Chromatographic analysis (LC-MS and GC-MS), antioxidant activity, antibacterial activity, total phenol, and total flavonoid determination of Cleome arabica L. growing in Jordan

Rami Q. Alkhatib, Afnan B. Almasarweh, Nour M. Abdo, Abdulraouf S. Mayyas, Mahmoud A. Al-Qudah, and Sultan T. Abu-Orabi

Department of Biotechnology and Genetic Engineering, Faculty of Science and Arts, Jordan University of Science and Technology, Jordan; Department of Public health and Community Medicine, Faculty of Medicine, Jordan University of Science and Technology, Jordan; Department of Conservation Science, Queen Rania Faculty of Tourism and Heritage, The Hashemite University, Jordan; Department of Chemistry, Faculty of Science, Yarmouk University, Jordan; Department of Medical Analysis, Faculty of Science, Tishk International University, Erbil, Iraq

ABSTRACT
The pivotal Cleome arabica L. species belongs to the Cleome genus with fascinating secondary metabolites consisting of main classes of phenols, flavonoids, and glucosinolate derivatives. The current study was carried out on three crude extracts of Cleome arabica L. plant of Jordanian origin. In terms of the valuable resources for natural new drug development, the phytochemical contents, antioxidants, and antibacterial activities, in addition to the oil composition of this species were identified. Forty-six compounds representing 96.98% of the total composition were detected from the essential oil obtained by hydrodistillation (0.06%). The oil composition was dominated by nitrogen-sulfur-containing compounds (35.66%), esters (19.64%), and oxygenated sesquiterpenes (11.92%). Butanol extract had significantly the best scavenging activities of DPPH (P < .0001), ABTS (P < .0001), and hydroxy radicals (P < .0001) while the Aq.Methanol extract exhibits the highest flavonoid content (173.5 ± 0.002 mg Quercetin/g dry extract) and had significantly the most ferrous chelating effect (P = .0011). Also, most of the tested extracts showed antibacterial activities against bacterial strains. Our findings exhibited that the Cleome arabica L. plant from Jordan origin might be a good candidate as potential biologically compounds that could be used as a source of natural antioxidants and antibacterial agents.

Introduction
Plants, in general, were used for many decades in the treatment and prevention of various dietary and pathogen–related diseases. Several studies showed that the biological activities including antioxidant, antimicrobial, anticancer, anti-inflammatory, and anti-diabetic activities of these plants are related to the presence of phenols, flavonoids, alkaloids, terpenoids, carotenoids, vitamins, and tannins. These compounds showed hydrogen donors capacity, free radical scavengers, and metal chelating potential. Also, they have been known as antimicrobial agents.

Cleome, known as a spider flower, is the largest genus of the Cleomaceae family comprising about 200 species spread around the world, especially in North Africa. Cleome species have traditionally been known for their medicinal treatments against earache, skin diseases, rheumatism and headache. Phytochemical screening studies carried out on Cleome species revealed the presence of different important secondary products including flavonoids, saponins, coumarins, terpenoids, phenolics, and alkaloids. The
essential oils extracted from different organs of Cleome species are substantially composed of monoterpenene hydrocarbons, oxygenated monoterpenoids, sesquiterpene hydrocarbons, oxygenated sesquiterpenoids and fatty acids, in addition to isothiocyanate and nitrile compounds, which can be derived by degradation of glucosinolates.\cite{10-12} In Jordan, three wild types of Cleome species are found including Cleome arabica L., Cleome trinervia Fresen and Cleome drasarifolia (Forssk.) Del.\cite{4}

Cleome arabica L. is an annual, erect, and simple or branched with 30–50 cm in length. It is distributed in deserts, ruderal sites and wadies, lower Jordan valley, Dead Sea area and Edom.\cite{4} Previous studies showed that Cleome arabica L. plant from different origins has anti-inflammatory,\cite{13-15} anti-cancer,\cite{16,17} antimicrobial and analgesic activities,\cite{15} antihypercholesterolemic,\cite{18} antioxidant,\cite{9,18} antifeedant and insecticidal activities.\cite{19,20} Earlier chemical investigations from Cleome arabica L. have revealed the separation of different chemicals such as cleomin, phenolic compounds alkaloids, steroid derivatives, damarane triterpene, glucosylated and rhamnosylated flavonols.\cite{21-24}

As far as we know, the chemical composition of essential oil and biological activities of Jordanian Cleome arabica L. has not been performed. Therefore, our study aimed to analyze the chemical composition of essential oil and to identify the major components of butanol, aq. methanol and water extracts of the Cleome arabica L. by LC-MS, in addition, we aimed to demonstrate the antioxidant and antibacterial activities of the Cleome arabica L. plant grown in Jordan.

Materials and methods

Chemical and reagents

Helium (high purity 99%), n-alkanes (C8-C20) GC grade AR., 5% diphenyl, 95% dimethyl polysiloxane (DP-5) grade AR, ABTS, DPPH, Folin-Ciocalteu reagent, Sodium carbonate, Sodium hydroxide, aluminum chloride, gallic acid, Quercitin, ascorbic acid, BHA, methanol, potassium persulfate, ferrous chloride, ferrous sulfate, hydrogen peroxide, salicylic acid, ferrozine, Sodium nitrite, and EDTA were purchased from Sigma-Aldrich (Buchs, Switzerland). For antibacterial activities, DMSO (Alpha Chemica, India), PBS (Capricon Scientific), MHB and MHA were used.

Plant material

Cleome arabica L. plant was collected from the Aqaba region (near Al-Yutom Valley Customs Center, 29°35’45.5”N 35°09’50.5”E) during the flowering period. Then, it was identified by a specialized taxonomist (Figure 1). Later, the collected sample was given a voucher specimen number (YU/2/CC/1002) and deposited in the Yarmouk University herbarium, Irbid, Jordan.

Figure 1. Cleome arabica L. plant grown in Aqaba region (Al-Yutom Valley Customs Center, the southern part of Jordan during flowering period.
**Preparation of crude extracts**

Freshly plant samples were air-dried at room temperature for a month. Then, it was ground to a powder and the fatty acids were removed using petroleum ether (40–60°C) in Soxhlet extraction. The remaining plant material was dried and then extracted with methanol. A rotary vacuum evaporator was used to concentrate the methanol extracts and dried them. Then, the dried extract was separated between CHCl₃ and H₂O (1:1 v/v) solvent system. After that, the dried CHCl₃ fraction was separated between 10% aqueous methanol and hexane. n-butanol was used to extract the polar organic compounds. Thus, the obtained three extracts (10% aqueous methanol, n-butanol and water) were directly used for testing.

**Essential oil extraction**

The essential oil from fresh aerial parts of the *Cleome arabica* L. plant was extracted following the protocol described in the literature.¹¹,²⁵ Briefly, small pieces of fresh aerial parts of the *Cleome arabica* L. plant (200 g) were hydrodistilled for 4 hours using a Clevenger-type apparatus. Then, anhydrous sodium sulfate was used for drying the oil. Then, the oil is directly stored in GC-grade n-hexane at 4°C for (GC-MS) analysis.

**LC-MS analysis of phytochemicals**

LC-MS analysis procedure was conducted according to Abu-Orabi et al.²⁶ Briefly, A Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UHPLC system (Bremen, Germany) was used for screening flavonoids and phenolics compounds of interest in both positive (M + H) and negative (M-H) electrospray ionization modes. Briefly, Chromatographic separation was conducted using a 120, C18 reversed phase column [100 x 2.1 mm, 1.8 μm (120 Å)] at 30°C, autosampler temperature 8.0°C with total run time 20.0 min using gradient grade. The elution gradient consisted of mobile phase A (water/methanol (90:10%) with 5 mM ammonium formate and 0.1% formic acid)) and solvent B (methanol with 5 mM ammonium formate and 0.1% formic acid). MS/MS analysis was performed in negative ion mode with an ion spray voltage of −4,500 V.

**Gas chromatographic analysis of the essential oils**

Chemical extractions procedure was described by Al-Qudah et al.²⁷ Briefly, 1 μL extracted oil diluted in 10.0 μL GC grade n-hexane was analyzed by GC-MS (Model Varian Chrompack CP-3800 GC/MS, Saturn, Netherlands) system equipped with a DB-5 GC capillary column (5% diphenyl, 95% dimethyl polysiloxane, for quantitative analysis, Hewlett-Packard HP-8590 GC equipped with optima-5 column (5% diphenyl, 95% dimethyl polysiloxane) (30 m x 0.25 mm, 0.25 μm film thickness) and a split-splitless injector (split ratio 1:50) was used. The relative percent concentrations of the detected compounds were calculated using relative peak areas. A hydrocarbon mixture of n-alkanes (C₈–C₂₀) was analyzed separately under the same chromatographic conditions. Essential oils with different chemical constituents were identified by comparing their mass spectra with those found in the database library (Wiley 275 library, New York, USA).

**Phytochemical screening**

The phytochemical screening of butanol, Aq. MeOH and water extracts from *Cleome arabica* L. were performed using a standard procedure.²⁸
**Total phenolic contents (TPC) and total flavonoid contents (TFC)**

Follin-Ciocalteu method and aluminum chloride assay were conducted to quantify TPC and TFC, respectively.\(^{[27,29]}\) For TPC analysis, 0.5 mL of the extract was mixed with 2.5 mL of 0.2 N Follin-Ciocalteu reagent and 2 mL of Na\(_2\)CO\(_3\) (75 g/L). The absorbance of the final solution was then recorded at 765 nm wavelength. The TPC content of the plant was represented as (mg GA/g dry extract). For TFC analysis, aliquots of (1 mL) extracts of (1 mg/mL) concentration were taken in different volumetric flasks (10 mL). Then, 4 mL of distilled water was added to each flask, followed by the addition of 0.3 mL of sodium nitrite (5% NaNO\(_2\), w/v). After 15 min, the absorbance was measured at 510 nm. The TFC content for different extracts of the plant was represented as (mg quercitin/g dry extract). Methanol was used as blank.

**Antioxidants activity**

Antioxidant activity of the *Cleome arabica* L. plant was investigated for butanol, Aq. MeOH and water crude extracts. DPPH, ABTS, FIC and Hydroxyl radical (HO•) scavenging assays were used to determine the antioxidant activities of the extracts according to the literature.\(^{[27,29]}\) IC\(_{50}\) value, the concentration of the substrate that causes 50% loss of the radical activity, was used for the interpretation of the results from all used methods. The IC\(_{50}\) values were calculated using the linear regression method of the tested crudes. Measurements were performed in triplicates. Percentage of inhibition in all assays was calculated as:

\[
\text{Scavenging effect (\%) = (Ac – AS/Ac) \times 100.}
\]

where Ac represents the absorbance of the blank and As represents the absorbance in the presence of the extract.

**Antibacterial activity**

The antibacterial activity of the *Cleome arabica* L. extracts was evaluated using both gram-positive and gram-negative bacterial strains (provided by ATCC). Gram-positive bacteria included *Staphylococcus aureus* (ATCC 29213), which was used as standard and *Staphylococcus aurus* (BAA-41), which was used as a resistant isolate. Whereas gram-negative bacteria included *Escherichia coli* (ATCC 25922), which was used as a standard and *Escherichia coli* (BAA-2452), which was used as a resistant strain. Broth micro-dilution method with some modifications was used to determine the Minimum inhibitory concentration (MIC) of the *Cleome arabica* L. extract against susceptible bacteria as described by Kueté et al.\(^{[30]}\) Briefly, each extract was dissolved in 10% (DMSO) and diluted in MHB to reach a 100 mg/mL concentration. After that, 50 µl of inoculums (standardized to 1 \times 10^5 CFU/mL by adjusting the optical density to (0.08–0.13) at 600 nm) was added into a 96-well plate containing 50 µl of different concentrations. The bacterial culture (10% DMSO and broth) without the plant extract was considered as a positive control, while the culture containing the broth-only was considered as a negative control. The plates were incubated at 37°C for 24 h. For the minimum bactericidal concentration (MBC) determination, 10 µl from each well was added to 90 µl of phosphate buffer saline (PBS) and diluted eight times. Then 10 µl of each well was placed on MHA medium and incubated at 37°C for 24 h. The lowest concentration exhibited no growth was corresponded to MBC.

**Statistical analysis**

Data were analyzed using PC SAS (v. 9.2; SAS Institute, Cary, NC, USA). Mean and standard error were calculated for each antioxidant and fraction. One-way ANOVA (analysis of variance) was used to assess significant differences between different methods of the extraction at different concentrations at \(\alpha = 0.05\). A post-hoc Tukey–Honest test was used to estimate pairwise comparisons for significant results from the One-way ANOVA. Graphs of different parameters were made using Graph Pad Prism.
7. Linear Pearson correlation for each of butanol, methanol, and water extracts was conducted between total phenolic content and antioxidant activity. R Gui version 4.0.3 was used to visualize the correlation matrix and pair-wise scatter plots using the chart correlation function.

Results and discussion

**LC-MS profiling**

The results of the LC-MS/MS analysis of *Cleome arabica* L. are shown in (Table 1). LC-MS analysis of the three extracts of the plant revealed the presence of 30 compounds in different amounts. The most abundant detected compounds were isoorientin, hesperidin and nevirapine. The relative amount (%) of each compound in butanol, Aq. MeOH and water extracts were, respectively: 47.33, 31.29 and 11.08 for isoorientin, 18.40, 4.45 and 25.40 for hesperidin and 2.49, 11.90 and 18.36 for nevirapine. In comparison with other studies, four compounds including β-sitosterol, 11-α-acetylbachych-carpone -22(23)-en, 17-α-hydroxycabralactone and amblyone were detected in *Cleome arabica* L. from Tunisia. [19] The variation in the content of the same species possibly depends on the plant’s origin (the site from where the plant sample was collected).

**Chemical composition of essential Oil of Cleome arabica L. growing in Jordan**

The chemical profile of the oil retention indices (RI) and the percentage area under the GC peak of the oil constituents are shown in (Table 2). A total of 46 compounds representing 96.98% of the total composition were investigated. The principal components of the plant oil were 2-Methyl butyl isothiocyanate (27.11%), ethyl dodecanoate (16.57%), isopropyl isothiocyanate (6.94%) and

| No. | Rt (min) | Compound          | Butanol extract | Aq. MeOH extract | Water extract |
|-----|---------|-------------------|-----------------|------------------|--------------|
| 1   | 0.96    | Gallic Acid       | 0.44            | -                | 0.91         |
| 2   | 1.94    | Luteolin          | 0.60            | 0.05             | -            |
| 3   | 2.22    | 2,5-Dihydroxybenzoic acid | 6.35          | -                | 0.10         |
| 4   | 2.64    | 4-Hydroxybenzoic acid | 1.23            | 0.19             | 0.18         |
| 5   | 3.34    | Caffeic Acid      | 0.68            | -                | 0.46         |
| 6   | 3.39    | Vanillic acid     | 2.87            | -                | 1.47         |
| 7   | 3.99    | Cleomiscosin B    | 1.61            | 0.16             | 1.05         |
| 8   | 4.51    | p-Coumaric acid   | 0.6             | 0.15             | 0.28         |
| 9   | 4.84    | Cleomiscosin A    | 0.85            | -                | 2.17         |
| 10  | 4.93    | Hesperidin        | 18.4            | 4.45             | 25.4         |
| 11  | 4.95    | Vitexin           | 3.35            | 0.63             | 3.32         |
| 12  | 5.16    | Naringenin-4’-galactoside | 0.96          | -                | 0.47         |
| 13  | 5.19    | Ferulic acid      | 1.08            | 0.96             | 0.38         |
| 14  | 6.38    | Benzyl isothiocyanate | 0.35           | 3.55             | 10.57        |
| 15  | 6.57    | Isoorientin       | 47.33           | 31.29            | 11.08        |
| 16  | 6.74    | Cleomiscosin C    | -              | 0.57             | -            |
| 17  | 6.87    | Cleomaldeic acid  | 1.24            | 0.16             | 0.28         |
| 18  | 7.01    | Cleomiscosin D    | 0.78            | 0.42             | 0.5          |
| 19  | 7.78    | Hyperoside        | 0.51            | 0.17             | -            |
| 20  | 8.67    | Ellagic acid      | 0.37            | -                | 0.15         |
| 21  | 12.25   | β-sitosterol      | 0.14            | 0.13             | -            |
| 22  | 12.28   | Kaempferol        | -              | 1.82             | -            |
| 23  | 12.9    | Naringenin        | -              | 0.78             | -            |
| 24  | 13.46   | Sakuranetin       | -              | 1.18             | -            |
| 25  | 13.81   | kaempferitin      | -              | -                | 1.97         |
| 26  | 14.56   | Kumatakenin       | 2.48            | 33.72            | -            |
| 27  | 21.54   | Nevirapine        | 2.49            | 11.9             | 18.36        |
| 28  | 29.02   | Ursolic acid      | -              | 1.16             | 3.12         |
Table 2. Chemical composition of the essential oil of aerial parts of Cleome arabica L. collected from Jordan.

| NO | \( \text{RI}_{\text{exp}}^* \) | \( \text{RI}_{\text{rep}} \) | Compound | Peak area % | Identification |
|----|-------------------------------|-----------------|----------|-------------|----------------|
| 1  | 802                           | 800             | n-Octane | 0.27        | a, b           |
| 2  | 828                           | 810             | 5-Methyl-2-nobornene | 0.32        | a, b           |
| 3  | 832                           | 821             | Methyl pentanoate  | 0.49        | a, b           |
| 4  | 840                           | 840             | Isopropyl isothiocyanate | 0.94        | a, b, c        |
| 5  | 850                           | 853             | 3E-Hexenol | 0.68        | a, b           |
| 6  | 872                           | 877             | 4Z-Hexenol  | 0.73        | a, b           |
| 7  | 885                           | 885             | Isopropyl –2-methyl butyrate | 0.69        | a, b           |
| 8  | 902                           | 902             | Heptanal   | 0.32        | a, b, c        |
| 9  | 947                           | 947             | Isobutyli isothiocyanate | 0.97        | a, b, c        |
| 10 | 965                           | 962             | Cyclohexyl formate | 0.73        | a, b           |
| 11 | 970                           | 965             | 2-Methyl-(3E)-octen-5-yne | 6.48        | a, b           |
| 12 | 996                           | 999             | Yamogi alcohol | 0.28        | a, b           |
| 13 | 1030                          | 1026            | 1-p-menthene | 0.31        | a, b           |
| 14 | 1056                          | 1055            | 2-Methyl butyl isothiocyanate | 27.11       | a, b           |
| 15 | 1060                          | 1059            | y -Terpinene | 0.66        | a, b, c        |
| 16 | 1068                          | 1063            | α – Methyl benzene methanol | 1.19        | a, b           |
| 17 | 1102                          | 1106            | trans-Verticiral | 0.28        | a, b           |
| 18 | 1105                          | 1108            | Phenyl ethyl alcohol | 3.41        | a, b           |
| 19 | 1123                          | 1127            | Chrysanthanone | 0.42        | a, b           |
| 20 | 1153                          | 1150            | 2-(12)-Propenyl phenol | 1.18        | a, b           |
| 21 | 1215                          | 1216            | trans-carveol | 0.37        | a, b, c        |
| 22 | 1240                          | 1238            | trans-chrysanthenyl acetate | 0.19        | a, b           |
| 23 | 1284                          | 1280            | Dihydroedulan II | 3.6         | a, b           |
| 24 | 1294                          | 1294            | trans-thio rose oxide | 0.64        | a, b           |
| 25 | 1305                          | 1305            | Isomentyl acetate| 0.72        | a, b           |
| 26 | 1310                          | 1311            | Sesamol     | 0.92        | a, b           |
| 27 | 1338                          | 1330            | E-Patchenol | 1.12        | a, b           |
| 28 | 1386                          | 1375            | α–Yangene   | 2.02        | a, b           |
| 29 | 1407                          | 1407            | Cycloexchelene | 0.38        | a, b           |
| 30 | 1409                          | 1409            | α-Gurjunene | 0.47        | a, b           |
| 31 | 1413                          | 1419            | E-Caryophyline | 0.91        | a, b, c        |
| 32 | 1461                          | 1471            | 3Z-Jasmonyl lactone | 0.69        | a, b           |
| 33 | 1483                          | 1488            | E-β-Ionone  | 0.81        | a, b           |
| 34 | 1508                          | 1509            | Farenal     | 1.54        | a, b           |
| 35 | 1512                          | 1513            | γ-Cadinene  | 0.41        | a, b           |
| 36 | 1540                          | 1532            | γ – Dehydro-ar-Himachalene | 0.60        | a, b           |
| 37 | 1575                          | 1566            | Dodecanolic acid | 2.75        | a, b           |
| 38 | 1584                          | 1594            | Ethyl dodecanoate | 16.57       | a, b, c        |
| 39 | 1616                          | 1607            | Geranyl isovalerate | 1.49        | a, b           |
| 40 | 1640                          | 1648            | Khusilal    | 1.98        | a, b           |
| 41 | 1670                          | 1668            | E-Amyl cinnamaldehyde | 0.88        | a, b           |
| 42 | 1673                          | 1669            | β-Atlantone | 0.84        | a, b           |
| 43 | 1712                          | 1713            | (2E,6Z)-Famesal | 1.24        | a, b           |
| 44 | 1768                          | 1763            | β – Acroadienol | 1.59        | a, b           |
| 45 | 1886                          | 1880            | 3Z-Hexenyl cinnamate | 0.47        | a, b           |
| 46 | 1912                          | 1913            | (5E,9E)-Famesyl acetone | 0.32        | a, b           |

Total 96.98

Monoterpenoid hydrocarbons 1.29
Oxygenated monoterpenoids 4.87
Sesquiterpene hydrocarbons 4.79
Oxygenated sesquiterpenoids 11.92
Esters 19.64
Nitrogen-Sulfur containing compounds 35.66
Carboxylic acids 2.75
Other 16.06

(*) \( \text{RI}_{\text{exp}} \) refers to the retention index experimentally calculated using C8-C20 n-alkanes on DB-5 column.\( \text{RI}_{\text{rep}} \): Retention Index on DB-5 column in reference to n-alkanes as reported by Adams and literatures.
(a) Linear retention index. (b) Identification based on comparison of mass spectra.
(c) Co-injection with standard sample.
2-Methyl-(3E)-octen-5-yne (6.48%). The detected oil constituents of the plant were classified as follows: monoterpenic hydrocarbons (1.29%), oxygenated monoterpenes (4.87%), sesquiterpene hydrocarbons (4.79%), oxygenated sesquiterpenes (11.92%), nitrogen-sulfur containing compounds (11.92%), esters (19.64%), one carboxylic acid (2.75%) and other compounds (16.06%).

Essential oils are mixture of natural products that give plants their smell and flavor. Several Cleome species from different origins have been analyzed by many researchers for their essential oils composition. For example, it was reported that oxygenated diterpenes and (Z)-Phytol were the major compounds of the oil isolated from Indian Cleome rutidosperma DC. Essential oils analysis of Cleome amblyocarba Barratte & Mur, Cleome rupicola vicary and Cleome ramosissma Webb ex Parl. grown in Saudi Arabia exhibited their enrichment with a variety of compounds dominated by isothiocyanate and cubenol. Also, it was reported that the essential oils of two Jordanian Cleome species namely, Cleome droserefolia and Cleome trinervia were rich with terpenoids and sulfur and nitrogen containing compounds. The chemical composition variations of the essential oils present in different Cleome species could be attributed to the plant’s origin, which represents several factors affecting the plant chemical composition, such as climatic change, genetic variability, seasonal variation and the nature of the soil. The presence of high amount of sulfur-nitrogen containing compounds in the Cleome arabica L. plant is supported by the presence of glucosinolates that can produce nitrile and isothiocyanate compounds via hydrolysis. It is worth mentioning that this is the first study analyzing the essential oil composition of Cleome arabica L. plant in Jordan.

**Phytochemical analysis of Cleome arabica L extracts**

Plants contain a wide variety of phytochemical compounds including phenols, flavonoids and terpenoids. In this study, the phytochemical screening of the Aq. MeOH and butanol extracts from Cleome arabica L. plant revealed the presence of tannins, flavonoids, saponins and glycosides, whereas, the water extract from the same plant contained only saponins and glycosides. However, the phytochemical tests applied on the three crude extracts showed the absence of the alkaloids, anthraquinones and terpenoids (Table 3). In previous studies, several extracts including methanol, ethanol, acetone and butanol have been used for plant extraction exhibiting differences in the content of phytochemical compounds and antioxidant activities among them. These differences could be related to the influence of plant materials by the solubility of solvent, degree of phenols polymerization and the interaction of phenol compounds with other plant constituents. Our data of phytochemical analysis showed an agreement with the studies reported that Cleome arabica L. plant from different origins contain several types of phytochemical compounds. In addition, our data showed that there are qualitative and quantitative differences in the phytochemical compounds detected in the three extracts (Aq. MeOH, butanol and water) from the same Cleome arabica L. plant. This can be attributed to the differences in the polarity of the extraction solvents, which could bring a wide variation in the levels of the extracted bioactive compounds.
**Total phenolic and total flavonoid contents**

Total phenolic content (TPC) of the three extracts were identified using Folin-Ciocalteu method and calculated as (mg gallic acid (GA)/g dry weight of extract). The total phenolic content was calculated using the following regression equation:

\[
Y = 2.00 \times 10^{-3} X - 2.98 \times 10^{-2}, \quad R^2 = 0.9698 \text{ (based on the calibration curve)}
\]

where \(Y\) is the absorbance at 765 nm and \(X\) is the TPC in the extract. Table 3 shows the amount of TPC in the three extracts of *Cleome arabica* L. The results showed that butanol extract had the highest TPC (539.46 ± 12.00 \times 10^{-3} mg GA/g dry weight), then Aq. MeOH (321.8 ± 9.00 \times 10^{-3} mg GA/g dry weight), and the lowest TPC was determined in the water extract (189.46 ± 21.00 \times 10^{-3} mg GA/g dry weight). Total flavonoid content (TFC) of the three extracts was determined using the aluminum chloride assay and calculated as (mg Quercetin/g dry weight of extract). TFC was identified according to the following regression equation based on the calibration curve.

\[
Y = 3.00 \times 10^{-4} X + 3.92 \times 10^{-2}, \quad R^2 = 0.9956,
\]

where \(Y\) is the absorbance at 510 nm and \(X\) is the TFC in the extract. The obtained results showed that Aq. MeOH extract had the highest content of flavonoid (173.5 ± 2.0 \times 10^{-3} mg quercetin/g dry weight), then butanol (146.9 ± 5.0 \times 10^{-4} mg quercetin/g dry weight), whereas water extract had the lowest content of flavonoid (4.66 ± 1.0 \times 10^{-3} mg quercetin/g dry weight) (Table 4). Variation in the amount of phenolic and flavonoid compounds between extracts could be attributed to the differences in solubility and polarity of solvents used during extraction.[37]

**Antioxidant activity**

Free radicals scavenging and metal-chelating activities of the three *Cleome arabica* L. extracts were demonstrated using 2,2-diphenyl-1-picylhydrazyl (DPPH), 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), hydroxyl (HO) radicals and ferrous ion chelating (FIC) assays. The inhibition (%) was calculated. Then, the \(IC_{50}\) value was determined (low \(IC_{50}\) value indicates high radical scavenging activity). Butanol extract significantly had the highest scavenging activity of DPPH radical (\(P < .0001\)) (Figure 2a), ABTS radical (\(P < .0001\)) (Figure 2b) and HO radical (\(P < .0001\)) (Figure 2c). The high radical scavenging % of butanol extract could be attributed to its high phenolic content. In contrast, Aq. MeOH significantly had the highest FIC activity among other extracts (\(P = .0011\)) (Figure 2d).

**Relationship between phenolic content and antioxidant activity of butanol, Aq.MeOH and water extracts**

The relationship between the total phenolic content and the scavenging % of DPPH, ABTS and HO radicals and FIC effect showed that there was a strong positive linear correlation between them. The correlation coefficients (R) for DPPH, ABTS, HO and FIC were, respectively: 0.79, 0.65, 0.90 and 0.69 in butanol extract (Figure 3a), 0.99, 0.76, 0.92 and 0.99 in Aq. MeOH extract (Figure 3b) and 0.85, 0.88, 0.93 and 0.80 in water extract (Figure 3c). Phenolic and flavonoid are major compounds that exhibit a wide spectrum of chemical and biological activities including radical scavenging properties.[38] Our

| Table 4. Total phenolic and total flavonoid contents of crude extract fractions of *Cleome arabica* L. |
|---------------------------------------------|
| Extract      | TPC (mg GA/g dry weight) | TFC (mg Quercetin/g dry weight) |
|---------------|--------------------------|---------------------------------|
| Aq. MeOH      | 321.80 ± 9.00*10^{-3}    | 173.50 ± 2.00*10^{-3}           |
| Butanol       | 539.46 ± 12.00*10^{-3}   | 146.90 ± 0.50*10^{-3}           |
| Water         | 189.46 ± 21.00*10^{-3}   | 4.66 ± 1.00*10^{-3}             |

*Our
results showed that there is a positive linear correlation between phenolic content of extracts and their antioxidant activities and these results agree with several studies that reported that there is a strong positive linear relationship between high content of phenolic compounds and their biological activities of plants.\[9,31,39\]

**Antibacterial activity**

The phenomenon of bacterial resistance to antibiotics leads to the emergence and spread out of diseases for which no treatment yet exists. So, the search for novel antibacterial agents from medicinal plants has become a highly relevant and important subject for the research.\[40\] In this study, the effectiveness of the three extracts (Aq. MeOH, butanol, and water) of *Cleome arabica* L. plant from Jordan against four bacterial strains was determined using microdilution method. The results showed that all the tested extracts had antibacterial activity against gram-positive bacteria (*Staphylococcus aureus*) at 25 and 50 mg/mL compared with the positive control (+ve) (Tables 5, Tables S1 and S2). On the other hand, butanol and water extracts showed antibacterial activity at 50 mg/mL against gram-negative bacteria (*Escherichia coli*) (Tables 5, Tables S3 and S4). The lower sensitivity of gram-negative than gram-positive bacteria toward the *Cleome arabica* L. extracts may be due to the cell wall impermeable to foreign substances (antibacterial agents) which is composed of structural lipopolysaccharides. Moreover, gram-negative bacteria exhibit multiple efflux pumps preventing the intracellular accumulation of antibacterial agents.\[40\] Thus, the antibacterial activity could be associated with levels of tannins present. Tannins present
Figure 3. Linear Pearson correlation for (a) butanol, (b) methanol, and (c) water extracts between total phenolic content and antioxidant activity. The right upper part represents Pearson’s r coefficient for each pair, and the red stars represent the strength of significant results. The left bottom shows the bivariate scatter plots. Each antioxidant histogram distribution is shown in the middle diagonal line.
Table 5. Antibacterial activities of three different extracts of Cleome arabica L. against both gram-positive and gram-negative bacteria.

| Extract      | Conc (mg/ml) | Staphylococcus aureus (ATCC 29213)control | Staphylococcus aureus (BAA-41)resistant | Escherichia coli (ATCC 25922)control | Escherichia coli (BAA-2452)resistant |
|--------------|--------------|------------------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|
| Aq. Methanol | 50           | -                                        | +                                     | -                                    | +                                    |
|              | 25           | -                                        | -                                     | +                                    | +                                    |
|              | 12.5         | +                                       | -                                     | +                                    | +                                    |
|              | +ve          | +                                       | +                                     | +                                    | +                                    |
|              | -ve          | -                                       | -                                     | -                                    | -                                    |
| Butanol      | 50           | -                                        | -                                     | -                                    | -                                    |
|              | 25           | -                                        | -                                     | +                                    | +                                    |
|              | 12.5         | +                                       | -                                     | +                                    | +                                    |
|              | +ve          | +                                       | +                                     | +                                    | +                                    |
|              | -ve          | -                                       | -                                     | -                                    | -                                    |
| Water        | 50           | -                                        | -                                     | -                                    | -                                    |
|              | 25           | -                                        | -                                     | +                                    | +                                    |
|              | 12.5         | +                                       | +                                     | +                                    | +                                    |
|              | +ve          | +                                       | +                                     | +                                    | +                                    |
|              | -ve          | -                                       | -                                     | -                                    | -                                    |

(-) No bacterial growth.
(+ ) Bacterial growth.

in Cleome arabica L. extracts may react with the bacterial proteins forming a stable water-soluble compound resulting in killing the bacterial cells through a direct damage that occurs to their cell membranes. In general, the antibacterial activity of the Cleome arabica L. extracts is based on their contents of phenols, flavonoids, tannins, and glycosides.\(^{40,41}\)

Conclusion

In this study, the analysis of chemical composition of essential oil isolated from Jordanian origin Cleome arabica L. plant lead to identify 46 compounds dominated by esters and sulfur-nitrogen containing compounds. The major ones were 2-Methyl butyl isothiocyanate, Ethyl dodecanoate, Isopropyl isothiocyanate and 2-Methyl-(3E)-octen-5-yne. Phytochemical screening of different extracts from aerial parts of Cleome arabica L. revealed their enrichment with tannins, flavonoids, saponins, and glycosides. All tested extracts showed the presence of phenolic and flavonoid compounds but with different quantities. In addition, all extracts showed radical scavenging potential and ferrous chelating effect with little differences in the scavenging percentage among them. Also, most of the tested extracts showed antibacterial activities for both gram-negative and gram-positive bacterial strains. To the best of our knowledge, this is the first study analyzing the essential oil and identified the phytochemical constituents and antioxidant and antibacterial activities of Cleome arabica L. grown in Jordan. However, more research has to be conducted to isolate the bioactive compounds of essential oil and extracts to determine their potential for therapeutic uses against different diseases and to synthesize natural antioxidants and antibacterial agents.

Abbreviations

ABTS, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Aq. MeOH, aqueous methanol; BHA, butylated hydroxyanisole; DMSO, dimethylsulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylene diamine tetra acetic acid; FIC, ferrous ion chelating activity; GA, gallic acid; GC-MS, gas chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MBC, minimum bactericidal activity; MHA, Mueller Hinton Agar; MHB, Mueller Hinton Broth; MIC, minimum inhibitory concentration; PBS, phosphate buffered saline; TFC, total flavonoids content; TPC, total phenolic content.
Acknowledgments

The authors thank Prof. Jamil Lahham from the Biological Sciences Department, Yarmouk University for identifying the plant used in this study.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was funded by the Deanship of Scientific Research at Jordan University of Science and Technology (grant number 20200253) and the Deanship of Scientific Research and Graduate Studies at Yarmouk University for the grant number (15/2018) awarded to M. A. Al-Qudah from the department of chemistry.

ORCID

Mahmoud A. Al-Qudah [http://orcid.org/0000-0001-7179-8249]

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection was performed by RA, AB and MA. Data analysis was performed by NA and MA. Interpretation of data was conducted by all authors. The first draft of the manuscript was written by RA, AB, and MA, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

[1] Agbor, G. A.; Moumbegna, P.; Oluwasola, E. O.; Nwosu, L. U.; Njoku, C. C.; Kanu, S.; Abudei, F. A. Antioxidant Capacity of Some Plant Foods and Beverages Consumed in the Eastern Region of Nigeria. J. AJTCAM. 2011, 8, 362-369. DOI: 10.4314/ajtcam.v8i4.4.
[2] Rice-Evans, C. A.; Miller, N. J.; Bobwell, P. G.; Bramley, P. M.; Pridham, J. B. The Relative Antioxidant Activities of Plant Derived Polyphenolic Flavonoids. J. Free Radical Res. 1995, 22, 375–383. DOI: 10.3109/10715769509145649.
[3] Cowan, M. M. Plant Products as Antimicrobial Agents. J. Clin Microbiol. Rev. 1999, 12, 564–582. DOI: 10.1128/CMR.12.4.564.
[4] Zohary, M.; Feinbrun-Dothan, N. Flora Palaestina. Jerusalem, Israel Academy of Sciences and Humanities, 1966.
[5] Abdullah, W.; Elsayed, W. M.; Abdelshafeek, K. A.; Nazif, N. M.; Singab, A. Chemical Constituents and Biological Activities of Cleome Genus: A Brief Review. J. IFPRR. 2016, 8, 777–787.
[6] Sungwarl, S.; Supanee, P. Biological Activity of Cleome Spp. Extracts against the Rice Weevil, Sitophilus Oryza L. J. Agricultural Sci. 2006, 37, 232–235.
[7] Jane, R.; Patil, S. Cleome Viscosa: An Effective Medicinal Herb for Otitis Media. J. IJSN. 2012, 3, 153–158.
[8] Aboushoer, M. I.; Fathy, H. M.; Abdel-Kader, M. S.; Goetz, G.; Omar, A. A. Terpenes, and Flavonoids from an Egyptian Collection of Cleome Droserifolia. J. Natural Product Research. 2010, 24, 687–696. DOI: 10.1080/14786410903292433.
[9] Aicha, M.; Nadia, Z.; Sihem, H.; Abdelmalik, B. Antioxidant Activity and Phenolic Compounds Contents of Spider Flower (Cleome Arabica Spp. Arabica), A Well Acclimated Species in the Algerian Desert Areas. J. ESJ. 2017, 13, 102–118. DOI: 10.19044/esj.2017.v13n12p102.
[10] Aparadh, V.; Karadge, B. Fatty Acid Composition of Seed Oil from Some Cleome Species. J. Phcog. 2010, 2, 324–327.
[11] Al-Humaidi, Y. J.; Al-Qudah, M. A.; Al-Saleema, S. M.; Alotaiba, M. S. Antioxidant Activity and Chemical Composition of Essential Oils of Selected Cleome Species Growing in Saudi Arabia. J. JIC. 2019, 14, 29–37.
[12] Muhaidat, R.; Al-Qudah, M. A.; Samir, O.; Jacob, J. H.; Hussein, E.; Al-Tarawneh, I. N.; Bsol, E.; Orabi, S. T. A. Phytochemical Investigation and in Vitro Antibacterial Activity of Essential Oils from Cleome Droserifolia (Forsk.) Delile and Cleome Trinervia Fresen. (Cleomaceae). J. Bot J. Afr. S. 2015, 99, 21–28. DOI: 10.1016/j.j sajb.2015.03.184.
[13] Bouriché, H.; Arnhold, J. Effect of Cleome Arabica Leaf Extract Treated by Naringinase on Neutrophil Degranulation. J. Acta Horticulturae. 2010, 854, 15–22. DOI: 10.17660/ActaHortic.2010.854.1.
[14] Bouriche, H.; Selloum, L.; Tigrine, C.; Boudoukh, C. Effect of Cleome Arabica Leaf Extract on Rat Paw Edema and Human Neutrophil Migration. *J. Pharmaceutical Biology*. 2003, 41, 10–15. DOI: 10.1076/phbi.41.1.10.14698.

[15] Khlifi, A.; L.; P.; Lobo, J. C.; Melo, D.; Ben Ayache, S.; Flamini, G.; Mbbp, O.; Oleszek, W.; Achour, L. Leaves of *Cleome Amblyocarpa* Barr. And Murb. And *Cleome Arabica* L.: Assessment of Nutritional Composition and Chemical Profile (LC-ESI-MS/MS), anti-inflammatory and Analgesic Effects of Their Extracts. *J. Ethnopharmacol.* 2021, 6(269), 113739. Epub 2021 Jan 12. PMID: 33359854. DOI: 10.1016/j.jep.2020.113739.

[16] Khlifi, A.; Chrlia, A.; Ben, L.; Ben, J.; Thou, A.; Adouni, K.; Flamini, G.; Achour, L. Gas chromatography-mass spectrometry (GM-MS) Analysis and Biological Activities of the Aerial Part of *Cleome Amblyocarpa*. *J. Environmental Science and Pollution Research*. 2020, 27, 22670–22679. DOI: 10.1007/s11356-020-08764-7.

[17] Tigrine, C.; Bulzomi, P.; Leone, S.; Bouriche, H.; Kameli, A.; Marino, M. *Cleome Arabica* Leaf Extract Has Anticancer Properties in Human Cancer Cells. *J. Pharm. Biol.* 2013, 12, 1508–1514. DOI: 10.3189/138802013.2013.796563.

[18] Samout, N.; Bouzenna, H.; Ettaya, A.; Elfeki, A.; Najla, H. Antihypercholesterolemic Effect of *Cleome Arabica* L. on High Cholesterol Diet Induced Damage in Rats. *J. EXCELL*. 2015, 14, 791–800.

[19] Ladhari, A.; Laarif, A.; Omezzine, F.; Haouala, R. Effect of the Extracts of the Spiderflower, *Cleome Arabica*, on Feeding and Survival of Larvae of the Cotton Leafworm, *Spodoptera Littoralis*. *Insect Sci. J.* 2013a, 13, 1–14. DOI: 10.1673/031.013.6101.

[20] Ladhari, A.; Omezzine, F.; DellaGreca, M.; Zarrelli, A.; Zuppolini, S.; Haouala, R. Phytotoxic Activity of *Cleome Arabica* L. and Its Principal Discovered Active Compounds. *J. Bot. Afr. S.* 2013b, 88, 341–351. DOI: 10.1016/j.sajb.2013.08.016.

[21] Jente, R.; Jakupovic, J.; Olatunji, G. A. A. Centromedoid Diterpene from *Cleome Viscosa*. *J. Phytochemistry*. 1999, 29, 666–667. DOI: 10.1016/S0031-9422(99)85142-3.

[22] Tsichritzis, F.; Abdel-Mogip, M.; Jakupovic, J. Dammarane Triterpenes from *Cleome Africana*. *J. Phytochemistry*. 1993, 33, 423–425. DOI: 10.1016/S0031-9422(93)85532-V.

[23] Djerdane, A.; Yousfi, M.; Brunel, J. M.; Stocker, P. Isolation and Characterization of a New Steroid Derivative as a Powerful Antioxidant from *Cleome Arabica* in Screening the in vitro Antioxidant Capacity of 18 Algerian Medicinal Plants. *J. Food Chem Toxicol.* 2010, 48, 2599–2606. DOI: 10.1016/j.jfct.2010.06.028.

[24] Takhi, D.; Ouinter, M.; Yousfi, M. Study of Antimicrobial Activity of Secondary Metabolites Extracted from Spontaneous Plants from the Area of Laghouat, Algeria. *J. Advances in Environmental Biology*. 2011, 5, 469–476.

[25] Al-Qudah, M. A.; Al-jaber, H. I.; Abu, M. H.; Abu, S. T. Flavonoid and Phenolic Compounds from Salvia Palaestina L. Growing Wild in Jordan and Their Antioxidant Activities. *J. Phytochemistry*. 2014, 99, 115–120. DOI: 10.1016/j.jphytochem.2014.01.001.

[26] Abu-Orabi, S.; Al-Qudah, M.; Saleh, N.; Bataineh, T.; Obeidat, S. M.; Al-Sheraideh, M. S.; Al-Jaber, H. I.; Tashtoush, H. I.; Lahham, N. J. Antioxidant Activity of Crude Extracts and Essential Oils from Flower Buds and Leaves of *Cistus Creticus* and *Cistus Salviifolius*. *J. Arabian Journal of Chemistry*. 2020, 13, 6256–6266. DOI: 10.1016/j.arabjc.2020.05.043.

[27] Al-Qudah, M. A.; Saleh, A. M.; Alhawassi, N. L.; Al-jaber, H. I.; Rizvi, A.; Afifi, F. U. Composition, Antioxidant and Anticancer Activities of the Essential Oil from Fresh and Air-Dried Aerial Parts of Pallenis Spinosa. *J. Chem Biodivers*. 2017, 14, 8. DOI: 10.1002/cbdv.201700146.

[28] Siddiqui, A.; Ali, M. Pratcal Pharmaceutical Chemistry. 1st Edition. *CBS Publish and Distributors*. New Delhi, 1997, 126–131.

[29] Al-Qudah, M. A.; Saleh, A.; Orabi, S. T. A.; El-Qlah, A. A.; Al-Maseed, E.; Al-Jaber, H. I.; Abu Orabi, S. T. Volatile Components Analysis, Total Phenolic, Flavonoid Contents, and Antioxidant Activity of Phlomis Species Collected from Jordan. *J. Journal of Essential Oil Bearing Plants*. 2018, 21, 583–599. DOI: 10.1080/0972060X.2018.1489739.

[30] Kuete, V.; Nana, F.; Ngameni, B.; Tsfack, A.; Keumedjio, F.; Tchaleu, B. Antimicrobial Activity of the Crude Extract, Fractions and Compounds from Stem Bark of Ficus Ovata (Moraceae). *J. 2009*, 124, 556–561.

[31] McNell, M. J.; Porter, R. B. R.; Rainford, L.; Dunbar, O.; Francis, S.; Laurieri, N.; Delgoda, R. Chemical Composition and Biological Activities of the Essential Oil from *Cleome Rutidosperma* DC. *J. Fitoterapia*. 2018, 129, 191–197. DOI: 10.1016/j.jfitote.2017.08.006.

[32] Medini, F.; Fellah, H.; Ksouri, R.; Abdelly, C. Total Phenolic, Flavanoid and Tannin Contents and Antioxidant and Antimicrobial Activities of Organic Extracts of Shoots of the Plant *Limonium Delicateum*. *J. Integrative Medicine Research*. 2014, 8, 216–224. DOI: 10.1016/j.jtmsci.2014.01.003.

[33] Al-Muniri, R. M. S.; Hossain, M. A. Evaluation of Antioxidant and Cytotoxic Activities of Different Extracts of Folk Medicinal Plant *Haplophyllum Tuberculatum*. *J. Egyptian Journal of Basic and Applied Sciences*. 2017, 4, 101–106. DOI: 10.1016/j.ejbas.2017.04.003.

[34] Galvez, G.; Martin-Cordero, P.; Houghton, A.; Ayuso, M. J. Antioxidant Activity of Methanol Extracts Obtained from *Plantago Species*. *J. Journal of Agriculture and Food Chemistry*. 2005, 6, 1927–1933. DOI: 10.1021/jf048076s.

[35] Ismail, S. I.; Ito, H.; Selloum, L.; Bouriche, H.; Yoshida, T. Constituents of *Cleome Arabica* Leaves and Tigs. *J. Environmental Science*. 2005, 59, 53.

[36] Cascon, S. C.; Brown, K. S. Biogenetically Significant Triterpenes in A Species of Meliaceae: Carbalea Polytrichya *A. Juss. J. Tetrahedron*. 1972, 28, 315–323. DOI: 10.1016/0040-4020(72)80138-8.
[37] Dhawan, D.; Gupta, J. Comparison of Different Solvents for Phytochemical Extraction Potential from *Datura Metel* Plant Leaves. *J. International Journal of Biological Chemistry*. 2016, 11, 17–22. DOI: 10.3923/IJBC.2017.17.22.

[38] Quettier-Deleu, C.; Gressier, B.; Vasseur, J.; Dine, T.; Brunet, C.; Luyckx, M.; Cazin, M.; Cazin, J. C.; Bailleul, F.; Trotin, F. Phenolic Compounds and Antioxidant Activities of Buckwheat (*Fagopyrum Esculentum Moench*) Hulls and Flour. *J. Ethnopharmacol.* 2000, 72, 35–42. DOI: 10.1016/s0378-8741(00)00196-3.

[39] Tawaha, K.; Alali, F. Q.; Gharaibeh, M.; Mohammad, M.; Elelimat, T. Antioxidant Activity and Total Phenolic Content of Selected Jordanian Plant Species. *J. Food Chem.* 2007, 104, 1372–1378. DOI: 10.1016/j.foodchem.2007.01.064.

[40] Toda, M.; Okubo, S.; Hiyoshi, R.; Shimamura, T. The Antibacterial Activity of Tea and Coffee: Their Extracts and Preparations. *J. International Journal of Food Properties*. 1989, 8, 123–1989. DOI: 10.1080/10942910701675928.

[41] Nascimento, G. G. F.; Locatelli, J.; Freitas, P. C.; Silva, G. L.; Piracicaba, U. M. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-resistant Bacteria. *J. Microbiol.* 2000, 31, 247–256. DOI: 10.1590/S1517-8382200000400003.