according to the similarity of its binding mode in the binding site to that of the lead compound. The binding pattern of the ideal pose for each of the tested molecules G and F and the corresponding C-DOCKER energy (Kcal/mole) were considered in our study to prioritize their virtual affinity to the binding site, in comparison to the ideal pose of the lead molecule and the Glimepiride as reference drug. The docking study revealed that the G molecule have higher docking scores relative to the lead molecule and the ligand (Glimepiride). Accordingly, the performed molecular modeling studies indicated that the G molecules are predicted to be promising active hits as antidiabetic through α-glucosidase inhibitor. Close examination of the highest ranked poses of compound G revealed that the high inhibitory activities of this compound may be attributed to the docked compounds showing similar binding mode to lead compound and high value of the C-DOCKER interaction energy which was matched with their antidiabetic activity (Table 12). The docking scores of these compounds may attributed to the seven hydrogen bonding interaction with the essential amino acid residues in the active site of α-glucosidase enzyme. Compound G made seven H-bond donors, one with Arg526, two with Asp443, three with Asp542, and one Tyr299.

Table 12 C-DOCKER interaction energy and three-dimensional interaction diagrams of compounds (Gand Glimepiride)

Compounds	3D interaction diagram	Glucose loading after 1 h	Glucose loading after 2 h	C-DOCKER interaction energy (Kcal/mol)	RMSD value (A°)
G		120	100	107.5	0.43
Glimepiride		130	110	106.5	0.65
Lead comp		–	–	100	0.526

Conclusion

The methanolic extract of *C. africana* led to isolation and identification of one compound (G) identified as 3-ethylsulfonyl-2,3-dimethoxypropyl GLs. Eight isothiocyanate and two thione compounds were identified from total GLs by enzymatic hydrolysis. Molecular docking studies proved that the molecule of G compound has a promising active hits and can be used as antidiabetic through glucosidase inhibitor, also different extracts exhibited an anti-inflammatory and hepatoprotective activity.

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Conflicts of interest

There are no conflicts of interest.

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