IJPSR (2014), Volume 5, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 March 2014; received in revised form, 13 May 2014; accepted, 28 June 2014; published 01 September 2014

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *CLEOME BRACHYCARPA* VAHL. GROWING IN EGYPT

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Keywords:

Cleome brachycarpa, α-flavonol glycoside, Methylated flavonoid, Buchairol, Teucladiol, Brine shrimp

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ABSTRACT: Flavonoid and sesquiterpene constituents of the aerial part of *Cleome brachycarpa* were chemically investigated and screened for their potential cytotoxicity. A flavonol glycoside; 3, 5, 4` trihydroxy-3`-methoxy flavone - 7 – *O* - α - L -rhamnopyranose (1```→ 2``) - *O* – β - D-glucopyranoside (1) and a megastigmane glucoside; (+)-(6S, 9R) roseoside (2) were isolated for the first time from the n-butanol fraction of the 90 % ethanol extract of *Cleome brachycarpa*. Four methylated flavonoids were isolated from the chloroform fraction and identified as 5,4`- dihydroxy-3,6,7,3' tetramethoxyflavone (3), 5,5`- dihydroxy-3,6,7,3',4', pentamethoxy-flavone (4), 5-hydroxyl -3,6,7,3',4',5'-hexamethoxyflavone (5) and 5, 7,3',4' tetrahydroxyflavone (6) In addition, two sesquiterpene oxides, namely buchairol (7) and teucladiol (8) were isolated from the n-hexane fraction for the first time. Structure elucidation was achieved using spectroscopic techniques, including IR, UV, ESI-MS, EI-MS and 1D, and 2D-NMR. Each of the three fractions and isolated compounds were screened for their cytotoxicity and lethality using the brine shrimp (*Artemia salina*) assay. The results showed high lethality for all the examined samples, which might be very useful as antiproliferative and antitumor.

INTRODUCTION: Different localities in Egypt are characterized by the presence of a considerable number of medicinal plants that are highly used in folk treatments. *Cleome brachycarpa* Vahl belongs to family Cleomaceae or Capparaceae [1]. Cleomaceae is a small family of flowering plants in the order Brassicales, comprising more than 300 species belonging to 9 genera of which *Cleome* is the largest genus with about 180 - 200 species of medicinal, ethnobotanical and ecological importance.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(9).4008-14

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).4008-14

Plants of this family are herbs, shrubs or trees, sometimes-woody climbers. Nine species of genus *Cleome* are distributed in Egypt [1, 2]. Species of *Cleome* were reported to exhibit several bioactivities and uses, such as antimicrobial [3] hepatoprotective immunomodulatory [4], anticancer [5], antiviral [6] and antioxidant [7]. Different chemical classes such as flavonoids [8, 9, 19] coumarino-lignans [11] steroids [12], dammarane-type triterpenes [13,14], trinortriterpenoid dilactone [15, 16], sesquiterpenes [17, 18], bicyclic diterpene [19], and betaines were isolated from different species of genus *Cleome*.

Leaves of *Cleome brachycarpa* Vahl. are used for the treatment of rheumatism and as anti-inflammatory [8, 20], antidermatosis (scabies and leucoderma) [20], carminative [20, 21] and anti-emetic [22]. Chemically, flavonoids [8, 9], trinortriterpenoid dilactone, deacetoxybrachycarpone, cabralealactone,

ursolic acid were reported in the plant [23]. The present study aimed to evaluate the main chemical constituents and screening the cytotoxicity of the aerial part of *Cleome brachycarpa* Vahl. growing in Egypt.

MATERIALS AND METHODS:

Plant Material: The aerial part of *Cleome brachycarpa* Vahl was collected from Wadi El Gemal National park, Red Sea coast, Egypt, in March 2010. The plant was kindly identified by Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen has been deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Misr International University, Cairo, Egypt.

General Experimental Procedure: UV spectra were determined using a Hitachi 340 spectro-photometer; IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The ^{1}H- and ^{13}C-NMR measurements were obtained with a Bruker NMR spectrometer operating at 500 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in DMSO-d_6 or $CDCl_3$ solution, and chemical shifts were expressed in (ppm) concerning TMS, and coupling constants (*J*) in Hertz. ^{1}H-^{13}C HMBC NMR experiments were carried out using a Jeol AMX-500 high field spectrometer equipped with software Master nova version 5.1.1-3092 program for NMR. ESI-MS (positive ion acquisition mode) was carried out on a TSQ700 triple quadruple instrument (Finnigan, Santos, CA, USA). TLC was performed on precoated TLC plates with silica gel 60 F254 (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany). Column chromatography was carried out using Silica gel 60 (Merck, 40-63 and 63-200 µ) and Sephadex LH-20 (Sigma, 25-100 µ). Developed chromatograms were visualized by spraying with 1% vanillin/H_2SO_4 [24] or anisaldehyde/ H_2SO_4 reagent [24] followed by heating at 100 ºC for 5 min.

Extraction and Isolation:

Extraction and Fractionation: The air-dried powdered material (1 Kg) was exhaustively extracted with 90% ethanol (3 × 5 L). The combined ethanol extract was concentrated under vacuum at 40 ℃ to give a brown residue (175 g). The obtained residue was suspended in distilled H_2O (700 ml) and defatted with petroleum ether, then partitioned successively with *n*-hexane, chloroform, and *n*-Butanol. Each fraction was concentrated under *vacuum* at a temperature not exceeding 40 °C to afford (12, 20 and 25 g), respectively.

Isolation of Compounds from n-Butanol Fraction: The *n*-Butanol fraction (18 g) was purified by chromatography on Sephadex LH-20 (100 × 5 cm, 400 g) using solvents CH_2Cl_2−MeOH (4:1), CH_2Cl_2−MeOH (3:2), CH_2Cl_2−MeOH (1:4) and MeOH to yield four fractions [A (2 g), B (1.8 g), C (3.5 g) and D (4 g)]. Fraction B was further fractionated over a silica gel column (60 g) and eluted with EtOAc and EtOAc/MeOH mixtures in a gradient elution system. Fractions eluted with 15% MeOH in EtOAc gave compound-1 as a light yellow amorphous powder (200 mg) after purification by sephadex LH-20 column with CH_2Cl_2−MeOH (65:35) as eluent. Fraction D was subjected to column chromatography over silica gel (80 g) and eluted with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$ (up to 30%) to give three subfractions. The first subfraction (2 g) was further purified by repeated chromatography on Sephadex LH_{20} column (50 g), using H_2O- MeOH mixture to give Compound-2 as an amorphous powder (25 mg).

Isolation of Compounds from the Chloroform Fraction: Chloroform fraction (20 g) was further fractionated by CC (Sephadex LH-20, 400 g) using CH_2Cl_2−MeOH (4:1), and increasing the polarity till 100% MeOH as eluents afforded, three subfractions [E (7.5 g), F (3.2 g), and G (3.7 g)]. Subfraction E (7.5 g,) was fractionated on a silica gel column (170 g) using 100% $CHCl_3$ as $_a$ solvent system and increasing the polarity with MeOH.

Similar eluates were pooled together, and eluates with 8% MeOH-$CHCl_3$ (4 g) were subjected to column chromatography on silica gel (100 g). The column was eluted with *n*-hexane with increasing the polarity with EtOAc. Repeated chromatography on LiChroprep® RP-18 column (50 g) and elution with 20% H_2O-MeOH mixture showed three major spots. Chromatography on sephadex LH20 using 20% H_2O-MeOH as a developing solvent gave compound 3 (20 mg), compound 4 (25 mg) and compound 5 (40 mg). Fraction 3 (3.0 g) obtained

from chloroform fraction was chromatographed over SephadexLH-20 CC using CH_2Cl_2−MeOH (7:3) as eluent to give 25 mg of light yellow amorphous powder of compound 6.

Isolation of Compounds from the n-hexane Fraction: The *n*-hexane fraction (6.0 g) was chromatographed over silica gel column (100 g), eluted with *n*-hexane and *n*-hexane/ EtOAc mixtures in a gradient elution. Fraction H eluted with *n*-hexane: EtOAc (1:3) showed a major violet spot with vanillin- sulphuric acid spray reagent. It was further purified on a silica gel CC eluted with *n*-hexane with increasing the polarity with EtOAc to produce subfraction I (100 mg) and subfraction J (75 mg). Each subfraction was further purified on sephadex LH-20 CC with CH_2Cl_2: MeOH (7:3) as eluent to give compound 7 from subfraction I (30 mg) and compound 8 from subfraction J (23 mg).

Compound-1: mp 224-226 °C; HR_f 38 (solvent system: 35% MeOH in EtOAc). UV δ_{max} (MeOH): 354, 268 and 254; (NaOMe) 398, 266 and 249 nm; ($AlCl_3$) 398, 362 and 269 nm; ($AlCl_3$/HCl) 401, 359 and 269 nm; (NaOAc) 412 and 262 nm; ESI-MS (m/z 625 $[M + 1]^+$ and 647 $[M + Na]^+$ calc. for $C_{28}H_{32}O_{16}$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.46 (1H, d, *J* = 1.8 Hz, H-6), 6.85 (1H, d, *J* = 1.8 Hz), 8.05 (1H, d, *J* = 2.0 Hz, H-2`), 6.90 (1H, d, *J* = 8.5 Hz, H-5`), 7.75 (1H, dd, *J* = 8.5, 2.0 Hz, H-6`), 3.85 (3H, s, $OCH_3$-3`), 5.52 (1H, d, *J* = 7.6 Hz, C-1``), 4.32 (1H, dd, *J* = 7.6, 9.0 Hz, H-2``), 3.20 (1H, t, *J* = 9.0 Hz, H-3``), 3.14 (1H, t, *J* = 10.2 Hz, H-4``), 3.18 (1H, m, H-5``), 3.48 (1H, dd, *J* = 11.5, 6.5 Hz, H-6``a) 3.67 (1H, dd, *J* = 11.5, 3.2 Hz, H-6``b), 5.58 (1H, brs, C-1```), 3.40 (1H, m, H-2```), 3.70 (1H, m, H-3```), 3.35 (1H, m, H-4```), 3.94 (1H, m, H-5```), 0.72 (3H, d, *J* = 6.1 Hz, H-6```); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 161.50 (C-5), 99.85 (C-6), 163.42 (C-7), 94.77 (C-8), 155.80 (C-9), 105.77 (C-10), 122.55 (C-1`), 115.40 (C-2`), 148.15 (C-3`), 153.15 (C-4`), 116.65 (C-5`), 123.53 (C-6`), 56.20 (OCH_3-3`), 101.20, (C-1``), 77.68 (C-2``), 74.35 (C-3``), 71.80 (C-4``), 78.29 (C-5``), 62.50 (C-6``), 98.70 (C-1```), 70.76 (C-2```), 70.15 (C-3```), 73.40 (C-4```), 70.24 (C-5```), 17.75 (C-6```).

Compound-2: R_f = 0.38, solvent system: 50% H_2O−MeOH; [α]D + 78.1 (c 0.025, MeOH); IR (film) δ_{max} 3398, 2968, 2929, 1652, 1436, 1373, 1072 and 1033 cm^{-1}; UV δ_{max} (MeOH): 238 nm; ESI-MS (m/z 409 $[M + Na]^+$ calc. for $C_{19}H_{30}O_8$); ^{1}H NMR (500 MHz, CD_3OD): δ_H 2.19 (1H, d, *J* = 16.8 Hz, H-2a), 2.68 (1H, d, *J* = 16.8 Hz, H-2b), 5.82 (1H, s, H-4), 5.81 (1H, d, *J* = 15.6 Hz, H-7), 5.78 (1H, d, *J* = 15.6 Hz, H-8), 4.41 (1H, dq, *J* = 6.6 and 6.0 Hz, H-9), 1.20 (3H, d, *J* = 6.6 Hz, CH_3-10), 0.92 (3H, s, CH_3-11), 1.02 (3H, s, CH_3-12), 1.72 (3H, d, *J* = 1.2 Hz, CH_3-13), 4.48 (1H, d, *J* = 7.8 Hz, H-1`), 3.32 (1H, dd, *J* = 7.8, 9.3 Hz, H-2`), 3.26 (1H, t, *J* = 9.3 Hz, H-3`), 3.44 (1H, t, *J* = 10.5 Hz, H-4`), 3.29 (1H, m, H-5`), 3.65 (1H, dd, *J* = 12.3, 6.2 Hz, H-6`a) 3.80 (1H, dd, *J* = 12.3, 2.5 Hz, H-6`b); $^{13}$C NMR (100 MHz, $CD_3OD$): $\delta_C$ 41.55 (C-1), 52.0 (C-2), 206.15 (C-3), 127.54 (C-4), 166.40 (C-5), 82.13 (C-6), 132.10 (C-7), 134.60 (C-8), 77.65 (C-9), 20.56 (C-10), 22.40 (C-11), 24.57 (C-12), 21.15 (C-13), 101.25 (C-1`), 74.70 (C-2`), 77.57 (C-3`), 71.23 (C-4`), 78.50 (C-5`), 62.84 (C-6`).

Cytotoxicity Evaluation:

Brine Shrimp Lethality Bioassay: Brine shrimp (*Artemia salina*) lethality bioassay [25, 26] was carried out to investigate the cytotoxicity of the three fractions (n-hexane, $CHCl_3$ and n-butanol) as well as the eight isolated compounds from *Cleome brachycarpa*. In each experiment, 10 mg of each of the *n*-Hexane, chloroform and *n*-Butanol fractions and 5 mg of isolated compounds [1-8], were dissolved in DMSO and solutions of varying concentrations (10-500 µg/ml) were obtained by serial dilution technique using DMSO, 0.5 ml of each sample was added to 5 ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted.

Experiments were conducted along with control (solvent-treated). Vincristine sulfate was used as a positive control.

Determination of Lethality Concentration: The percentage of lethality was calculated by comparing the mean survival larvae of fractions and pure isolated compounds treated tubes and control. An approximate linear correlation was observed when the logarithm of concentration versus the percentage of mortality was plotted, and the values of LC_{50} were calculated using Microsoft Excel® 2007.

Statistical Analysis: The lethal concentration of tested samples resulting in 50% mortality of the brine shrimp LC_{50} were determined from the 24 h counts, and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (Microsoft Office Excel 2007), and finally the LC_{50} was derived from the best-fit line obtained.

RESULTS AND DISCUSSION: The 90% ethanol extract of the air-dried aerial part of *C. brachycarpa* was successively partitioned between *n*-hexane, chloroform and *n*-Butanol to give the corresponding soluble fractions. A combination of normal phase Si gel, reversed phase RP-18 Si gel and Sephadex LH 20 column chromatography of each fraction led to the isolation of compounds [1 and 2] from the *n*-Butanol fraction, [3-6] from chloroform fraction and [7 and 8] from the *n*-hexane fraction.

Compound [1] was obtained as an amorphous yellow powder. It gave an intense yellow colour with ammonia vapor and yellowish brown color with vanillin/ H_2SO_4 spraying reagents. The positive-ion ESI-MS showed a pseudo-molecular ion peak at m/z 625 $(M + H)^+$ and 647 $(M + Na)^+$ which in conjunction with the ^{13}C-NMR spectral data, indicates that its $[M]^+$ was 624, suggesting the molecular formula $C_{28}H_{32}O_{16}$. The UV absorption maxima recorded in MeOH showed two absorptions at 268 and 354 nm, characteristic for a flavonol skeleton [27] **Fig. 1**.

The ^{1}H- and ^{13}C-NMR spectra of [1] showed two *meta*-coupled protons (AB-spin system) at δ_H 6.46 and 6.85 (each, 1H, d, J = 1.8 Hz, δ_C 99.85 (C-6), 94.77 (C-8) of ring A; characterized the 6- and 8-protons of a flavonoid with 5, 7 dihydroxy A-ring. It also showed three aromatic protons at δ_H 8.05 (1H, d, J = 2.0 Hz, H-2`, $\delta_C$ 115.40), $\delta_H$ 6.90 (1H, d, $J$ = 8.5 Hz, H-5`,δ_C 116.65) and 7.75 (1H, dd, J = 8.5, 2.0 Hz, H-6`, $\delta_C$ 123.53) and represented ABX spin-pattern able assigned to a disubstituted B-ring of 3`,4'-oxygenated [28] confirmed an aglycone with A-ring functionality at C-5 (δ 161.50) and C-7 (δ 163.42), and B-ring at C-3`(δ 148.15) and C-4` (δ 153.15).

The UV bathochromic shift with NaOMe (δmax 398nm), $AlCl_3$/HCl δmax 401, 359, and 269 nm) indicated 3, 5, a 4`free hydroxyl group. The position of the methoxyl group at C3` was indicated from HMBC of its protons at δ_H 3.85 with C3` at $d_C$ 147.4. In addition, the $^1$H- and $^{13}$C-NMR spectra of [1] showed a methoxyl signal at $\delta_H$ 3.85 (3H, s, $\delta_H$ 56.20, $OCH_3$-3`) and two sugars as concluded from the two anomeric protons at δ_H 5.52 (1H, d, J = 7.6 Hz, δ_C 101.20, C-1``) and 5.58 (1H, brs, δ_C 98.70, C-1```). In ^{13}C-NMR, the methyl carbon signals at δ_C 17.75, C-6```, and proton signals at δ_H 0.72 (3H, d, J = 6.1 Hz, H-6```, indicated that [1] contained a methyl pentose sugar. The chemical shifts of the sugar carbon were in agreement with those reported for *O*-α-L-rhamnopyranosyl (1```→ 2``)-*O*-β-D-glucopyranoside [28, 29]. The sugar units are attached to C-7 position based on the lack of bathochromic shift with NaOAc and the characteristic correlations observed between the glucosyl anomeric proton (δ_H 5.56) and methine carbons at (C-6 and C-8) and quaternary carbon at (δ_C 155.80, C- 9). Consequently, the structure of [1] was established as 3, 5, 4` trihydroxy-3`-methoxy flavone -7-*O*-α-L-rhamnopyranosyl (1```→ 2``)-*O*-β-D-glucopyranoside **Fig. 1**, and was in strong agreement with that previously isolated from *Cleome droserifolia* [8].

Compound [2] was isolated as an amorphous powder. The molecular formula was determined as $C_{19}H_{30}O_8$ based on the quasi-molecular ion peaks observed at 409 $(M + Na)^+$ by ESI-MS. The UV spectrum of [2] (λ_{max} 238 nm) and the IR absorption (ν_{max} 1652 cm^{-1}) indicated the presence of an α,β-unsaturated ketone **Fig. 1**.

The IR spectrum also exhibited strong absorptions at 3398 cm^{-1}, indicating the existence of hydroxyl functionalities. The ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H-^{13}C-HMBC showed signals for two olefinic protons at δ_H 5.86 (1H, d, J = 15.2, Hz, H-7, δ_C 132.10) and 5.79 (1H, d, J = 15.2 Hz, H-8, δ_C 134.60), which indicated the presence of a *trans* double bond; an oxymethine proton at δ_H 4.41 (1H, dq, J = 6.6 and 6.0 Hz, H- H-9, δ_C 77.65) and a secondary methyl group at δ_H 1.20 (3H, d, J =6.6 Hz, Me-10, δ_C 20.56), as an $AMXY_3$-type spin system.

Additional signals were found to be due to three tertiary methyl groups at δ_H 0.92 (3H, s, Me-11, δ_C 22.40), 1.02 (3H, s, Me-12, δ_C 24.57) and one being vinylic at δ_H 1.72 (3H, d, *J* = 1.2 Hz, Me-13, δ_C 21.15), a vinyl proton at δ_H 5.82 (1H, s, H-4, δ_C 127.54) and one methylene proton signals at δ_H 2.19 and 2.68 (2H, each d, *J* = 16.8 Hz, H-2, δ_C 50.75) were also observed. ^{1}H-^{13}C-HMBC showed correlations between CH_3-13 and C-4, C-5 and C-6 which confirmed the location of the conjugated ketone function at C-3, δ_C 206.15, and the correlations between CH_3-11 and CH_3-12 and C-1 (δ_C 41.55), C-2 (δ_C 52.0) and C-6 (δ_C 82.13) also clarified the six-membered ring moiety of [2]. Signal multiplicities, chemical shifts and coupling constants in the ^{1}H- and ^{13}C-NMR spectra of [2] revealed the resonance of an anomeric proton and carbon signals at (δ_H 4.48, d, *J* = 7.8 Hz, H-1`, δ_C 101.25) which were inconsistent with the presence of an β-D- glucopyranosyl unit. A loss of 162 mass units from the molecular-ion in the ESI-MS at m/z 225 (M + H- glucose) $^+$ and m/z 207 (M + H- glucose - H_2O) $^+$ suggested the presence of a glucose moiety in [2] **Fig. 1**. The linkage position of the sugar moiety was unambiguously determined to be at C-9 by the long-range correlation from (δ_H 4.48, H-1') of the sugar moiety to δ_C 77.65 (C-9) of the aglycone unit in the ^{1}H-^{13}C-HMBC spectrum of [2].

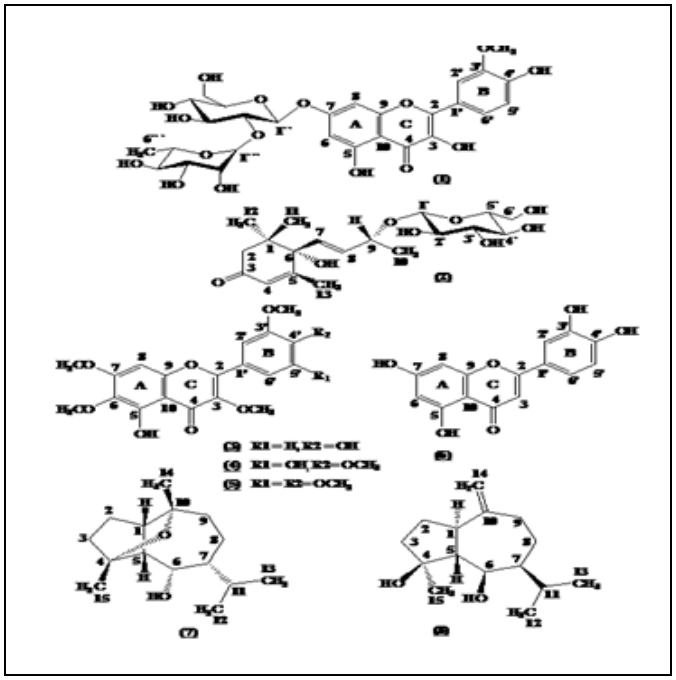

FIG. 1: STRUCTURE OF ISOLATED COMPOUNDS FROM *CLEOME BRACHYCARPA*

These spectroscopic data suggested that [2] is a megastigmane glucoside that was very similar to roseoside (+) - (6*S*, 9R) – 9 – *O* - β-D-glucopyranosyloxy-6- hydroxy-3-oxo-aionol)). Compared to the ^{13}C-NMR spectral data of (6*S*, 9*R*)-roseoside [27], the chemical shift values assigned to C-6, C-7, C-8, and C-9 in [2] were similar to those reported for the aglycone moiety of (+)-(6*S*,9*R*) of roseoside [30]. An upfield shift of C-9 (ca. 74 ppm) is indicative for the (9*S*)-configuration whereas compounds with (9*R*)-configuration exhibit a lower field signal (ca. 77 ppm). This empirical rule could be verified thorough comparison with literature data for diastereomeric 3-oxo-α-ionol glucosides [31]. The chemical structure of [2] was proposed as: (+)-(6*S*,9*R*)-9-*O*-β-D-glucopyranosyloxy-6-hydroxy-3-oxo-α-ionol **Fig. 1** and is identical to (6*S*,9*R*)-roseoside) by literature comparison [30-32].

All the assignments of the isolated compounds [2-8] **Fig. 1**, were supported by ^{1}H-C^{13} NMR, ESI-MS, HMQC and HMBC experiments as well as literature comparison which allowed the identification of compound [3] and [4] as 5,4`-dihydroxy-3,6,7,3' tetramethoxy flavone and 5-hydroxyl-3, 6, 7, 3' ,4', 5' - hexamethoxyflavone, respectively previously reported from *C. brachycarpa* [9]. Whereas, Compound 6 was identified as 5, 7, 3', 4' tetrahydroxyflavone (luteolin) previously isolated from *Cleome* species [8]. Compounds [4] and [7] were identified as 5, 3`-dihydroxy-3, 6, 7, 5', 4', pentamethoxy-flavone and buchairol, respectively previously isolated from *Cleome droserifolia* [8, 18, 33]. Compounds [8] was identified as 6-hydroxynardol (teucladiol) which was isolated before from *Teucrium leucocladum* [34]. Compounds (1, 2, 4, 7 and 8) have been isolated for the first time from *Cleome brachycarpa*. Although Sharaf *et al.*, [8] reported the absence of compound [4] from *C. brachycarpa* collected from EI-Taaif, Saudi Arabia in August but the current study reported its presence for the first time in the sample collected from Wadi El Gemal National park, Red Sea coast, Egypt, in March which may be attributed to seasonal, environmental or soil variations.

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity, which in most cases correlates

reasonably well with cytotoxic and anti-tumor properties [25, 26]. In the present study, the brine shrimp lethalities of the *n*-Hexane, chloroform, *n*-Butanol fractions of the 90% ethanol extract of *Cleome brachycarpa* and isolated compounds [1-8], were determined [25, 26]. The LC_{50} values of the brine shrimp lethality testing after 24 h of exposure of test samples and that of the positive control, vincristine sulfate are given in **Table 1**.

In comparison with vincristine sulfate, the cytotoxicity assay of the chloroform and *n*-hexane fractions of *Cleome brachycarpa* showed significant brine shrimp lethalities with LC_{50} values 15.10 and 26.50 µg respectively. Also, the *n*-Butanol fraction exhibited moderate brine shrimp lethality and the LC_{50} (52.10 µg) value was found to be lower than 100 µg [25, 26]. The isolated compounds; (5, 4, 7 and 8) exhibited significant brine shrimp lethalities with LC_{50} values (LC_{50}<30 µg/ml) 10.10, 12.75, 18.20 and 21.80 µg respectively, while compounds; (3, 1, 2 and 6) showed moderate brine shrimp lethalities with LC_{50} values 37.00, 40.31, 45.25 and 79.50 µg respectively.

The degree of lethality was found to be directly proportional to the concentration of the tested samples. The LC_{50} values of the tested samples were obtained by plotting the percentage of the *Shrimp nauplii* (larva) killed versus the logarithm of concentrations of the extracts or isolated compounds and the best-fit line was obtained from the data using regression analysis.

The presence of significant lethality of *C. brachycarpa* to brine shrimp is indicative of the presence of potent cytotoxic components, which suggested that it might be used as antiproliferative and antitumor.

TABLE 1: BRINE SHRIMP LETHALITY DATA OF EXTRACTS AND ISOLATED COMPOUNDS OF *CLEOME BRACHYCARPA*

Samples	LC_{50} (µg/ml, 24h)	Samples	LC_{50} (µg/ml, 24h)
Vincristine sulphate (Std.)	3.65	Compound-3	37.00
n-Hexane fraction	26.50	Compound-4	12.75
Chloroform fraction	15.10	Compound-5	10.10
n-butanol fraction	52.10	Compound-6	79.50
Compound-1	40.31	Compound-7	18.20
Compound-2	45.25	Compound-8	21.80

CONCLUSION: The present study reported the isolation of a flavonol glycoside, a megastigmane glucoside, four methylated flavonoids, and two sesquiterpene oxides. Although, the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. The results indicated for the first time that the aerial parts of *Cleome brachycarpa* extracts and the purified compounds exhibited significant cytotoxic activity (LC_{50} values < 100 µg/ml) using brine shrimp lethality assay and are considered as a source of natural agents that could be used as antiproliferative, antitumor and could provide leads to interesting pharmaceuticals of plant origin.

ACKNOWLEDGEMENT: The authors would like to acknowledge Dr. Mohamed El Gibaly for identification of plant material.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Afifi MS: Phytochemical and biological investigation of *Cleome brachycarpa* Vahl. Growing in Egypt. Int J Pharm Sci & Res 2014; 5(9): 4008-14. doi: 10.13040/IJPSR.0975-8232.5(9).4008-14.