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Biological and Chemical Study of *Cleome paradoxa* B.Br.

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ABSTRACT

The methanolic extract of *Cleome paradoxa* B.Br. and its different fractions (viz. hexane, chloroform, ethyl acetate and n-butanol) were tested for their antidiabetic activities. The ethyl acetate and hexane fractions exhibited the highest activities, which further subjected to chemical investigation. This study resulted in isolation of two compounds from the ethyl acetate fraction and three compounds from the hexane fraction. These compounds were identified using different spectroscopic methods as quercetin, quercetin-3-O-*β*-D-glucopyranoside (isoquercetrin), *α*-amyrin, sitgmasterol and sitgmasterol-3-O-*β*-D-glucpyranoside. The major compound isolated from ethyl acetate fraction, isoquercetrin, was assessed for its antidiabetic activity.

Keywords: *Cleome paradoxa, antidaiabetic, antihyperglycemic, isoquercetrin.*

INTRODUCTION

Different species of *Cleome* were reported to posses analgesic (1), anti-inflammatory (2), anticonvulsant (3), antioxidant (4), anthelmintic (5), antibacterial (6, 7) and insecticidal activities (8). *Cleome droserifolia* growing in Egypt exhibited antidiabetic activity (9). Certain species of *Cleome* contain essential oils (10, 11) that show insect repellent activity (12). Classes such as flavonids (8, 13) alkaloids (8, 14), sterols (8), sesquiterpenes and triterpenes (15) are the main active constituents isolated from different species of genus *Cleome*. Reviewing the available literature, nothing was found concerning the chemical or the biological investigation of *Cleome paradoxa* B.Br. The present study is aiming to investigate its antidiabetic activity and its main chemical constituents.

MATERIALS AND METHODS

General

Electro-thermal 9100 was used for determination of melting point, were incorrected. Shimadzu-IR -435 and Beckman Du-7 spectrophotometers were used for recording IR and UV spectra, respectively. NMR analysis was measured on ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz): Varian Mercury-VX-300 spectrometer. TLC was performed on precoated silica gel plates using solvent systems S_1: n-hexane-EtOAc (4:1); S_2: $CHCl_3$-MeOH (9:1) and S_3: $CHCl_3$-MeOH (4:1), the chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour, as well as spraying with anisaldehyde-sulphuric acid spray reagent.

Plant material

The aerial parts of *Cleome paradoxa* B.Br. were collected from Madina-Southwards, Kingdom of Saudi Arabia, in November 2005 and was identified by staff of the Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. A specimen was deposited in the herbarium of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (# CP 1039).

Test animals

Sprague Dawley rats (100-150 g) were obtained from the animal house of National Research Center, Dokki, Giza, Egypt. They were maintained in standard environmental conditions of temperature (25 ± 2 ^{0}C), relative humidity (55 ± 10%) and they were kept in cages and maintained in well-ventilated room under natural light and dark cycle. They were fed with standard rat diet and water *ad libitum*.

Extraction

Dried aerial parts of *C. paradoxa* (750 g) were extracted with MeOH using Ultra-Turrex (3 X 3 l). The combined extracts were evaporated under reduced pressure (≤ 60^{o}C) to give 93.2 g of green residue. The MeOH extract was fractionated into n-hexane (8.7 g), chloroform (2.9 g), ethyl acetate (4.5 g) and n-butanol (10.6 g).

Toxicity (LD 50%)

LD 50% of the methanolic extract was determined following Karber method (16).

Antihyperglycemic activity

The MeOH extract of *C. paradoxa* and its fractions (viz., *n*-hexane, chloroform, ethyl acetate and *n*-butanol) in addition to compound 2 were *in-vivo* tested for their antihyperglycemic activity against alloxan (Sigma-Aldrich Chemical Co., St. Luis, MO, USA) induced hyperglycemia (17) in Sprague Dawley rats (100-150 g). The animal were injected intraperitoneally with a single dose of alloxan (150 mg/kg b. wt.), then they were divided into eight groups, the first group was kept as diabetic non-treated negative control, the second to seventh groups received daily along the time of the experiment oral dose of the tested samples (200 mg/kg of the extract and 10 mg/kg of isoquercetrin, 2), the last group was given metformin (Chemical Industries Development, CID, Giza, Egypt) orally in a dose of (150 mg/kg) as standard drug. Blood samples were taken at zero time (G_0) and after 4 and 8 weeks (G_t) from the retro-orbital venous plexus, the serum of the blood samples were isolated by centrifugation, then the blood glucose level was estimated using Biomerieux kits according to the method described by Trinder (18). The percentage of change of blood glucose level was calculated [% of change = $(G_0 - G_t) \times 100 / G_0$], the data obtained were statistically analyzed using student's t-test (19), the results were represented in table 1.

Fractionation and Isolation

The ethyl acetate fraction (4 g) was chromatographed on a VLC column of Si gel G 60 (7 x 10 cm) using $CHCl_3$ (100 ml), $CHCl_3$- EtOAc mixtures (1 L, 10 % increment of EtOAc each 100 ml), EtOAc (100 ml) and EtOAc-MeOH mixtures (1 L, 2% increment of MeOH each 100 ml till 20 % MeOH). Fractions 100 ml each were collected and monitored by TLC (see experimental) and similar fractions were pooled together.

Fractions eluted with $CHCl_3$-EtOAc, 3:2 and 1:1 (200 mL) afforded compound **1** (23 mg) upon rechromatography on a sephadex LH-20 column (MeOH). Fractions eluted between 4-8 % MeOH/EtOAc (200 mL) afforded compound **2** (250 mg) upon rechromatography on a sephadex LH-20 column (MeOH).

The hexane fraction (8.7 g) was chromatographed on a VLC columns of Si gel G 60 (7x 10 cm) using C_6H_{12}

Table 1. Antihyperglycemic activity of methanolic extract, hexane, chloroform, ethyl acetate, n-butanol fractions of *C. paradoxa* and isoquercetrin (n=10).

Tested samples	Blood glucose level (mg/dl)				
	Zero	4 weeks		8 weeks	
	M ± S.E	M ± S.E	% of change	M ± S.E	% of change
Diabetic untreated (1 ml saline)	256.4±9.3	266.5±13.7	+ 3.9	265.3±11.2	+ 3.47
Alcohol extract (200 mg/kg)	269.8±10.3	186.2±7.6*	− 30.9	139.1±5.4*	− 48.4
Hexane fraction (200 mg/kg)	251.4±10.2	218.3±9.6	− 13.2	164.9±7.1*	− 34.4
Chloroform fraction (200 mg/kg)	249.6±9.4	211.7±7.5	− 15.2	183.5±6.2*	− 26.5
Ethyl acetate fraction (200 mg/kg)	265.9±8.6	208.2±7.3*	− 21.7	144.7±6.1*	− 45.6
Butanol fraction (200 mg/kg)	259.8±11.3	231.2±8.5	− 11	178.3±6.1*	− 31.4
Isoquercetrin (10 mg/kg)	253.8±9.3	196.5±6.4*	− 22.6	148.2±4.9*	− 41.6
Metformin (150 mg/kg)	261.3± 7.5	142.8±9.7*	− 45.4	89.3±4.1*	− 65.82

** Statistically significant from zero time at $p < 0.01$*

(100 ml), C_6H_{12}-$CHCl_3$ mixtures (1 L, 10 % increment of $CHCl_3$ each 100 ml), $CHCl_3$ (100 ml), $CHCl_3$-EtOAc mixtures (1 L, 10 % increment of EtOAc each 100 ml), EtOAc (100 ml) and EtOAc-MeOH mixtures (1 L, 2 % increment of MeOH each 100 ml till 20% MeOH). Fractions 100 ml each were collected and monitored by TLC (see experimental) to afford three main fractions. Fraction eluted with C_6H_6-$CHCl_3$ (1:1) afforded compounds **3** (50 mg) and **4** (42 mg) upon further purification on a Si gel column (C_6H_6-EtOAc (19:1). Fractions eluted with $CHCl_3$-EtOAc (1:9) to 2 % MeOH/EtOAc afforded compound **5** (63 mg) upon purification on a Si gel column ($CHCl_3$-MeOH, 49:1).

Compound 1: Yellow amorphous crystal; R_f 0.8 (TLC, S_2); mp 314-315 °C, UV λ_{max} nm (MeOH): 255 and 370, MeOH/NaOMe: 273 and 412, MeOH/$AlCl_3$: 271 and 449, MeOH/$AlCl_3$/HCl: 265 and 420, MeOH/NaOAc: 273 and 385, MeOH/NaOAc/H_3BO_3: 260 and 386; ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 6.18 and 6.41 (2H, *d*, *J* = 1.8 Hz, H-6 and H-8), 7.67 (1H, *d*, *J* = 2.4 Hz, H- 2'), 6.87 (1H, *d*, *J* = 8.7 Hz, H-5'), 7.52 (1H, *dd*, *J* = 8.7, 2.1 Hz, H-6').

Compound 2: Yellow amorphous crystal; R_f 0.4 (TLC, S_3); mp 240-242 °C; UV λ_{max} nm (MeOH): 257 and 356, MeOH/NaOMe: 273 and 407, MeOH/$AlCl_3$: 274 and 425, MeOH/$AlCl_3$/HCl: 269 and 403, MeOH/ NaOAc: 273 and 386, MeOH/NaOAc/H_3BO_3: 261 and 373; ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 6.19 and 6.39 (2H, *d*, *J* = 2.1 Hz, H-6 and H-8), 6.83 (1H, *d*, *J* = 7.2 Hz, H-5'), 7.55 (2H, 2*d* overlapped, H-2', 6'), 5.43 (*d*, *J* = 7.2 Hz, H-1''). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C: 156.1 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.3 (C-7), 93.5 (C-8), 156.3 (C-9), 103.9 (C-10), 121.5 (C-1'), 115.2 (C-2'), 144.7 (C-3'), 148.4 (C-4'), 116.2 (C-5'), 121.2 (C-6'), 101.0 (C-1''), 74.1 (C-2''), 76.5 (C-3''), 70 (C-4''), 77.4 (C-5''), 61 (C-6'').

Compound 3: White amorphous powder; R_f 0.7 (TLC, S_1).

Compound 4: White amorphous powder; R_f 0.5 (TLC, S_1).

Compound 5: White amorphous powder; R_f 0.5 (TLC, S_2); ^{1}H-NMR (DMSO-d_6), δ_H 0.65 and 0.95 (each 3H, *s*, H-18 and H-19), 0.78 and 0.82 (each 3H, d, *J* = 6.6 and 6.9, H-26 and H-27), 0.89 (3H, *d*, *J* = 6, H-21), 0.87 (3H, t, H-29), 4.39 (1H, *m*, H-3), 5.33 (1H, broad *s*, H-6), 4.82 (2H, *d*, *J* = 4.2, H-22 and H-23) and 4.21 (1H, *d*, *J* = 7.8 Hz, H-1').

RESULTS AND DISCUSSION

The dried aerial parts of *C. paradoxa* were extracted with MeOH, then fractionated into *n*-hexane, chloroform, ethyl acetate and *n*-butanol. LD_{50} of the methanolic extract was determined as 6.8 g/kg body weight, which indicated that the plant is very safe (20).

In a bioguided fractionation, the antihyperglycemic activity of the methanolic and related fractions thereof were determined. The antihyperglycemic activity of the methanolic extract after eight weeks of IP administration was determined as 48.4 % change relative to the diabetic untreated animals (table 1). Following the significant reduction of blood glucose level produced by the methanolic extract, the antihyperglycemic activity of all fractions was determined as 34.4, 26.5, 45.6 and 31.4 % change, respectively, compared to diabetic untreated animals (table 1). The antihyperglycemic activity among the tested fractions was in the following order MeOH, ethyl acetate, hexane, n-butanol and chloroform fractions (200 mg/kg). The recorded reduction produced by metformin (standard drug) was 65.82 % (150 mg/kg). In addition, the activity of compound 2 (the major compound of ethyl acetate fraction) was determined as 41.6 % change at a dose of 10 mg/kg. All the recorded values were found to be statistically significant.

In this study five compounds (**1–5**) were isolated from n-hexane and ethyl acetate fractions obtained from MeOH extract of *C. paradoxa* B.Br. using chromatographic purification on silica gel and/or sephadex columns using suitable solvent systems.

Compound **1** was identified as quercetin from the analysis of its UV spectra in MeOH before and after addition of the different shift reagents (21). Identification of compound **1** was further confirmed by ^{1}HNMR analysis and by co-chromatography against reference sample.

UV spectrum of compound **2** in MeOH showed absorption bands at λ_{max} 257 and 356 nm indicating a 3-substituted flavonol skeleton (21). The behavior of compound **2** with the different shift reagents and its ^{1}H-NMR spectrum were similar to that of compound **1**, which confirmed the presence of a quercetin skeleton (21) and further confirmed by acid hydrolysis. ^{1}H-NMR spectrum of **2** showed signals corresponding to those of quercetin (see expermintal). One anomeric proton at δ_H 5.43 (1H, *d*, *J* = 7.2 Hz, H-1''), indicated the presence of one sugar moiety with *β*-configuration (Mabry et al., 1970), in addition to the rest protons at δ_H 3.11- 3.61. From ^{1}H- and ^{13}C-NMR spectra, the sugar moiety was identified as *β*-D-glucose (22). The aforementioned data confirmed the identification of compound **2** as quercetin-3-*O*-*β*-D-glucopyranoside (isoquercetrin).

The significant antihyperglycemic activity of isoquercetrin (2) at dose of 10 mg/kg, was previously reported for some flavonol glycosides or flavonoids rich fraction from several plants as the possible active

constituents responsible for the antihyperglycemic activity (23–25). The result presented here was supported by the data reported by Addae-Mensah and Munenge, (26). However, other constituents could be also responsible for the hypoglycemic of *C. paradoxa* B.Br., which can not be excluded. This hyposesis could be explained by the reduction of glucose level by *n*-hexane fraction by 34.4 % relative to diabetic control group.

Compounds **3** and **4** were identified as *a*-amyrin and stigmasterol, respectively, using IR and by co-chromatography with authentic substances.

Compound **5** gave a positive Liebermann and Molish tests indicating its steroidal and glycosidal nature. ^{1}H-NMR spectrum of compound **5** showed two singlets at δ_H 0.65 and 0.95 each equivalent to 3H, corresponding to tertiary CH_3-18 and CH_3-19, three doublets at δ_H 0.78, 0.82 and 0.89 each equivalent to 3H, assigned for secondary CH_3-26, CH_3-27 and CH_3-21, and a triplet at δ_H 0.87 (3H) corresponding to primary CH_3-29. Signals at δ_H 5.33 (1H) and 4.82 (2H) were assigned to the olefenic H-6, H-22 and H-23. The doublet at δ_H 4.21 was assigned to the anomeric proton of the sugar molecule with a coupling constant 7.8 Hz which indicate its β configuration. By comparing the previous data of **5** with the reported spectral data (27, 28), compound **5** was identified as sitgmasterol-3-*O*-β -D-glucopyranoside. The significant antihyperglycemic activity of *C. paradoxa,* reflect the needs for further extensive toxicological studies in various animal models to explore its safety.

REFERENCES

1. Parimaladevi B., Boominathan R. and Mandal S. C. Studies on analgesic activity of *Cleome viscose* in mice. *Fitoterapia* **74**: 262–266, (2003).
2. Puchchakayala G., Podili L., Bobbala D., Thirupathi K., Boini K.M., Yellu N. R., Bobbala R.K., Gottemukkala K.M. and Pragada R.R. Antinociceptive and anti-inflammatory effects of *Cleome chelidonni* Linn. Roots in experimental animals. *Pharmacognosy-Magazine* **4**: 32–36(2008).
3. Akah P.A., Nwambie A.I., Gamaniel K.S. and Wambebe C. Experimental study of the plants used for treatment of infantile convulsion in Nigeria. *Brain Research Bulletin* **44**: 611–613(1997).
4. Narendhirakannan R. T., Subramanian S. and Kandaswamy M. Evaluation of antioxidant potential of *Cleome gynandra* L. leaf extract on the components of the lymphoid organs in adjuvant induced arthritis in rats. *International Journal of Biological Chemistry* **1**: 1–10(2007).
5. Bose A., Gupta J.K., Dash G.K., Ghosh T. and Devbhuti D. Evaluation of anthelmentic activity of *Cleome rutidosperma* DC, In: Singh V.K., Govil J.N., Ahmad K., Sharma R.K. (Eds.).*Recent Progress in Medicinal Plants Series, Natural Products* (vol. **I**), Studium press, pp. 79–83(2007).
6. Bigdeli M., Hashkavaii A., Rustaiyan A.A. A review on biological effect of Cleome l. and identification the composition of *Cleome coluteoides* essential oil. *Journal of Medicinal Plants* **3**(12), 9–14, (2004).
7. Williams L.A.D., Vasques E., Reid W., Porter R. and Kraus W.W. Biological activities of an extract from *Cleome viscose* L. (Capparaceae). *Naturwissenschaften* **90**: 468–472(2003).
8. El-Sayed N.H., Emam S.S., Mogahed M.I., Yousef A.K. and Mabry T.J. Flavonoids and other constituents from *Cleome Africana* and their insecticidal activities. *Revista Latinoamericana de Quimica* **27**: 9–12(1999).
9. El-Naggar E.B., Bartoskova L., Zemlicka M., Svajdlennka E., Rabiskova M., Strnadova V. and Necas J. Antidiabetic effect of *Cleome droserifolia* aerial parts: lipid peroxidation-induced oxidative stress in diabetic rats. *Acta Veterinaria Brno* **74**: 347–352(2005).
10. Mazloomifar H., Tehrani M.S., Rustaiyan A. and Masoudi S. Essential oil of *Cleome coluteoides* Boiss. From Iran. *Journal of Essential Oil Research* **15**: 337–338(2003).
11. Mirza M., Navaei M.N. and Dini M. Chemical composition of the oil of *Cleome iberica* DC. *Flavour and Fragrance Journal* **20**: 434–435(2005).
12. Ndungu M., Chhabra S.C. and Lwande W. *Cleome hirta* essential oil as livestock tick (*Rhipicephus appendiclatus*) and maize weevil (*Sitophilus zeamais*) repellent. *Fitoterapia* **70**: 514–16(1999).
13. Fushiya S., Kishi Y., Hattori K., Batkhuu J., Takano F., Singab A.N.B and Okuyama T. Flavonoids from *Cleome droserifolia* suppress NO production in activated macrophages *in vitro*. *Planta Med.* **65**: 404–407(1999).
14. Edeoga H.O., Okwu D.E. and Mbaedie B.O. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**: 685–688(2005).
15. El-Askary H.I. Terprnoids from *Cleome droserifolia* (Forssk.) Del. *Molecules* **10**: 971–977(2005).
16. Karber J. Beitrag zur kollektiven Behandlung pharmakologischer Reihen Versuche. *Arch Exp Pathol Pharmak* **162**: 480–483(1931).
17. Eliasson S.G. and Samet J.M. Alloxan induced neuropathies: lipid changes in nerve and root fragments. *Life Sci.* **8**: 493–498(1969).
18. Trinder P. Estimation of serum glucose and triglycerides by enzymatic method. *Ann. Clin. Biochem.* **6**: 24–27(1969).
19. Snedecor W.G., Cochran G.W. Statistical Methods. 10th ed., Lowa State, University Press, USA(1982).
20. Buck W.B., Osweiled G.D., Van Gelder A.G. Clinical & Diagnostic Vetrinary Toxicology2nd Ed., Kendall/Hunt Publishing Company, Iowa(1976).
21. Mabry T.J., Markham K.R. and Thomas M.B. The Systematic Identification of Flavonoids. Springer Verlag, New York, pp.280(1970).
22. Agrawal P.K. Carbon-13 NMR of flavonoids. Elsevier, New York, (1989).
23. Pathak D., Pathak K., Singla A.K., Flavonoids as medicinal agents. Recent advances. *Fitoterapia* **62**: 371–389(1991).
24. Ahmad M., Akhtar M.S., Malik T., Gilani A.H. Hypoglycaemic action of the flavonoid fraction of *Cuminum nigrum* seeds. *Phytother. Res.* **14**: 103–106(2000).
25. Hnatyszyn O., Mino J., Ferraro G., Acevedo C. The hypoglycemic effect of *Phyllanthus sellowianus* fractions in streptozotocin-induced diabetic mice. *Phytomedicine* **9**: 558–559(2002).
26. Addae-Mensah I., Munenge R.W. Quercetin-3-Neohesperidoside (rutin) and other flavonoids as the active hypoglycaemic agents of *Bridelia ferruginea*. *Fitoterapia* **60**: 359–362(1989).
27. Good J.L. and Akisha T. Analysis of Sterols. Blackie Academic and Professional Press, Champan and Hall, 1st Ed. (1997).
28. Leitao S.G., Kaplan M.A.C., Manache F.D., Akisha T. and Tamura T. Sterols and sterol glucosides from two *Aegiphila* species. *Phytochemistry* **31**: 2813–2817(1992).