**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-rhamnopyranosyl (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′-pentamethoxyflavone (4), 5-hydroxy-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. 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. Plants of this family are herbs, shrubs or trees, sometimes-woody climbers. Nine species of genus *Cleome* are distributed in Egypt. Species of *Cleome* were reported to exhibit several bioactivities and uses, such as antimicrobial, hepatoprotective immunomodulatory, anticancer, antiviral and antioxidant. Different chemical classes such as flavonoids, coumarino-lignans, steroids, dammarane-type triterpenes, trinortriterpenoid dilactone, sesquiterpenes, bicyclic...
diterpene, 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, antidermatosis (scabies and leucoderma), carminative, and anti-emetic. Chemically, flavonoids, trinortriterpenoid dilactone, deacetoxybrachycarpone, cabraleafactone, ursolic acid were reported in the plant. The present study aimed to evaluate the main chemical constituents and screening the cytotoxicity of the aerial part of *Cleome brachycarpa* 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 spectrophotometer; 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) with reference to 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 quadrupole 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$_2$SO$_4$ or anisaldehyde/H$_2$SO$_4$ reagent followed by heating at 100°C for 5 min.

**Extraction and isolation:**

**Extraction and fractionation:** The air-dried powdered material (1 Kg) was exhaustively extracted with 90% ethanol (3 x 5 L). The combined ethanol extract was concentrated under vacuum at 40°C to give a brown residue (175 g). The obtained residue was suspended in distilled H$_2$O (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$_2$Cl$_2$-MeOH (4:1), CH$_3$Cl$_2$-MeOH (3:2), CH$_2$Cl$_2$-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 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-I as a light yellow amorphous powder (200 mg) after purification by sephadex LH-20 column with CH$_2$Cl$_2$-MeOH (65:35) as eluent. Fraction D was subjected to column chromatography over silica gel column (60 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 LH20 column (50 g), using H$_2$O- MeOH mixture to give Compound-2 as 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$_2$Cl$_2$-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 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$_2$O-MeOH mixture showed three major
spots. Chromatography on sephadex LH20 using 20% H2O-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 CH2Cl2–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 CH2Cl2: 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; HRf 38 (solvent system: 35% MeOH in EtOAc). UV £max (MeOH): 354, 268 and 254; (NaOMe) 398, 266 and 249 nm; (AlCl3) 398, 362 and 269 nm; (AlCl3/HCl) 401, 359 and 269 nm; (NaAc) 412 and 262 nm; ESI-MS (m/z 625 [M + Na]+ and 647 [M + Na]+ calc. for C28H32O6); 1H NMR (500 MHz, DMSO-d6): £dH 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, OCH3-3`), 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``); 13C-NMR (100 MHz, DMSO-d6): δC 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 (OCH3-3`), 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**: Rf = 0.38, solvent system: 50% H2O–MeOH; [α]D + 78.1 (c 0.025, MeOH); IR (film) λmax 3398, 2968, 2929, 1652, 1436, 1373, 1072 and 1033 cm⁻¹; UV £max (MeOH): 238 nm; ESI-MS (m/z 409 [M + Na]+ calc. for C19H30O8); 1H NMR (500 MHz, CD3OD): δH 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, CH3-10), 0.92 (3H, s, CH3-11 ), 1.02 (3H, s, CH3-12), 1.72 (3H, d, J = 1.2 Hz, CH3-13), 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); 13C NMR (100 MHz, CD3OD): δC 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, CHCl3 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 sulphate was used as 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 logarithm of concentration versus percentage of mortality was plotted and the values of LC50 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 24hrs 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 amorphous yellow powder. It gave intense yellow colour with ammonia vapor and yellowish brown colour with vanillin/ H$_2$SO$_4$ 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 (Figure 1).

The $^1$H- and $^{13}$C-NMR spectra of [1] showed two meta-coupled protons (AB-spin system) at $\delta_H$ 6.46 and 6.85 (each, 1H, d, $J$ = 1.8 Hz, $\delta_C$ 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 $\delta_H$ 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', $\delta_C$ 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 flavone with a-ring functionality at C-5 ($\delta$ 161.50) and C-7 ($\delta$ 148.15), and B-ring at C-3'($\delta$ 148.15) and C-4' ($\delta$ 153.15).

The UV bathochromic shift with NaOMe ($\lambda_{max}$ 398nm), AlCl$_3$/HCl $\lambda_{max}$ 401, 359 and 269 nm) indicated 3, 5, 4'-free hydroxyl group. The position of the methoxyl group at C3' was indicated from HMBC of its protons at $\delta_H$ 3.85 with C3' at $\delta_C$ 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 anumeric protons at $\delta_H$ 5.52 (1H, d, $J$ = 7.6 Hz, $\delta_C$ 101.20, C-1') and 5.58 (1H, brs, $\delta_C$ 98.70, C-1'''). In $^{13}$C-NMR, the methyl carbon signals at $\delta_C$ 17.75, C-6'', and proton signals at $\delta_H$ 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 was 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 ($\delta_H$ 5.56) and methine carbons at (C-6 and C-8) and quaternary carbon at ($\delta_C$ 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 (Figure 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$_8$ on the basis of the quasi-molecular ion peaks observed at 409 (M + Na)$^+$ by ESI-MS. The UV spectrum of [2] ($\lambda_{max}$ 238 nm) and the IR absorption ($\nu_{max}$ 1652 cm$^{-1}$) indicated the presence of an $\alpha$,$\beta$-unsaturated ketone (Figure 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-HMBHC showed signals for two olefinic protons at $\delta_H$ 5.86 (1H, d, $J$ = 15.2, Hz, H-7, $\delta_C$ 132.10 ) and 5.79 (1H, d, $J$ = 15.2 Hz, H-8, $\delta_C$ 134.60), which indicated the presence of a trans double bond; an oxymethine proton at $\delta_H$ 4.41 (1H, dq, $J$ = 6.6 and 6.0 Hz, H- H-9, $\delta_C$ 77.65) and a secondary methyl group at $\delta_H$ 1.20 (3H, d, $J$ =6.6 Hz, Me-10, $\delta_C$ 20.56), as an AMXY$_3$-type spin system.

Additional signals were found to be due to three tertiary methyl groups at $\delta_H$ 0.92 (3H, s, Me-11, $\delta_C$ 22.40), 1.02 (3H, s, Me-12, $\delta_C$ 24.57) and one being vinylic at $\delta_H$ 1.72 (3H, d, $J$ = 1.2 Hz, Me-13, $\delta_C$ 21.15), a vinyl proton at $\delta_H$ 5.82 (1H, s, H-4, $\delta_C$ 127.54) and one methylene proton signals at $\delta_H$ 2.19 and 2.68 (2H, each d, $J$ = 16.8 Hz, H-2, $\delta_C$ 50.75) were also observed. $^1$H-$^{13}$C-HMBHC 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, $\delta_C$ 206.15, and the correlations between CH$_3$-11 and CH$_3$-12 and C-1 ($\delta_C$ 41.55), C-2 ($\delta_C$ 52.0) and C-6 ($\delta_C$ 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 ($\delta_H$ 4.48, d, $J$ = 7.8 Hz, H-1`, $\delta_C$ 101.25) which were in consistent with the presence of an $\beta$-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$_2$O) $^+$ suggested the presence of a glucose moiety in [2] (Figure1). The linkage position of the sugar moiety was unambiguously determined to be at C-9 by the long-range correlation from ($\delta_H$ 4.48, H-1`) of the sugar moiety to $\delta_C$ 77.65 (C-9) of the aglycone unit in the $^1$H-$^{13}$C-HMBC spectrum of [2].

![Structure of Isolated Compounds from Cleome Brachycarpa](image)

FIGURE 1: STRUCTURE OF ISOLATED COMPOUNDS FROM CLEOME BRACHYCAPRA

These spectroscopic data suggested that [2] is a megastigmane glucoside that was very similar to roseoside (+) - (6S, 9R) - 9 - O - $\beta$-D-glucopyranosylxyloxy-6-hydroxy-3-oxo-a-ionol). Compared to the $^{13}$C-NMR spectral data of (6S, 9R)-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 (+)-(6S,9R) of roseoside 30. An upfield shift of C-9 (ca. 74 ppm) is indicative for the (9S)-configuration whereas compounds with (9R)-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: (+)-(6S,9R)-9-O-$\beta$-D-glucopyranosyloxy-6-hydroxy-3-oxo-α-ionol (Figure1) and is identical to (6S,9R)-roseoside) by literature comparison 30-32.

All the assignments of the isolated compounds [2-8] (Figure 1), were supported by $^1$H-$^{13}$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' - hexamethoxy flavone, 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 buchailor, respectively previously isolated from Cleome drosenifolia 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 El-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 hours of exposure of test samples and that of the positive control, vincristine sulphate are given in (Table 1).
In comparison with vincristine sulphate, 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. In addition, 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. 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 nauplius (larva) killed versus the logarithm of concentrations of the extracts or isolated compounds and the best-fit line was obtained from the data by means of regression analysis. The presence of significant lethality of *C. brachycarpa* to brine shrimp is an indicative of the presence of potent cytotoxic components, which suggested that it might be used as antiproliferative and antitumor.

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**CONCLUSION:** The present study reported the isolation of a flavonol glycoside, a megastigmame 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 (LC50 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.

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**REFERENCES:**