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Original Article

Studies on Chemical Composition, Antimicrobial and Antioxidant Activities of *Cleome brachycarpa* (Forssk.) Vahl ex DC. and *Cleome quinquenervia* DC

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ABSTRACT

Herbal medicines are now in huge prospects in the developing and developed nation for basic health treatment. They are inexpensive and has minimal side effects. Cleome species are one of the potential sources of secondary metabolites which have high medicinal value and huge utility in healthcare development. In this study, in vitro antimicrobial and antioxidant activities of essential oils and ethanol extracts from various parts of two cleome species named *Cleome brachycarpa* (Forssk.) Vahl ex DC. and *Cleome quinquenervia* DC. were investigated. Antibacterial activities were determined using the MIC method against pathogenic bacteria and fungi responsible for common infections and antioxidant effects were determined by DPPH assays. Essential oils of both plants were performed better antimicrobial activities than their extracts. Also, in two tested plant oils and extracts, the antifungal properties showed better performance than antibacterial effects. In the DPPH radical scavenging assay, C. *quinquenervia* leaf and *C. brachycarpa* flower and oils showed the highest activities with IC_{50} values of 69 and 75 µg/ml, respectively. In the present study, the essential oils obtained by hydro distillation of the organs of *C. quinquenervia* and *C. brachycarpa* were analyzed by GC-MS. A total of 48 compounds were identified in *C. quinquenervia* essential oil and 19 compounds in *C. brachycarpa* essential oil. The dominant constituents assessed in the essential oil of *C. quinquenervia* were p-Caryophylline-(I3) (% 29), Dibutyl phthalate (% 13), β_Element (% 11). In the case of *C. brachycarpa*, the main components of the total oil were: were Ent-sandaracopimaradien-3β-ol (68.02%), Cembrene (6.98%), Juniper camphor (4.2%).

INTRODUCTION

Nowadays people are once again tried to use herbal sources as medicine. Many diseases caused by free radicals and microbial infections are significantly cured with natural essential oils and extracts of plants. *Cleome* species have traditionally known for their different medicinal properties. This genus has been used in folk medicine for a long time because of its ethno medicinal properties including anthelmintic, carminative, anticonvulsant, antidiarrheal, antimicrobial, and wound healing effects [1]. Cleome is the genus from family Cleomaceae comprising 180 to 200 species of herbaceous annual or perennial plants and shrubs widely distributed in tropical and subtropical regions [2]. Cleomaceae is a small family of flowering plants, comprising more than 300 species belonging to 9 genera of which Cleome is the largest genus species of medicinal, ethnobotanical, ecological importance. Cleome is known by various names such as spider flower and mountain bee plant [3]. *C. brachycarpa* and *C. quinquenervia* are two of the sixteen native *Cleome* species of Iran and is commonly found in the southern and eastern provinces of the country [4].

C. brachycarpa is a perennial herb, often presenting with a woody base, erect or suberect, up to 50 cm tall, branched mostly from below very rarely

subglabrous or apparently glaucous. Leaves are 3-5 foliated, lower petiole upper most ternate to simple and subsessile; leaflets are oblong, obovate or linear lanceolate, 5-15 mm long, 2-4 mm broad, petiole up to 25 mm long. Flowers are yellow and 6-8 mm across. A minute scale like appendage present at the base towards inner side [5]. It is useful for scabies, rheumatism and inflammation and used as an analgesic agent against pain in lower abdomen in the indigenous systems of medicine in West Africa. It is a fodder for sheep and goats [6]. The essential oil content in the aerial parts of *C. brachycarpa* reported in the literature and thunbergol, α-eudesmol, elemol was reported as a major components of the oil [7].

When we reviewed the existing literature we found out that the evaluate the antibacterial and antioxidant activities of from various organs oil and extracts of *C. brachycapra* and *C. quinquenervia* have not been examined.

MATERIALS AND METHODS

Plant Material

Fresh aerial parts of *C. brachycarpa* were collected from Torbatejam Khorasan Iran, in March 2018. Flower, leaf and stem of *C. quinquenervia* were collected at the same time and location from an altitude of 1300 m. The plant was kindly identified by Dr. Zarei (Medicinal and Natural Products Chemistry Research Center, Shiraz, Iran). A voucher specimen has been deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.

Ethanol Extract

Five hundred gram of each dried powder of two plants aerial parts were extracted individually with ethanol for five days. Then, they were filtered to get the crude extracts which were then evaporated to dryness under vacuum and the dried extracts of ethanol were used for *in vitro* biological assays.

Essential Oils

Individually, 100 grams of air-dried powdered sample of flower, leaf, stem and total aerial parts of two plants were immediately submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours. At the end of distillation, the oils were collected, dried with anhydrous Na2SO4, measured, and transferred to glass vials for further in vitro analysis. After that the oil was stored at refrigerator at 4 °C, following the protocol of European Pharmacopoeia [8,9].

Antimicrobial Assay

In vitro antimicrobial bioassay screening of the oil and extract samples was tested by using the disc-diffusion method [10], and determining the minimal inhibitory concentration (MIC) using the macro dilution broth technique. The extract was screened against 6 bacterial and 3 fungal strains. The bacteria that were used in this study were *Escherichia coli* (PTCC 1396), *Klebsiella bacter* (PTCC 1053), *Escherichia albertii* (PTCC 1399), *Staphylococcus aureus* (PTCC 1431), Staphylococcus epidermis (PTCC 1435) and *Corynebacterium glutamicum* (PTCC 1532). The fungal strains that were used in this study were *Aspergillus niger* (PTCC 5154) *Fusarium solani* (PTCC 5284) and *Alternaria alternata* (PTCC 5224). All microorganisms were obtained from the Persian type culture collection (PTCC), Tehran, Iran. Each extracts and oils were tested in duplicate and the experiments were repeated 4 times. Briefly, an overnight culture of approximately 5×10^5 CFU/ml was inoculated into tubes containing test compound dilutions in concentrations from 4 t0 512 mg/ml and incubated at 36 °C for 24 h. The MIC was defined as the lowest concentration of test sample able to restrict bacterial growth to a level lower than 0.05 at 650 nm.

Antioxidant Activity

DPPH radical scavenging activity of extracts and essential oils was obtained against the stable free radical DPPH as described previously [11]. Briefly, three different dilutions of samples, in the range 50–120 µg/ml, were incubated with a methanolic solution of DPPH 100 lM. After 30 min of incubation at room temperature, the absorbance at 517 nm was measured by a spectrophotometer (Bio-Tek, Model Uvikon XL). The percentage of inhibition (%I) of the radical was calculated according to the change of absorbance of the DPPH solution for each dilution of oils or extracts and IC_{50} values were determined according to the following formula:

$\%I = [(A_{DPPH} – A_P)/A_{DPPH}] \times 100$

Where A_{DPPH} and A_P were the absorbance of the DPPH solutions containing ethanol and plant extracts or oils, respectively.

RESULTS AND DISCUSSION

Antimicrobial Activity

The Antimicrobial activity of various organs and total aerial parts oils and extracts of *C. brachycapra* and *C. quinquenervia* are reported in Table 1. The obtained essential oils had a yellow color and the extracts were dark green. In the flower part essential oils of *C. brachcapa*, the best MIC result was obtained against Escherichia albertii (4 mg/ml) and Salmonella typhi (8 mg/ml), while the best performance of *C. quinquenervia* flower was showed against the fungi especially Fusarium solani (4 mg/ml). In the leaf essential oils of two plant species the best MIC results was obtained against *Salmonella typhi* (4 mg/ml) by *C. brachcapa* leaves oils and *Alternaria alternata* (4 mg/ml). Also in the stem and total parts oils of the two plants, the best antibiotic efficiency was seen against fungal strains. On the other hand, ethanol extract of two studied species showed less antimicrobial activities then their oils. In comparing two extract results, The MIC obtained by *C. brachcapa* ethanol extract against *Fusarium solani* was better (8 mg/ml). Some investigations reported that there is a connection between the chemical structures of the most abundant compounds in the tested oils and extracts of plants and the antimicrobial activity [12].

Antioxidant Activity

The antioxidant activity of ethanolic extract, leaves and flower part oils of *C. brachycarpa* and *C. quinquenervia* measured with DPPH assays and as shown in table 2, the highest IC_{50} was supplied by *C. quinquenervia* leaves Oil with 69.35 µg/ml, and *C. brachycapra* Flower Oils with 75.22 µg/ml. As can see in table 2, all tested samples has moderate antioxidant activities but screening oils samples show more antioxidant activities than extract samples of two *Cleome* species. Stem and total aerial parts essential oils had not shown any effects on DPPH solution so they are not seen in the table 2. The antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Moreover, essential oils been also able the scavenging free radicals. Increasing evidence has suggested that some important diseases may result from cellular damage caused by free radicals [13].

Essential Oil

Due to the sufficient amount of *C. quinquenervia*, the essential oil of this plant was extracted and its compounds were identified. The yield of essential oil obtained from the hydrodistillation process of the species *C. quinquenervia* was 0.5% (w / w). In total, 48 components were identified, representing 99.19% of the total amount. The major Compounds identified in the essential oil of the species were as follows: Caryophylline-(I3) (29%), Dibutyl phthalate (13%) and β. Elemene (% 11). The essential oil components are listed in Table 3 oil.

It has to be mentioned that in 2013, Mehdi Mirza et al. analyzed the essential oils of leaves, seeds and roots of *C. quinquenervia* through GC and GC-MS and reported their results as: The volatile constituents of the oil extracted from the root, leaf and seed of *C. quinquenervia* DC were isolated through a hydro distillation method and then analyzed by GC and GC-MS. Root, leaf and seed were found to contain 15, 52, and 42 components respectively. The major components of leaf oil were found to be β-pinene (31%), α-pinene (26.1%), trans-pinocarvyl acetate (6.6%). The root oil constituents were characterized by high amounts of α-eudesmol (29%), β-eudesmol (27.5%) and γ-eudesmol (13%). The main constituents of the seed oil were shown to trans-pinocarvyl acetate (12.5%), β-eudesmol (10.8%) and β- pinene (10.8%), α-eudesmol (9.6%) [1].

Also, the chemical composition of *C. brachycarpa* was studied after extraction. It was found that the amount of essential oil obtained was 0.5% (w / w) and 19 compounds were identified. The main compounds were Ent-sandaracopimaradien-3beta-ol (68.02%), Cembrene (6.98%), %) and Juniper camphor (4.2%). The essential oil components are listed in Table 4. Similar research has been carried out on different species of Cleome and their antibacterial and antioxidant properties, and they have had almost the same result as our research.

For instance, by studying the antioxidant properties of *C. gynandra* and measuring the antioxidants using DPPH, In the year 2020 introduced this plant as a very valuable nutrient in the field of medicine with high antimicrobial activity.

Also in 2019 reported on the antibacterial activity of methanolic extract of *C. coluteoides*, on *Staphylococcus aureus*, they observed that this plant has high antibacterial properties against this gram-positive bacterium [14].

Polyphenols are reactive species toward oxidation, hence their description as antioxidants in vitro [15].

In 2021, in a study carried out on the antioxidant properties of another *Cleome* species called *C. amblyocarpa*, the plant showed strong antioxidant potentials [16].

Research has shown that in this genus antibacterial properties are attributed to flavonoids of baikalin, apigenin and loteolin in plants.

Therefore, the occurrence of antibacterial properties in the species studied in this research can be attributed to its flavonoid and polyphenolic compounds [17].

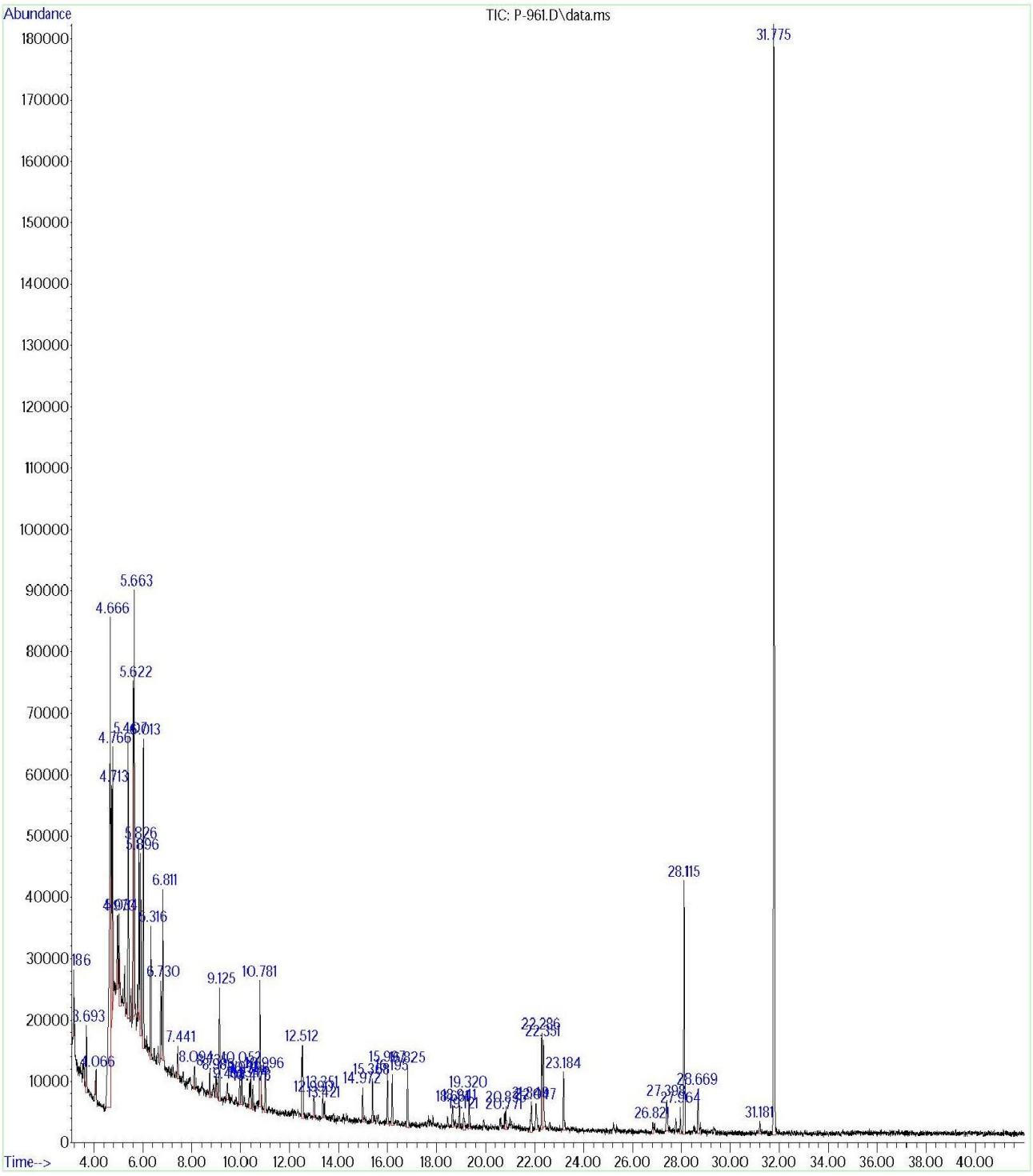

Fig. 1 Chromatogram of the chemical compounds identified via GC-MS of *C. quinquenervia*

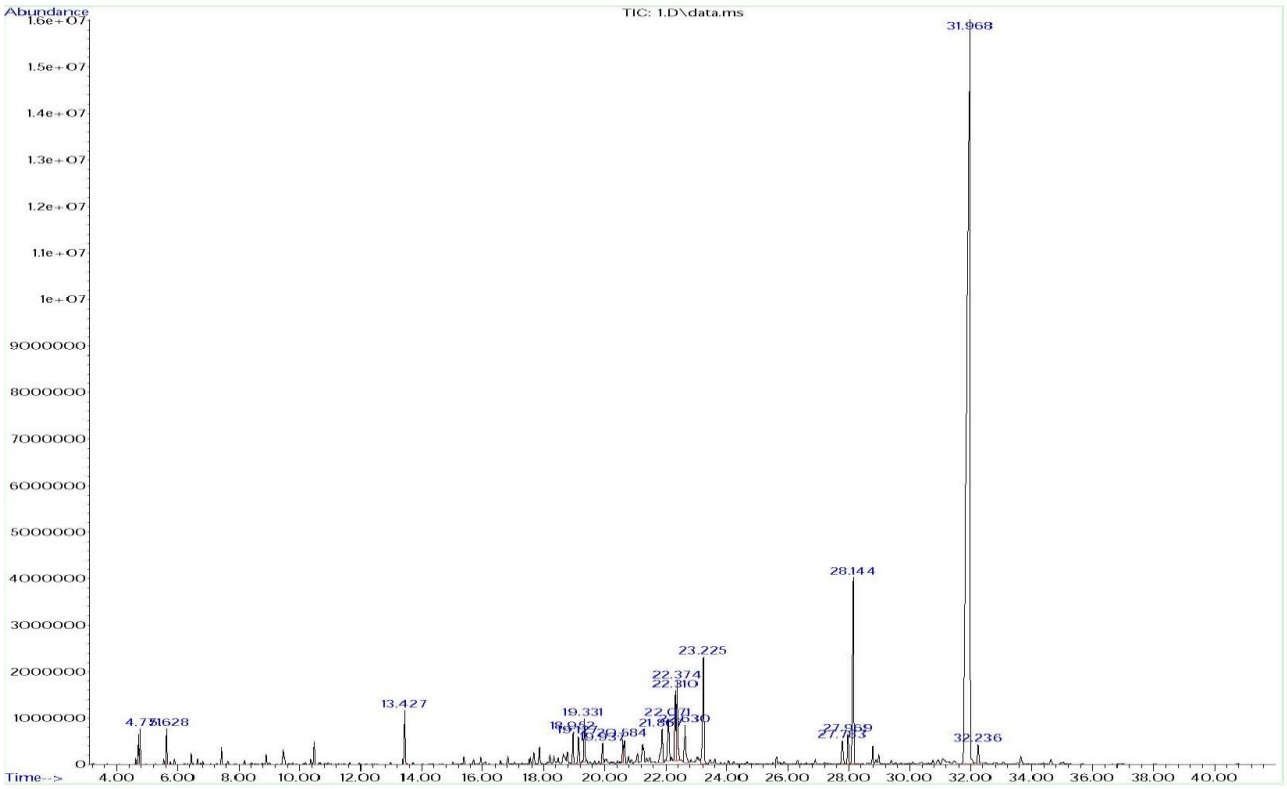

Fig. 2 Chromatogram of the chemical compounds identified via GC-MS of *C. brachycapa*

Table1 Antimicrobial activity (MIC mg/ml) of various essential oil and extracts of *C. brachycapra* and *C. quinquenervia.*

Microorganism	Essential oil Flower		Leaves		Stem		Ethanolic extract Total		Total	
	C. brachycarpa	*C. quinquenervia*	*C. brachycarpa*	*C. quinquenervia*	*C. brachycarpa*	*C. quinquenervia*	*C. brachycarpa*	*C. quinquenervia*	*C. brachycarpa*	*C. quinquenervia*
E. coli	512	512	512	256	256	32	256	64	256	32
K. bacter	64	128	256	64	128	256	128	64	128	64
E. albertii	4	256	32	256	32	256	32	128	64	16
C. glutamicum	128	128	64	64	128	128	64	64	32	32
S. typhi	8	512	4	128	256	512	32	128	128	128
S. epidermis	32	256	512	256	512	256	128	256	256	64
F. solani	128	4	32	32	8	8	32	8	64	8
A. alternata	128	16	128	4	4	16	32	32	64	16
A. niger	16	8	8	32	32	8	8	32	16	32

Table 2 Inhibition of DPPH –Free –Radical Scavenging Activity (IC_{50} µg/ml) of Crude Extract and Essential oil and Extracts of *C. brachycapra* and *C. quinquenervia*

Samples	DPPH IC_{50} (µg/ml)
C. brachycapra. Ethanol Extract	100.42 ± 6.15
C. brachycapra. leaves Oil	84.98 ± 4.1
C. brachycapra. Flower Oil	75.22 ± 8.247
C. quinquenervia Ethanol Extract	94.34 ± 8.44
C. quinquenervia leaves Oil	69.35 ± 6.94
C. quinquenervia Flower Oil	88.42 ± 12.19

Many studies have been carried out in recent years on the essential oils and extracts of medicinal plants and they have shown satisfactory results for medicinal use. In a different study in 2020, a group obtained iron nanoparticles from the aqueous extract of *Eucalyptus robusta* leaf and evaluated their antimicrobial activity on various pathogenic microorganisms.

Table 3 Chemical composition of *C. quinquenervia* Total

No	Compound	Tx	RI	%
1	Cyclooctane	3.186	-	3.84
2	Cyclohexane, ethyl	3.221	-	1
3	Decane, 5,6-dimethyl	3.460	-	0.28
4	Octane, 2-methyl	3.559	-	0.91
5	Ethylbenzene	3.582	-	0.99
6	p-Xylene	3.693	-	4.15
7	m-Xylol	4.060	-	1.27
8	5-Methyl-1,2,4-triazole-3-thiol	4.515	932	0.23
9	5-Methyl-1,2,4-triazole-3-thiol	4.707	932	3.19
10	Dodecane, 2-methyl-	5.261	945	3.08
11	Benzaldehyde	5.261	961	1.26
12	Cyclobutanone, 2,2-dimethyl	5.407	948	1.25
13	β-Pinene	5.622	979	3.19
14	1-Decene	5.826	989	0.36
15	4-Nonene, 5-methyl	5.891	981	0.43
16	3-Carene	6.730	1029	1.26
17	dl-Limonene	6.450	1022	-
18	1,8-Cineole	6.817	1033	0.28
19	Iso-Butoxyamine	7.435	1059	0.61
20	β-thujone	8.904	1119	0.28
21	Benzyl nitrile	9.446	1140	0.38
22	4-Terpineol	10.775	1191	0.35
23	m-Thymol	13.421	1391	0.50
24	Benzyl isothiocyanate	15.374	1467	1.06
25	1-Octadecene	15.595	1491	0.32
26	2 trans-Caryophyllen	16.819	1625	0.47
27	α-Humulen	17.664	1459	0.23
28	Octane, 1,1'-oxybis	17.734	1462	0.26
29	Calarene	17.845	1466	0.31
30	3,5-Cycloheptadienone	18.655	1789	0.64
31	β-Bisabolene	18.941	1711	0.64
32 33	β-Cubebene	19.116	1719	0.43
33	δ-Cadinene	19.326	1719	0.86
34	3,5-Cycloheptadienone	19.932	1789	0.37
35	Guaiene	20.573	1581	0.26
36	Hexadecane, 7,9-dimethyl	20.998	1599	0.26
37	Valencen	21.849	1892	1.05
38	Calarene	22.053	1846	1.11
39	β-Eudesmol	22.281	1857	2.19
40	β-Panasinsene	22.351	1859	2.99
41	(+)-6-Methyl-2-[4-methyl-3-cyclohexen-1-(R)-yl]-1,5-heptadien-4-(RS)-ol	22.613	1672	0.25
42	β-Guaiene	23.190	1897	1.57
43	Germacrene D	27.777	2019	0.48
44	(2R,5E)-caryophyll-5-en-12-al	27.958	2128	% 1.01
45	β_Elemene	28.115	2136	11
46	Dibutyl phthalate	28.669	2165	13
47	Adamantane, 1,3-dimethyl	28.762	2070	0.46
48	Caryophylline-(I3)	31.775	2331	29
Total				99.19

Table 4 Chemical composition of *Cleome brachycapa* `Total

No	Component	RT	Area%	KI
1	α-Pinene	4.771	0.72	939
2	β_Pinene	5.628	0.78	979
3	Thymol	13.427	1.52	1290
4	Bisabolene	18.952	0.95	1506
5	γ- Cadinene	19.127	1.06	1514
6	δ_Cadinene	19.331	1.3	1523
7	Elemol	19.937	0.68	1550
8	Muurol-5-en-4-beta-ol<cis>	20.584	0.74	1552
9	γ- Eudesmol	21.867	1.64	1632
10	tau-Cadinol	22.071	2	1640
11	β_Eudesmol	22.31	2.39	1651
12	α-Eudesmol	22.374	2.76	1654
13	Atlantone < β >	22.63	1.58	1670
14	Juniper camphor	23.225	4.2	1700
15	Juniper camphor, Acetate	27.783	0.92	1841
16	10-Isopropenyl-3,7-cyclodecadien-1-one	27.969	1.07	1930
17	Cembrene	28.144	6.98	1939
18	Ent-sandaracopimaradien-3beta-ol	31.968	68.02	2143
19	Sandaracopimarinal	32.236	0.7	2185
Total		99.97		

Antimicrobial activity was studied against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and it

was reported that the antimicrobial activity of FeNPs was related to the components in the extract [18]. In 2020, by studying the composition, phenolic content, antioxidant and antimicrobial activity of *Pistacia atlantica*, 95 compounds were reported in the essential oil of this compound. Also, in MIC test, the highest and lowest bactericidal concentration were reported against *Candida albicans* and *Escherichia coli*, respectively [19].

In a study by Kostova et al. in 2020, tangerine, grapefruit, cinnamon and lemon essential oils antioxidant and antimicrobial activities and all essences with antimicrobial activities were studied against microorganisms in order to report their chemical components. Additionally, they showed high antioxidant activities and the highest antioxidant activity was determined for grapefruit peel essential oil and lemon peel essential oil, tangerine peel essential oil and cinnamon essential oil had good antioxidant activity, respectively [20].

In a study on the antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States in August 2020, citrus species from Argentina and the United States were used to obtain their essential oils. Limonene was found to be the main compound with many components that vary according to different species. Moreover, in the study of antimicrobial activity and antioxidant activity, grapefruit and lemon essential oils consistently showed strong antimicrobial activity against all tested bacteria, and Mandarin essential oil from the United States showed the strongest antioxidant capacity in various analyses [21]. In a study in 2020 on the antioxidant and antimicrobial activities of two standard extracts of the new non-psychoactive *Cannabis sativa* L compound, the antioxidant properties were evaluated by several methods. Also, antimicrobial activity against gram-positive, gram-negative bacteria and *Candida albicans* yeast were evaluated.Both extracts showed significant antioxidant activity and strong antimicrobial properties against the tested strains. In this study, *Cannabis sativa* L extract was introduced as a promising new antibacterial agent for the treatment of widespread *Staphylococcus aureus* infections [22].

CONCLUSION

Both plant oils and extracts demonstrated good antimicrobial activity and moderate antioxidant activity. The antifungal properties showed better performance than antibacterial effects in overall. Further quantitative in vivo studies needs to be done on these two plants to isolated active phytochemicals and treatment diseases or use it as food preserving.

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