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Chemical Composition, Antimicrobial, and Antioxidant Potential of the Essential Oil from Aerial Parts of *Cleome rostrata* Bobrov, a Novel Study

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This study aimed to identify the chemical compounds present in the essential oil of *Cleome rostrata* for the first time. *C. rostrata* belongs to the *Cleome* genus of the Cleomaceae family. The study was conducted to investigate the phytochemical composition, antimicrobial activity, and antioxidant potential of the aerial parts of *C. rostrata*. GC-MS analysis identified a total of 39 compounds. The major components of *C. rostrata* aerial parts were found to be farnesan (10.49%), 4-methyl-dodecan-1-ol (14.07%), 2,7-dimethylnaphthalene (4.81%), phytane (6.05%), and tetradecane, 4-methyl- (4.81%). The methanol extract of the plant was evaluated for its total flavonoid content using the aluminum chloride colorimetric method. The methanol extract of *C. rostrata* exhibited high total flavonoid content, measuring 18.04 mg/g extract. The methanolic extract also exhibited significant antimicrobial activity against three fungal strains and three Gram-positive bacterial strains. Notably, the highest activity was observed against *Aspergillus niger* at a concentration of 16 mg/mL. Moreover, the extract demonstrated potent antioxidant activity, as evidenced by its IC_{50} value of 202.48 µg/L in the DPPH test. These findings highlight the potential of *C. rostrata* as a source of natural antioxidants and antimicrobial agents with potential applications in food preservation and pharmaceuticals.

Keywords**:** antimicrobial activity, antioxidant activity, chemical composition, *Cleome rostrata*, DPPH assay, essential oil, flavonoids

INTRODUCTION

Essential oils (EOs) are a mixture of volatile oily compounds produced by medicinal plants as secondary metabolites. According to the International Organization for Standardization, EOs are extracted from plant sources or fruits using steam or water distillation methods (Pan *et al*. 2019). The chemical compositions of EOs vary based on factors such as the plant, environment, and extraction method. Due to their antimicrobial, antioxidant, anti-inflammatory, and anti-cancer properties, EOs can be used as an alternative in the food and pharmaceutical industries (Alonso-Gato *et al*. 2021) EOs have become increasingly popular due to the rise in natural substance usage in advertisements – especially in food, cosmetics, and pharmaceuticals (Elgamal *et al.* 2021). EOs are the primary compounds found in aromatic plants (Elgamal

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et al. 2021). EOs have been a significant medicinal and biological source since ancient times (Al-Rowaily *et al*. 2020). EOs have been shown to have many biological potentials such as hepatoprotective, anticancer, anti-aging, antioxidant, antipyretic, anti-inflammatory, and antimicrobial properties (Edziri *et al.* 2013; Assaeed *et al*. 2020; Elshamy *et al.* 2021) The use of EOs and extracts of medicinal plants has been a significant part of traditional medicine for therapeutic purposes for thousands of years. Recently, the use of compounds that are generally considered harmless and known as generally recognized as safe (GRAS) has gained a lot of attention. Biologically active natural compounds derived from plants are among the most important GRAS compounds because they contain substances obtained from EOs and plant extracts. They can be used to preserve and protect food and pharmaceuticals and as a new therapeutic agent against microbial diseases and infections (Jobling 2000; Benli *et al.* 2007). However, currently, only about 25–50% of the common medicines of herbal origin are used worldwide. The number of pathogenic bacteria resistant to common antibiotics is increasing day by day. Therefore, discovering new therapeutic agents is required to control and eliminate resistant microbial infections, especially hospital infections (Duarte *et al.* 2007).

C. rostrata, originally described from Pamir-Alaj in Tajikistan, has expanded its distribution to Afghanistan and now to Iran (Flora Iranica). The plant shares similar characteristics with Iranian species (Hedge and Lamond 1970). In 1998, *C. rostrata* was first discovered in the east of Torbat Jam in Khorasan province, adding a new species to the flora of Iran (Saghafi 2000). *Cleome* plants, belonging to the Cleomaceae family, have been traditionally used as vegetables due to their sharp mustard-like taste in leaves and branches. *Cleome* seed oil is highly nutritious – containing proteins, fatty acids, dietary amino acids, and lipids. Additionally, *Cleome* species are rich in fiber and low in fat. The genus *Cleome* has been recognized as a valuable food and nutritional resource due to its significant role (Moyo *et al*. 2018). Moreover, *Cleome* species have medicinal properties, particularly in the treatment of fungal and bacterial infections. *Cleome gynandra*, a species of the *Cleome* genus, is employed as an anthelmintic agent in Ayurveda (Chand *et al*. 2022).

The aim of this study was to investigate the antioxidant and antimicrobial properties of *C. rostrata*, a plant species traditionally used for medicinal purposes. In comparison with previous studies on *Cleome brachycarpa* Vahl ex DC. and *Cleome quinquenervia* DC, which focused on the antimicrobial properties of their EOs, this study specifically evaluated the antioxidant and antimicrobial properties of *C. rostrata* using various test methods (Joukar *et al*. 2022). The results of the study revealed that the plant extract exhibited significant antioxidant activity, as indicated by its high levels of total flavonoids and effective DPPH (2-phenyl-1-picrylhydrazyl) scavenging activity. Moreover, the extract demonstrated potent antimicrobial activity against a variety of pathogenic bacteria and fungi, indicating its potential as a natural antimicrobial agent. This study contributes to the existing body of literature by highlighting the antioxidant and antimicrobial properties of *C. rostrata*, expanding our understanding of the biological activities of this plant species. Additionally, it emphasizes the potential utility of *C. rostrata* as a source of natural antioxidants and antimicrobial agents, which could have implications for the development of novel therapeutic interventions or functional food ingredients.

MATERIALS AND METHODS

Chemicals

MHA (Mueller-Hinton agar), SDA (Sabouraud dextrose agar), and Mueller-Hinton broth were obtained from Merck (Germany). Absolute methanol (≥ 99.9% purity) was obtained from Merck (Germany) and $AlCl_3$ from Fluka Chemie AG (Buchs, Switzerland). DPPH was obtained from Sigma Chemicals Co. *Klebsiella pneumonia* (PTCC 1053), *Escherichia albertii* (PTCC 1399), *Escherichia coli* (PTCC1399), *Corynebacterium glutamicum* (PTCC1532), *Staphylococcus aureus* (PTCC1431; Gram-positive), *Staphylococcus epidermidis* (PTCC1435; Gram-positive), *Fusarium solani* (PTCC5284), *Alternaria alternate* (PTCC5224), and *Aspergillus niger* (PTCC5154) were obtained from the Collection Center of Industrial Microorganisms Scientific and Industrial Research Organization (Esfahan, Iran). Ultra-pure water was obtained from a laboratory ultra-pure water production machine (ZU101) from Nanotechnology Researchers Company (Tehran, Iran). EO composition was analyzed using appropriate methods.

Plant Material

The whole plant organ of wild *C. rostrata* Bobrov was collected at the full growth stage from the North Khorasan province of Iran in June 2019. The taxonomic identification of the plant material was confirmed by Mahmood Zakaee, a plant taxonomist at the Department of Basic Science College of Ferdowsi University (Mashhad, Iran). The voucher specimen 35042 has been deposited in the herbarium center at Ferdowsi University (Mashhad, Iran). The fresh plants were washed, dried, and subsequently kept in a dry shade. The collected plant materials were stored in optimal and suitable conditions at a temperature of 25 °C for 3 d before being ground. To extract the EO from the plant, we used the distillation

method with water and the Clevenger EO extraction device. This research is a continuation of our previous study on *Cleome brachycarpa* and *Cleome quinquenervia* in 2022 (Joukar *et al.* 2022).

Essential Oils (EOs)

The extraction of EO from *C. rostrata* and the evaluation of its antimicrobial properties were performed following the protocol described in our previous study (Joukar *et al.* 2022). Briefly, 100 g of ground powder was subjected to the Clevenger balloon apparatus with a capacity of 3 L for 2 h. The EO yield obtained from the hydrodistillation method was determined to be 1.5 g per g of dry weight of the plant (Equation 1). The dehydrated EO was collected in a closed, dark container using anhydrous sodium sulfate (Na_2SO_4) and stored at 4 °C until further analysis. Subsequently, the EO was stored at 4 °C in a refrigerator for further analysis. The identification of oil constituents was performed by comparing their GC-MS (gas chromatography–mass spectrometry) spectra with authentic standards and utilizing the NIST database (2008). Additionally, the retention index (RI) was determined through the co-injection of an alkane standard solution (Souza *et al.* 2014).

$$\text{Yield } \% = \frac{\text{Weight of essential oil}}{\text{Weight of raw material}} \text{ x } 100 \qquad (1)$$

Essential Oil (EO) Composition

The determination of the EO was conducted using a Thermoquest-Finnigan Trace GC-MS instrument (Mass Lab Group; Manchester, UK) equipped with a DB-5 capillary column (30 m × 0.25 mm id, 0.25 mm film thickness) and an FID. Helium was used as the carrier gas with a constant flow rate of 1.1 mL/min. The oven temperature was increased from 100 to 238 °C at a rate of 4 °C/min, followed by holding it at 238 °C for 20 min, with a transfer line temperature of 200 °C. The detector and injector temperatures were set at 250 °C. The quadruple mass spectrometer, with an ionization voltage of 70 eV and an ionization current of 150 µA, analyzed compounds in the 45–465 amu range. The identification of FA components was achieved using a library search (NIST 2008 and Wiley 7.0) and comparing it with literature data. The quantification was carried out using GC-FID data, and the results were reported as weight percentages.

Methanolic Extraction

To extract *C. ros*trata, a methanolic extraction method was utilized. The aerial plant materials were dried at room temperature and then finely powdered using a laboratory mill, considering a medium level of grinding to ensure that the plant's compounds are not lost while allowing for proper penetration into all parts of the plant and subsequently mixed with methanol solvent in a closed Erlenmeyer flask. The ratio used was 1:5, with 1 g of *C. rostrata* material being mixed with 5 g of methanol solvent. The mixture was stirred using a magnetic stirrer (VELP Scientifica, Type ARE) at room temperature for 24 h and then extracted using the organic solvent of methanol. After the extraction process, the extracts were filtered using filter paper, and a rotary evaporator under vacuum (BUCHI: vacuum system B-169 and water bath B-480) was employed to remove the solvent from the extracts until the final stage. Subsequently, the resulting extracts were transferred to plates for further analysis.

Determination of Flavonoid Concentrations in the Plant Extracts

The concentration of flavonoids in plant extracts was determined using a method previously described by various researchers (Quettier-Deleu *et al*. 2000; Stanković 2011; Elshamy *et al*. 2020). In brief, the plant extract was dissolved in methanol at a concentration of 1 mg/mL and incubated with 2% $AlCl_3$ for 1 h at room temperature. The absorbance was measured at λ_{max} = 415 nm using a spectrophotometer, and the concentration of flavonoids was calculated using a standard curve of quercetin (0.215–25 mg/mL; y = 0.0237x – 0.0867). The experiment was performed in triplicate, and the results were reported as mg quercetin equivalents per mg of extract.

DPPH Radical Scavenging Assay of Methanolic Extract

To evaluate the scavenging ability of the methanolic extract, we employed the DPPH radical scavenging assay, following the protocol of Ballester-Costa *et al.* (2013). In brief, we added 30 µL of the extract to 270 µL of a DPPH solution (6×10^{-5} M) and monitored the reaction for 40 min at 525 nm using a spectrophotometer (BioTek, Synergy HT, PMT 49984, US). We selected concentrations of the extract based on preliminary experiments to construct a dose-response curve (12.5–400 µg/mL), and we used Trolox to create the calibration curve (5–175 mg/L; R^2 = 0.9997). The experiment was conducted in triplicate, and the results were expressed as mg Trolox equivalent (TE) per g of sample. Analyzes were performed in triplicate.

$$100 - \frac{Extract\ absorbance - Blank\ absorbance}{Control\ absorbance} \text{ x } 100 \qquad (2)$$

Antimicrobial and Antifungal Activity Minimum Inhibitory Concentration (MIC) Assays

The minimum inhibitory concentration (MIC) assay is used to determine the lowest concentration of an

$$100 - \frac{Extarct\ absorbance - Blank\ absorbance}{Control\ absorbance} \times 100 \qquad (2)$$

antibiotic or sample needed to prevent the visible growth of microorganisms. In this study, we collected three fungal strains (*Aspergillus niger*, *Alternaria alternata*, and *Fusarium solani*) plus six bacterial strains (*Klebsiella pneumoniae*, *Escherichia albertii*, *Escherichia coli*, *Corynebacterium glutamicum*, *Salmonella typhi*, and *Staphylococcus epidermidis*) from the Center of Industrial Microorganisms Scientific and Industrial Research Organization in Esfahan, Iran. The standards for antimicrobial evaluations on Gram-positive and negative bacteria and fungi were ampicillin, gentamicin, and fluconazole, respectively. The bacterial strains were cultured on Mueller-Hinton broth medium for 24 h at 37 °C, whereas the fungal pathogens were cultured on Sabouraud liquid broth medium at 25 °C for 48 h – 3 d before the experiments. All microorganisms were stored at –20 °C. To conduct the MIC assay, we used the microdilution method in a sterile 96-well plate. First, we added 95 µL of Mueller Hinton Broth and 5 µL of the desired microorganism to the first to eighth rows of the pellets. Then, we added 100 µL of the extract (the amount of dry matter of the extract was determined beforehand) to the first well of each row, followed by 95 µL of other successive dilutions of the extract to the other wells up to the eighth well. The ninth well (as the blank control) and the tenth well contained 95 µL of culture medium without extract or essence and 5 µL of microorganisms. The eleventh well was the antibiotic control according to the microorganism under investigation. The plate was incubated at 37 °C for 24 h, and the presence of turbidity compared to the control row indicated bacterial growth, whereas transparency indicated the absence of bacterial growth. The lowest concentration of the extract or EO that prevented turbidity was determined and reported as the MIC. If the bacterium did not grow at the MIC concentration on Petri dishes without extract or EO, then the extract or EO at the MIC concentration had a bactericidal effect. The wells in the pellet were compared, and the lowest concentration of the extract or EO that prevented turbidity was determined and reported as the MIC.

RESULTS AND DISCUSSIONS

In this study, we identified and reported for the first time the chemical EO compounds of *C. rostrata* using GC-MS (Table 1). The extraction efficiency of the EO was 1.5%, and we identified 39 different compounds representing 99.63% of the total EO. The main chemical compounds identified were hexane, 3-ethyl-4-methyl- (10.54%), farnesan (6.86%), phytane (6.05%), 2,7-dimethylnaphthalene

Table 1. Volatile components identified in *C. rostrata* essential oil.

No.	Compound	Cn	Cn+1	Tx	RI*	%	Formol
1	Propylcyclopentane	….	….	3.186	…..	2.19	C_8H_{16}
2	Ethylcyclohexane	….	….	3.221	…..	2.63	C_8H_{16}
3	Heptane, 2,3-dimethyl-	….	….	3.454	…..	0.82	C_9H_{20}
4	4-Methyl-dodecan-1-ol	….	….	3.501	…..	14.07	$C_{13}H_{28}O$
5	m-Xylene	….	….	3.687	…..	4.06	C_8H_{10}
6	Cumene	9	10	4.585	925	0.74	C_9H_{12}
7	2,6-dimethyl-octane	9	10	4.678	930	3.25	$C_{10}H_{22}$
8	5-Methylnonane	9	10	5.179	956	3.16	$C_{10}H_{22}$
9	Nonane, 2-methyl	9	10	5.267	961	5.46	$C_{10}H_{22}$
10	Cyclohexane, ethyl	9	10	5.756	986	2.62	C_8H_{16}
11	Heneicosane	11	12	9.831	1154	1.58	$C_{21}H_{44}$
12	Albocarbon	11	12	10.518	1181	0.74	$C_{10}H_8$
13	Azulene	11	12	10.641	1186	0.72	$C_{10}H_8$
14	1-hexyl-3-methyl- cyclopentane	11	12	10.798	1192	1.24	$C_{12}H_{24}$
15	10-Methylnonadecane	12	13	13.631	1299	0.88	$C_{20}H_{42}$
16	5-Methyldodecane	13	14	15.018	1354	0.66	$C_{13}H_{28}$
17	4-Methyl-tridecane	13	14	15.140	1358	1.1	$C_{14}H_{30}$
18	10-Methylnonadecane	13	14	15.263	1363	2.64	$C_{20}H_{42}$
19	Phytane	13	14	15.607	1377	6.05	$C_{20}H_{42}$

Table 1. Cont.

20	Cyclohexanemethanol	13	14	15.834	1385	0.64	$C_7H_{14}O$
21	1,1,3-trimethyl-2-(3-methylpentyl)- Cyclohexane	13	14	15.869	1387	0.86	$C_{15}H_{30}$
22	Cyclotetradecane	13	14	15.991	1392	1.56	$C_{14}H_{28}$
23	Decanedioic acid, didecyl ester	13	14	16.429	1309	1.12	$C_{14}H_{26}O_4$
24	1,5-dimethyl- naphthalene	13	14	16.091	1395	1.44	$C_{12}H_{12}$
25	1,3-Dimethylnaphthalene	13	14	16.726	1321	3.7	$C_{12}H_{12}$
26	2,7-Dimethylnaphthalene	13	14	16.807	1324	4.81	$C_{12}H_{12}$
27	1,2-Dimethylnaphthalene	13	14	17.204	1340	1.58	$C_{12}H_{12}$
28	Limonene epoxide	13	14	17.408	1349	3.16	$C_{10}H_{16}O$
29	5-Methyl-pentadecane	13	14	17.507	1353	1.27	$C_{16}H_{34}$
30	1,3-Dimethylnaphthalene	13	14	17.588	1356	0.84	$C_{12}H_{12}$
31	Tetradecane, 4-methyl-	13	14	17.635	1358	4.81	$C_{15}H_{32}$
32	Farnesan	13	14	17.734	1362	10.49	$C_{15}H_{32}$
33	3-(2-Methyl-propenyl)-1H-indene	14	15	18.801	1406	0.9	$C_{13}H_{14}$
34	3-Buten-2-one,-4-(3-methoxyphenyl)	14	15	19.215	1423	1.46	$C_{11}H_{12}O_2$
35	Azulene, 4,6,8-trimethyl-	14	15	19.314	1428	3.14	$C_{13}H_{14}$
36	5-Isopropyldihydro-3(2H)-furanone	14	15	19.891	1452	0.88	$C_7H_{12}O_2$
37	10-Methylnonadecane	16	17	25.358	1699	0.97	$C_{20}H_{42}$
38	Butyl phthalate	18	19	28.663	1865	0.76	$C_{16}H_{22}O_4$
39	δ-Guaijene	19	20	31.775	2031	0.63	$C_{15}H_{24}$
	Total identified					**99.63%**	

*Retention indices using a 5MS-HP column

(4.81%), and tetradecane, 4-methyl- (4.81%) (Table 1). Our previous research in 2022 identified 48 compounds in the EO of *Cleome quinquenervia* and 19 compounds in the EO of *Cleome brachycarpa*. The main compounds in *Cleome quinquenervia* EO were p-caryophyllene-(I3) (29%), dibutyl phthalate (13%), and β_element (11%). For *Cleome brachycarpa*, the main components were entsandaracopimaradien-3β-ol (68.02%), cembrene (6.98%), and juniper camphor (4.2%). In 2018, the *Cleome* species were subjected to phytochemical screening, which detected the presence of several significant secondary metabolites such as flavonoids, saponins, coumarins, terpenoids, phenolics, and alkaloids. Our observations indicated the presence of hydrocarbon compounds in *C. rostrata* (Jane and Patil 2012; Aicha *et al*. 2017). The quality and quantity of these compounds can vary depending on environmental factors such as geographical location and plant origin, which can also affect their activities and lead to different results reported in research. Since a significant content of petroleum-based compounds was found in *C. rostrata* as a new species, there is a high concern about contamination in the Khorasan province. Thus, further analysis should be considered to prove the chemical compounds in the harvesting medium or confirm the contaminations. However, the significant content of organic compounds (petroleum-based) compounds might address the new source of natural-based fuel source (plant-based fuel) and/or undiscovered sources of the oil field that contaminated the plant species of Khorasan province since there is no petroleum refinery industry and/or oil fields found in that area until 2023.

In general, hydrocarbons were observed as the major compounds in the genus *Cleome*. In *Cleome amblyocarpa*, hexahydrofarnesyl acetone (7.92%) was reported as the main compound (Abd-ElGawad *et al.* 2021). In *Cleome quinquenervia* `total 5-methyl-1,2,4-triazole-3-thiol (3.42%) and dodecane, 2-methyl- (3.08%) were reported as the main compounds (Joukar *et al.* 2022). In *Cleome spinosa*, among the aerial parts, the most abundant compounds were tetradecanoic acid (40.6%) and (Z)-phytol (6.58%) in the fruit oil – reported as the main constituents. Additionally, in the EO extracted from the leaves, (Z)-phytol (31.3%) was observed as a major

component, and in the EO from the aerial parts, (Z)-phytol (19.5%) was also detected (McNeil *et al.* 2010).

Additionally, in the volatile oil of *Cleome rutidosperma*, the most abundant compound was (Z)-phytol (65.1%) (McNeil *et al.* 2018). In the EO of *Cleome heratensis*, hexanal (7.57%-33.96%), α-farnesen (7.54%-10.9%), and hexadecane (2.34%-4.82%) were also reported as major compounds (Nasseri *et al.* 2019). In our present study, farnesan (10.49%), 4-methyl-dodecan-1-ol (14.07%), 2,7-dimethylnaphthalene (4.81%), phytane (6.05%), and tetradecane, 4-methyl- (4.81%) were identified as main constituents of the EO. The composition of EOs can vary depending on various factors such as the plant's growth stage, geographical location, and extraction method.

It is worth mentioning that these findings are specific to *Cleome* genus, and it is always interesting to explore the chemical diversity among different plant species. By studying the chemical constituents of plants, scientists can gain insights into their potential therapeutic properties and understand their ecological roles.

Antioxidant Capacity

The total flavonoid content of the methanolic extract was expressed in terms of quercetin equivalent. A high content of total flavonoids (18.04 mg/g extract) was observed. Various amounts of flavonoids have been reported in the genus *Cleome*. Nasseri *et al*. (2019) reported the highest amount of flavonoids in *Cleome heratensis* to be 4.4444 equivalents of rutin in mg per g of dry matter or mg RE/g DM in the aerial extract of the plant. In the leaves of *Cleome viscosa*, an average amount of flavonoid equivalent to 0.54 ± 0.04 mg/g was reported (Gupta *et al.* 2011).

Stanković (2011) reported that methanolic and acetone extracts of *Marrubium peregrinum* have a high concentration of flavonoids, which corresponded to the strong antioxidant activity of these extracts. Since flavonoids are attributed to the antioxidant activities of the majority of plant extracts (Cesoniene *et al*. 2012), the results demonstrated the high antioxidant potentiality of *C. rostrata*. However, the antioxidant capacity of *C. rostrata* was determined by DPPH free radicals (Figure 1). A significant decrease in DPPH radical absorption from the extract at high concentrations was observed. The IC_{50} value for the extract was 202.48 µg/L. In order to establish an appropriate dose-response curve, a careful selection of concentration ranges was conducted for the methanolic extract through trial and error. The optimum activity ranges were observed between 12.5–400 µg/mL. Our research findings revealed a positive correlation between the antioxidant function and the presence of total flavonoids. These findings support the previously reported high flavonoid content in *Cleome gynandra*, particularly in the DCMF (50.09%) and EAF (19.464%) fractions. The results obtained from studying *C. gynandra* provide a justification for the traditional use of these plants in various applications such as those pertaining to inflammatory diseases, cancer, and cellular aging. This suggests the therapeutic potential of *C. gynandra* and its relevance in traditional medicine practices (Meda *et al.* 2013).

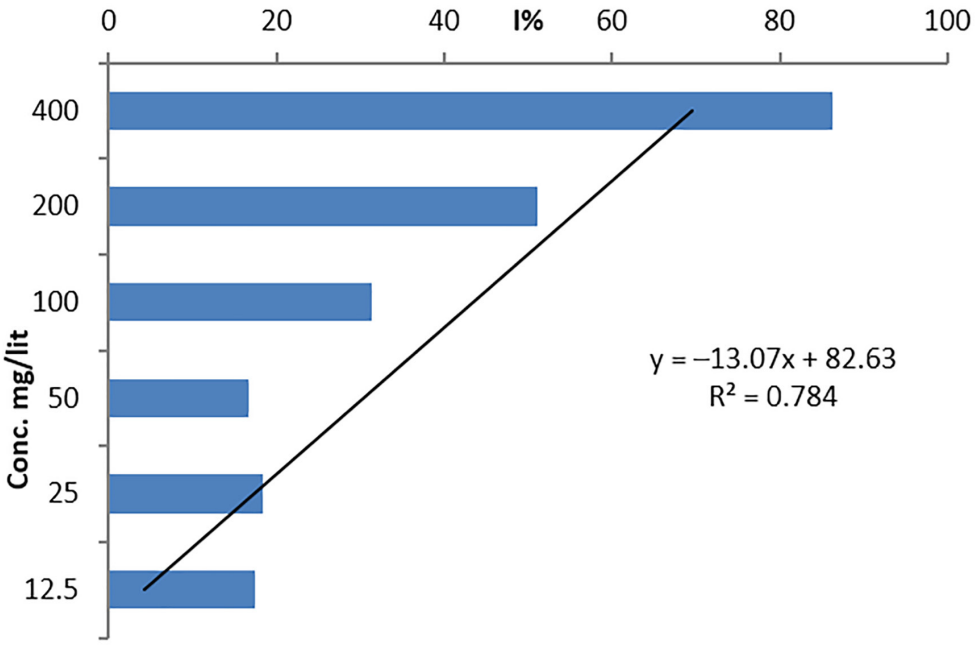

Figure 1. Antioxidant activity corresponded to DPPH free radical (µg/mL).

Antimicrobial and Antifungal Activity

This study is the first to investigate the chemical composition, antimicrobial, and antioxidant activities of the EO of *C. rostrata*. The methanolic extract of *C. rostrata* showed moderate antibacterial activity against three Gram-negative bacterial strains. It also demonstrated remarkable antimicrobial activity against the three studied fungal and three Gram-positive bacterial strains, with the highest activity observed in *A. niger* (Table 2). The high content of flavonoids in the plant extract likely contributed to its antimicrobial activity (Leven *et al*. 1979; Ahmed *et al.* 1998; Saravanakumar *et al*. 2009).

Previous research on *Cleome coluteoides* flower extract found that Gram-negative bacteria and fungi were resistant to plant extracts, whereas *C. coluteoides* flower extract had the highest inhibitory effects against all Gram-positive bacteria tested. The most sensitive bacterium was *Bacillus cereus* (Zarghami Moghaddam *et al*. 2021). In a similar study conducted on *Cleome rutidosperma*, the antimicrobial activity of its methanol extract was examined. The results showed promising outcomes, demonstrating an inhibition zone of 12 mm against *B. cereus* and *E. coli* bacteria. Moreover, the methanol extract also exhibited significant antioxidant activity. These findings are consistent with our research and indicate the potential activity of *Cleome* genus in terms of antimicrobial and antioxidant properties (Bose *et al*. 2007).

Our results on *C. rostrata* indicate that the plant extract has the greatest inhibition against fungi and Gram-positive bacteria. In 2006, tests on *Cleome viscosa* showed a wide range of antimicrobial activity – especially against *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* – but we observed only moderate activity against Gram-negative bacteria. *C. viscosa* leaf extract demonstrated moderate activity against pathogenic fungi, whereas our best result was the inhibition of *A. niger* fungus (Saradha and Rao 2010).

A report of antimicrobial activity in *Cleome chelidonii* and *Cleome gynandra* is consistent with our research. *C. gynandra* demonstrated maximum antibacterial activity against Gram-positive *Staphylococcus aureus* bacteria, and we also observed a good inhibitory effect against *S. aureus* in Gram-positive bacteria. Additionally, *Cleome chelidonii* showed good antifungal potential against *Candida albicans* (Sridhar *et al*. 2014).

CONCLUSIONS

This study is the first to investigate the chemical composition of the EO of *C. rostrata*, an endemic plant in Iran, as well as its antioxidant and antimicrobial activities. The high flavonoid content in *C. rostrata* may have contributed to its significant antioxidant activity. Additionally, the study revealed that *C. rostrata* has strong antimicrobial activity against various human pathogens. As a result, this research provides a promising foundation for future studies aimed at identifying and characterizing both known and unknown compounds found in *C. rostrata*. These compounds may have potential therapeutic applications for a variety of human disorders.

Table 2. Antimicrobial activity (MIC mg/mL) of various essential oils and extracts of *C. rostrata*.

Bacterial strain	Methanolic extract	Essential oil	*Ampicillin	*Gentamicin	*Fluconazole
Gram-negative bacteria					
E. coli	128 mg/mL	128 mg/mL	16 mg/mL	...	...
K. pneumonia	256 mg/mL	256 mg/mL	8 mg/mL	...	...
E. albertii	128 mg/mL	256 mg/mL	8 mg/mL	...	...
Gram-positive bacteria					
C. glutamicum	64 mg/mL	64 mg/mL	...	8 mg/mL	...
S.aureus	32 mg/mL	64 mg/mL	...	16 mg/mL	...
S. epidermis	128 mg/mL	128 mg/mL	...	16 mg/mL	...
Fungi					
F. solani	32 mg/mL	64 mg/mL	...	...	8 mg/mL
A. alternata	64 mg/mL	64 mg/mL	...	...	8 mg/mL
A. niger	16 mg/mL	16 mg/mL	...	...	8 mg/mL

*Approved standard

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REFERENCES

ABD-ELGAWAD AM, ELGAMAL AM, EI-AMIER YA, MOHAMED TA, EL GENDY AE, ELSHAMY AI. 2021. Chemical Composition, Allelopathic, Antioxidant, and Anti-inflammatory Activities of Sesquiterpenes Rich Essential Oil of *Cleome amblyocarpa* Barratte & Murb. Plants 10(7): 1294, 25. https://doi.org/10.3390/plants10071294

AHMED I, MEHMOOD Z, MOHAMMAD F. 1998. Screening of Indian medicinal for their antimicrobial properties. J Ethnopharmacol 62(2): 183–193. https://doi.org/10.1016/s0378-8741(98)00055-5

AICHA M, NADIA, Z. SIHEM H, ABDELMAIK B. 2017. Antioxidant Activity and Phenolic Compounds Contents of Spider Flower (*Cleome arabica* ssp. *arabica*), a Well Acclimated Species in the Algerian Desert Areas. European Scientific Journal 13(12): 1857–7881. https://doi.org/10.19044/esj.2017.v13n12p102

AL-ROWAILY SL, ABD-ELGAWAD AM, ASSAEED AM, ELGAMAL AM, GENDY AENGE, MOHAMED TA, DAR BA, MOHAMED TK, ELSHAMY AI. 2020. Essential oil of *Calotropis procera*: comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. Molecules 25(21): 5203. https://doi.org/10.3390/molecules25215203

ALONSO-GATO M, ASTRAY G, MEJUTO JC, SIMAL-GANDARA J. 2021. Essential oils as antimicrobials in crop protection. Antibiotics 10(1): 34. https://doi.org/10.3390/antibiotics10010034

ASSAEED A, ELSHAMY A, EL GENDY AEN, DAR B, AL-ROWAILY S, ABD-ELGAWAD A. 2020. Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis* exhibited antioxidant activity and allelopathic effect on weeds. Agronomy 10: 399. https://doi.org/10.3390/agronomy10030399

BALLESTER-COSTA C, SENDRA E, FERNÁNDEZ-LÓPEZ J, PÉREZ-ÁLVAREZ JA, VIUDA-MARTOS M. 2013. Chemical composition and *in vitro* antibacterial properties of essential oils of four *Thymus* species from organic growth. Industrial Crops and Products 50: 304–311.

BENLİ M, BINGOL Ü, GEVEN F, GUNEY K, YIGIT FATMAGÜL, YIIT N. 2007. Antimicrobial activity of some endemic plant species from Turkey. African Journal of Biotechnology 6(15): 1774–1778. https://doi.org/10.5897/AJB2007.000-2260

CESONIENE L, DAUBARAS R, VISKELIS P, SARKINAS A. 2012. Determination of the total phenolic and anthocyanin contents and antimicrobial activity of *Viburnum opulus* fruit juice. Plant Foods for Human Nutrition 67: 256–261. https://doi.org/10.1007/s11130-012-0303-3

BOSE A, MONDAL S, GUPTA JK, GHOSH T, DASH GK, SI S. 2007. Analgesic, anti-inflammatory, and antipyretic activities of the ethanolic extract and its fractions of *Cleome rutidosperma*. Fitoterapia 78(7–8): 515–520. https://doi.org/10.1016/j.fitote.2007.05.002

CHAND J, PANDA SR, JAIN S, MURTY USN, DAS AM, KUMAR GJ, NAIDU VGM. 2022. Phytochemistry and polypharmacology of cleome species: a comprehensive ethnopharmacological review of the medicinal plants. Journal of Ethnopharmacology 282: 114600. https://doi.org/10.1016/j.jep.2021.114600

DUARTE MCT, LEME EE, DELARMELINA C, SOARES AA, FIGUEIRA GM, SARTORATTO A. 2007. Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. Journal of Ethnopharmacol 111: 197–201. https://doi.org/10.1016/j.jep.2006.11.034

EDZIRI H, MASTOURI M, AOUNI M, ANTHONISSEN R, VERSCHAEVE L. 2013. Investigation on the genotoxicity of extracts from *Cleome amblyocarpa* Barr. and Murb, an important Tunisian medicinal plant, South African Association of Botanists 84: 102–103. https://doi.org/10.1016/j.sajb.2012.10.005

ELGAMAL ABDELBASET M, AHMED RANIA F, ABD-ELGAWAD AHMED M, EL GENDY ABD EL-NASSER G, ELSHAMY ABDELSAMED I, NASSAR MAHMOUD I. 2021. Chemical profiles, anticancer, and anti-aging activities of essential oils of *Pluchea dioscoridis* (L.) DC. and *Erigeron bonariensis* L. Plants 10(4): 667. https://doi.org/ 10.3390/plants10040667

ELSHAMY AI, AMMAR NM, HASSAN HA, AL-ROWAILY S, RAGAB TI, EL GENDY AE, ABD-ELGAWAD AM. 2020. Essential oil and its nanoemulsion of *Araucaria heterophylla* resin: chemical characterization, anti-inflammatory, and antipyretic activities. Industrial Crops and Products 148: 112272. https://doi.org/10.1016/j.indcrop.2020.112272

ELSHAMY A, ESSA AF, EL-HAWARY SS, EL GAWAD AMA, KUBACY TM, EL-KHRISY EEDA, YOUNIS IY. 2021. Prevalence of diterpenes in essential oil of *Euphorbia mauritanica* L, detailed chemical profile, antioxidant, cytotoxic, and phytotoxic activities. Chem

Biodivers 18(7): e2100238. https://doi.org/10.1002/cbdv.202100238

GUPTA PC, SHARMA N, RAO CV. 2011. Comparison of the antioxidant activity and total phenolic, flavonoid content of aerial part of *Cleome viscosa* L. International Journal of Phytomedicine 3(3): 386–391.

HEDGE IC, LAMOND J. 1970. Capparidaceae. In: Flora Iranica, No. 68. Rechinger KH ed. Graz.

JANE PR, PATIL SD. 2012. *Cleome viscosa*: an Effective Medicinal Herb for Otitis Media. International Journal of Science and Nature 3(1): 153–158.

JOBLING J. 2000. Essential oils: a new idea for post-harvest disease control. Good Fruit and Vegetables Magazine 11(3): 50–54.

JOUKAR M, LARIJANI K, FARJAM MH, GIVIANRAD MH, NEMATOLLAHI F. 2022. Studies on Chemical Composition, Antimicrobial, and Antioxidant Activities of *Cleome brachycarpa* Vahl ex DC. and *Cleome quinquenervia* DC. Journal of Medicinal Plants and By-products 12(3): 251–258. https://doi.org/10.22092/jmpb.2022.353596.1336

LEVEN M, VANDEN B, MERTENS DA, VLIETINICK F, LAMMENS A. 1979. Screening of higher plants for biological activities, antibacterial activity. Planta Medica 36(4): 311–321. https://doi.org/10.1055/s-0028-1097277

MCNEIL MJ, PORTER R.B, RAINFORD L, DUNBAR O, FRANCIS S, LAURIERI N, DELGODA R. 2018. Chemical composition and biological activities of the essential oil from *Cleome rutidosperma* DC. Fitoterapia 129: 191–197. https://doi.org/10.1016/j.fitote.2018.07.006

MCNEIL MJ, PORTER R.B, WILLIAMS LA, RAINFORD L. 2010. Chemical composition and antimicrobial activity of the essential oils from *Cleome spinosa*. Natural Product Communications 5(8): 1934578X1000500833. https://doi.org/10.1177/1934578X100050083

MEDA NTR, BANGOU MJ, BAKASSO S, MILLOGO-RASOLODIMBY J. 2013. Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *Maerua angolensis* of Burkina Faso. Journal of Applied Pharmaceutical Science 3(2): 36–42. https://doi.org/10.7324/JAPS.2013.30207.

MOYO M, AMOO OS, ADEYEMI AO, GRUZ J, ŠUBRTOVÁ M, JAROŠOVÁ M, TARKOWSKIAND P, DOLEŽAL K. 2018. Determination of Mineral Constituents, Phytochemicals, and Antioxidant Qualities of *Cleome gynandra*, Compared to *Brassica oleracea* and *Beta vulgaris*. Food Chemistry 5: 128. https://doi.org/10.3389/fchem.2017.00128

NASSERI MA, BEHRAVESH S, ALLAHRESANI A, KAZEMNEJADI M. 2019. Phytochemical and antioxidant studies of *Cleome heratensis* (Capparaceae) plant extracts. Bioresources and Bioprocessing 6: 5. https://doi.org/10.1186/s40643-019-0240-1

PAN ZH, ZHANG R, ZICARI S eds. 2019. Integrated processing technologies for food and agricultural by-products. Academic Press.

QUETTIER-DELEU C, GRESSIER B, VASSEUR J, DINE T, BRUNET C, LUYCKX M, CAZIN M, CAZIN JC, BAILLEUL F, TROTIN F. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. Journal of Ethnopharmacology 72(1–2): 35–42. DOI: 10.1016/s0378-8741(00)00196-3

SAGHAFI F. 2000. Flora of Iran. Capparaceae. Research Institute of Forests & Rangelands, Tehran, Iran [in Persian].

SARADHA JK, RAO BS. 2010. *In vitro* antibacterial activity of *Cleome viscosa* Linn. Int J Pharm Sci 1(2): 71–78.

SARAVANAKUMAR A, VENKATESHWARAN K, VANITHA J, GANESH M, VASUDEVAN M, SIVAKUMAR T. 2009. Evaluation of antibacterial activity, phenol, and flavonoid contents of *Thespesia populnea* flower extracts. J Pharm Sci 22(3): 282–286.

SOUZA MA, LEMOS MJ, BRITO DM, FERNANDES MS, CASTRO RN, SOUZA SR. 2014. Production and quality of menthol mint essential oil and antifungal and antigerminative activity. American Journal of Plant Sciences 5(21): 3311. http://dx.doi.org/10.4236/ajps.2014.5213

SRIDHAR N, SASIDHAR T, KANTHAL LK, SURYA KIRAN BVVS. 2014. *In vitro* antimicrobial screening of methanolic extraxts of *Cleome chelidonii* and *Cleonme gynandra*, Bangladesh Journal of Pharmacology 9(2): 161–166. https://doi.org/10.3329/bjp.v9i2.17759

STANKOVIĆ MS. 2011. Total phenolic content, flavonoid concentration, and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal of Science 33: 63–72. UDC 581.19:582.929.4:577.164.3

ZARGHAMI MOGHADDAM P, MOHAMMADI A, ALESHEIKH P, FEYZI P, HAGHBIN A, MOLLAZADE S, SABWTI Z, NAKHLBAND A, J KASAIAN A. 2021. Antibacterial, Antifungal, and Antioxidant Activity of *Cleome coluteoides*: an *In Vitro* Comparative Study between Leaves, Stems, and Flowers. Turk J Pharm Sci 18(1): 10–16. https://doi.org/10.4274/tjps.galenos.2019.59320