Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2024; 13(6): 176-182
Received: 17-08-2024
Accepted: 20-09-2024

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Seasonal variation of the essential oil from *Casimiroa edulis* using GC/MS and assessment of its antimicrobial potential

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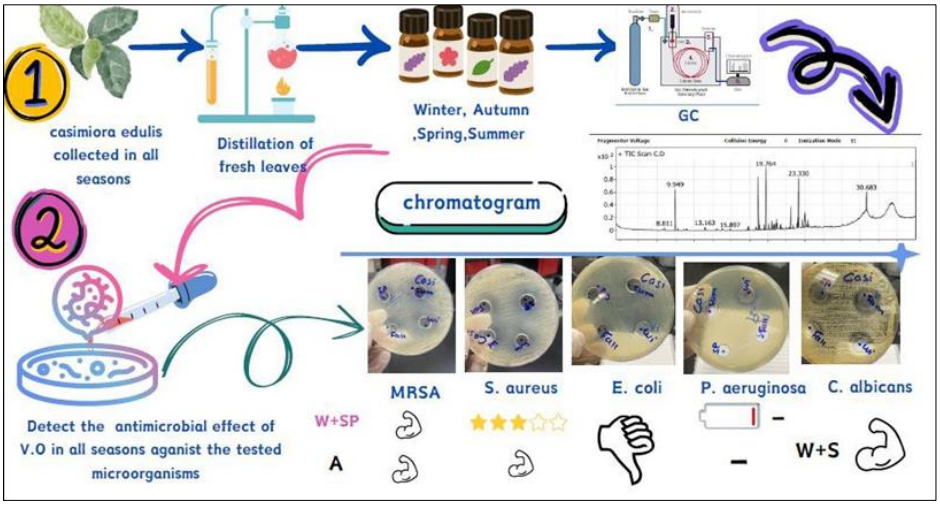
DOI: https://doi.org/10.22271/phyto.2024.v13.i6c.15176

Abstract
This study aims to analyze the composition of the essential oils of *Casimiroa edulis* leaves over four-season cycles using GC-MS to investigate the effect of climate variation on the chemical composition and their activities. The oil yield from hydrodistillation varied between 0.02% and 0.1% v/w, with the highest yield observed in spring and the lowest in Summer. GC-MS identified a total of ninety-two components in the oil samples collected during the four seasons. In all samples, sesquiterpene hydrocarbons were predominating (29.94-59.95%). The antimicrobial screening showed that the essential oils were active against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (No. ATCC 4175) using the agar diffusion method. It was concluded that the best time for collecting the plant and obtaining the oil with higher yield and promising biological activities is during autumn and the oil can be used as a candidate for antimicrobial therapy.



Keywords: *Casimiroa edulis*, seasonal variation, essential oil, antimicrobial

Introduction
Casimiroa edulis Llave (Rutaceae) is a species native to temperate regions of Mexico and Central America. Commonly known as "white sapote," it has been recognized since pre-Hispanic times for its notable sedative effects and its traditional use as a sleep aid [1]. Pharmacological studies of aqueous and alcoholic extracts from the seeds and leaves of *C. edulis* have demonstrated a wide range of different activities, including cardiovascular, anticonvulsant, sedative, anti-inflammatory, antimutagenic, diuretic, hypnotic, antihypertensive, muscle relaxant, and contractile effects. The pharmacological activities of the bioactive compounds from *Casimiroa* have been documented. Various species within this genus are known to contain notable secondary metabolites. Key constituents of *Casimiroa* species include; alkaloids, flavonoids, coumarins, limonoids, and N-benzoyltyramide derivatives [2]. Plants produce essential oils as a way to adapt to environmental stressors like water scarcity, intense radiation, high temperatures, and the presence of heavy metals [3]. Consequently, the composition and yield of these essential oils can vary both qualitatively and

quantitatively in response to factors such as seasonal changes, including variations in temperature, humidity, and rainfall [4, 5]. The effect of seasonal variation on the essential oils has been investigated in numerous plants [5-10]. Understanding this variation is crucial for determining the optimal time for harvesting and collecting plants to maximize the yield of active compounds and achieve the greatest effectiveness [11]. This study aims to analyze the composition of the essential oils extracted from *Casimiroa edulis* leaves across the four seasons, from January 2023 to October 2023. The goal is to examine how seasonal climate conditions affect the chemical profile, as well as the antimicrobial activities of the oils.

Experimental

Plant material

Fresh leaves of *Casimiroa edulis* were collected from the same trees, at three months intervals along the four season's *viz*., in January 2023 (winter), April 2023(spring), July 2023 (summer), and October 2023(autumn) from the Irrigation nurseries of Qalyubia governorate, Egypt. Plant identification was confirmed by Prof. Dr. Wafaa.M. Amer Professor of Taxonomy, Faculty of Science, Cairo University, Egypt. The voucher specimen was kept in the herbarium, under number Ca16022.

Essential oils extraction

Fresh leaves, 1kg, were subjected to hydrodistillation using a Clevenger-type apparatus for 6 hours. After the oil samples collection, any water traces were removed using anhydrous Na_2SO_4. The volumes of the obtained oil were measured in the apparatus's graduated tube as 0.2, 0.5, 0.3, and 1 ml for the summer, autumn, winter, and spring seasons, respectively. The oil yields were then calculated as percentages based on the weight of the fresh plant material used in the extraction, yielding 0.08%, 0.2%, 0.12%, and 0.4% v/w for the summer, spring, winter, and autumn seasons, respectively. The essential oil samples were kept in a sealed brown vial under refrigeration for gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis conditions

The hydrodistilled essential oils were analyzed for their chemical composition using GC-MS, following the method described by Cassel *et al*. (2009), and employing Clevenger's apparatus. The analyses were performed with a GC-MS system from Agilent Technologies, consisting of a 7890B gas chromatograph and a 5977A mass spectrometer detector, at the Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were prepared by diluting the oils with hexane in a 1:19 (v/v) ratio. A DB-624 column (30 m x 320 µm internal diameter, 1.8 µm film thickness) was used in the gas chromatograph. The carrier gas was hydrogen, with a flow rate of 3.0 ml/min, and a split ratio of 1:10. An injection volume of 0.5 µl was used, and the temperature program started at 40 °C for 1 minute, then increased at a rate of 7 °C/min until reaching 250 °C, where it was held for 5 minutes. The injector and detector temperatures were maintained at 250 °C. Mass spectra were obtained via electron ionization (EI) at 70 eV, covering a spectral range of m/z 30-440, with a solvent delay of 4 minutes. The identification of the various constituents was achieved by comparing their fragmentation patterns with those in the Wiley and NIST Mass Spectral Library, as well as the Adams Library (Adams, 2007). The retention indices (Kovats indices) of the essential oil components were determined by GLC analysis of a series of authentic n-alkanes under the same experimental conditions.

Determination of the antimicrobial activity

Microorganisms and culture media

A series of bacterial and fungal strains (available in the stock culture at the Microbiology Department, Faculty of Pharmacy, October 6 University) were used for susceptibility testing comprising of *Staphylococcus aureus* (No. ATCC 4175) and Methicillin Resistant *Staphylococcus aureus* (MRSA) as representative gram-positive bacterium while *Escherichia coli* (No. ATCC 10536) and *Pseudomonas aeruginosa* (No. CNCM A21) as gram-negative ones. Also, *Candida albicans* (No. ATCC 60193) was tested as a yeast strain. The tested bacteria and yeast were grown on the solid culture medium Trypticase soy agar and Sabouraud dextrose agar respectively prepared by solubilization in distilled water (pH 6.5±0.1 at 25 °C) followed by autoclaving at 121 °C for 20 min for sterilization.

Standards used in the study

Ofloxacin was used as a control for Gram-positive and Gram-negative bacteria while Fluconazole injection was used for *Candida albicans*.

Antimicrobial screening using agar diffusion method

The antimicrobial activity of *Casimiroa edulis* was evaluated using the cup plate technique according to (Shubha & Hiremath, 2010) [12].

Base layer in petri-dishes was obtained by pouring around 20–30 ml of Muller Hinton Agar solution to obtain a thickness of 4 mm. It was then kept for solidification. The overnight grown subculture of the used isolates was adjusted to 0.5 0 McFarland standard in definite volumes of nutrient broth at 37°C at least for 2–4 hours prior to plating. After incubation with the help of cotton swab, the organisms were streaked on Petri dish containing base layer medium. The sterile borer was used to prepare cups of 8 mm diameter, in the medium of each Petri dish. 50 µl of each extract were applied to the wells of all plates and then incubated at 30°C for 24 hours. The presence of inhibition zones around the cup indicated antibacterial activity then the zones were measured and recorded.

Results

Chemical composition of oils using GC-MS analysis

Ninety-two compounds were identified in the oil samples collected during the four seasons as shown in (Table 1). Among the seasonal cycles, the major chemical components of the samples were; β-pinene (42.73%), pseudolimonene (40.75%), caryophyllene (23.26%), β-caryophyllene (15.17%), and β- bourbonene (15.1%). The oil yields varied from a peak of 0.1% in the spring season to a low of 0.02% in the Summer season. Sesquiterpene hydrocarbons were predominating (29.94- 59.95%) in all the examined samples (Figure 1).

Table 1: Chemical composition of the essential oils from *Casimiroa edulis leaves* collected during the four seasons

No.	KI Reported	KI Calculated	Compound	Formula	January	April	July	October
1	939	939	α-Pinene	$C_{10}H_{16}$	0.72	2.19	3.28	4.02
2	979	979	β-Pinene	$C_{10}H_{16}$	12.58	-	35.2	42.73
3	993	993	β-Myrcene	$C_{10}H_{16}$	0.44	-	2.19	3.48
4	1010	1010	α-Thujene	$C_{10}H_{16}$	-	0.68	-	0.6
5	969	969	Sabinene	$C_{10}H_{16}$	-	2.38	-	-
6	1004	1004	Pseudolimonene	$C_{10}H_{16}$	-	40.75	-	-
7	974	974	Bicyclo [3.1.0] hexane, 6- isopropylidene-1-methyl-	$C_{10}H_{16}$	-	0.09	-	-
8	1026	1026	p-Cymene	$C_{10}H_{14}$	-	0.2	-	-
9	1030	1034	D-Limonene	$C_{10}H_{16}$	-	1.16	1.81	2.43
10	3001	3001	δ -Carene	$C_{10}H_{16}$	-	0130	-	0.58
11	428	429	Dihydropinene	$C_{10}H_{18}$	-	0.05	-	-
12	966	966	β-Thujene	$C_{10}H_{16}$	-	-	0.45	-
13	897	897	Sabinene	$C_{10}H_{16}$	-	-	7.52	-
14	920.7	921.4	5,5-Dimethyl-1- vinylbicyclo [2.1.1] hexane	$C_{10}H_{16}$	-	-	-	0.05
15	954	954	Camphene	$C_{10}H_{16}$	-	-	-	0.05
16	1030	1030	β -Phellandrene	$C_{10}H_{16}$	-	-	-	0.05
17	1063	1063	α -Terpinolene	$C_{10}H_{16}$	-	-	-	0.59
18	1044	1040	β -Ocimene	$C_{10}H_{16}$	-	-	-	0.08
19	1062	1062	γ -Terpinene	$C_{10}H_{16}$	-	-	-	1.07
20	1355	1355	7-Propylidene- bicyclo[4.1.0] heptane	$C_{10}H_{16}$	-	0.21	-	-
21	1334	1334	γ-Elemene	$C_{15}H_{24}$	1.96	-	-	0.06
22	1370	1370	α -ylangene	$C_{15}H_{24}$	1.08	-	-	-
23	1386	1386	β-Bourbonene	$C_{15}H_{24}$	15.1	3.01	3.51	1.37
24	1391	1391	β-Elemene	$C_{15}H_{24}$	1.3	-	-	-
25	1406	1405	β-ylangene	$C_{15}H_{24}$	1.33	-	-	-
26	1423	1423	Caryophyllene	$C_{15}H_{24}$	18.31	23.26	-	13.15
27	1715	1715	Valencene	$C_{15}H_{24}$	1.75	-	-	-
28	1456	1456	Humulene	$C_{15}H_{24}$	2.05	1.57	-	1.69
29	1433	1433	β-copaene	$C_{15}H_{24}$	1.62	6.03	0.57	1.24
30	1372	1370	Ylangene	$C_{15}H_{24}$	1.6	-	-	-
31	1481	1481	γ-Muurolene	$C_{15}H_{24}$	2.63	0.62	-	-
32	1504	1506	α-Muurolene	$C_{15}H_{24}$	1.47	0.38	-	-
33	1528	1529	δ -Cadinene	$C_{15}H_{24}$	1.18	-	0.51	2.29
34	1416	1416	β- Caryophyllene	$C_{15}H_{24}$	0.53	-	-	-
35	1405	1404	Junipene	$C_{15}H_{24}$	2.92	-	-	-
36	1491	1482	β-Guaiene	$C_{15}H_{24}$	5.12	-	-	-
37	1446	1445	δ-EIemene	$C_{15}H_{24}$	-	0.33	-	-
38	1355	1354	α -Cubebene	$C_{15}H_{24}$	-	0.21	-	0.47
39	1370	1370	Copaene	$C_{15}H_{24}$	-	0.27	-	0.78
40	1386	1387	β-Elemene	$C_{15}H_{24}$	-	0.9	-	-
41	1385	1385	Isogermacrene D	$C_{15}H_{24}$	-	0.2	-	-
42	1436	1440	Aromandendrene	$C_{15}H_{24}$	-	2.58	-	-
43	1507	1507	α-Farnesene	$C_{15}H_{24}$	-	2.4	2.9	-
44	1512	1512	γ-Cadinene	$C_{15}H_{24}$	-	0.41	-	-
45	1519	1519	β-Cadinene	$C_{15}H_{24}$	-	2.67	-	-
46	1492	1492	β-Germacrene	$C_{15}H_{24}$	-	0.33	-	-
47	1497	1497	Viridflorene	$C_{15}H_{24}$	-	0.12	-	-
48	1491	1491	Himachalene-1,4-diene	$C_{15}H_{24}$	-	0.39	-	-
49	1479	1479	γ-Gurjunene	$C_{15}H_{24}$	-	0.05	-	-
50	1413	1413	β-Caryophyllene	$C_{15}H_{24}$	-	-	15.17	-
51	1456	1456	α-Humulene	$C_{15}H_{24}$	-	-	1.47	-
52	1485	1485	Germacrene D	$C_{15}H_{24}$	-	-	13.6	5.24
53	1415	1415	α-Bergamotene	$C_{15}H_{24}$	-	-	1.5	-
54	1431	1431	Aromadendrene	$C_{15}H_{24}$	-	-	-	0.11
55	1495	1496	δ-Guajene	$C_{15}H_{24}$	-	-	-	0.3
56	1505	1505	Bicyclogermacrene	$C_{15}H_{24}$	-		1.74	3.24
57	1262	1262	ω-Decenol	$C_{10}H_{180}$	-	0.08	-	-
58	1100	1100	Linalool	$C_{10}H_{180}$	-	-	-	0.47
59	1142	1142	2-Cyclohexen-1-ol, 1- methyl-4-(1-methylethyl)-, trans-	$C_{10}H_{180}$	-	-	-	0.07
60	1138	1138	trans-Pinocarveole	$C_{10}H_{180}$	-	-	-	0.38
61	1178	1178	Terpinen-4-ol	$C_{10}H_{180}$	-	-	-	2.11
62	1212.8	1212.8	Myrtenol	$C_{10}H_{180}$	-	-	-	0.07
63	1229	1229	Nerol	$C_{10}H_{180}$	-	-	-	0.12
64	1181	1181	α -Terpineol	$C_{10}H_{180}$	-	-	-	0.87

65	1062	1062	Linalool oxide Cis	$C_{10}H_{1802}$	-	-	-	0.14
66	1070	1070	cis-Sabinenhydrate	$C_{10}H_{180}$	-	0.11	-	-
67	1080	1080	trans-Linalool oxide	$C_{10}H_{1802}$	-	0.07	-	-
68	1722	1722	cis-Farnesol	$C_{15}H_{26O}$	-	-	-	0.2
69	1514	1514	Cubedol	$C_{15}H_{26O}$	0.72	-	-	0.29
70	1514	1514	4-epi-cubedol	$C_{15}H_{26O}$	0.98	-	-	-
71	1646	1646	Ledene oxide-(II)	$C_{15}H_{24O}$	0.66	-	-	-
72	1722	1722	trans- Farnesol	$C_{15}H_{24O}$	5.44	-	-	-
73	1573	1573	Spathulenol	$C_{15}H_{24O}$	1.35	-	-	-
74	1594	1595	Isoaromadendrene epoxide	$C_{15}H_{24O}$	2.38	-	-	0155
75	1582	1583	Caryophyllene oxide	$C_{15}H_{24O}$	12.7	-	01.5	3101
76	1639	1638	τ -Cadinol	$C_{15}H_{26O}$	1.93	3131	-	3151
77	1583	1582	Elemol	$C_{15}H_{26O}$	-	1.05	-	0.51
78	1690	1690	Eudesma-4(15),7-dien-1- β -ol	$C_{15}H_{24O}$	-	0.05	-	-
79	1694.5	1694.5	(1R,7S,E)-7-Isopropyl-4,10- dimethylenecyclodec-5-enol	$C_{15}H_{24O}$	-	0.17	-	-
80	1644	1644	Isospathulenol	$C_{15}H_{24O}$	-	1.15	1.34	-
81	1682	1682	14-Hydroxy caryophyllene	$C_{15}H_{24O}$	-	0.29	-	-
82	1778	1778	β-Costol	$C_{15}H_{24O}$	-	0.74	-	-
83	1565	1566	Nerolidol	$C_{15}H_{26O}$	-	-	3.15	1.15
84	1592	1591	Viridiflorol	$C_{15}H_{26O}$	-	-	-	0.24
85	1643	1644	Cubenol	$C_{15}H_{26O}$	-	-	-	0.8
86	1648	1648	τ -Muurolol	$C_{15}H_{26O}$	-	-	1.15	-
87	1660	1660	α -Cadinol	$C_{15}H_{26O}$	-	-	-	1.26
88	2299	2298	Aromadendrene oxide-(2)	$C_{15}H_{24O}$	-	-	-	0.2
89	1593	1593	5-Cyclodecen-1-ol	$C_{15}H_{24O}$	-	-	-	0.09
90	1663	1663	Methyl 10, 11- tetradecadienoate	$C_{15}H_{26O2}$	-	0.06	-	-
91	1654	1654	Perilla alcohol angelate	$C_{15}H_{22O2}$	-	0.06		
92	1658	1658	Perilla alcohol tiglate	$C_{15}H_{22O2}$	-	0.06		
Total identified percentage					99.85	99.64	99.81	99.8

(-) dash indicates absence of the compound, January, April, July, and October = oil samples hydrodistilled from leaves collected in winter, spring, summer, and autumn.

Table 2: Effect of seasonal variation on the relative percentages of the different classes of constituents of the essential oil of the leaves of *Casimiroa edulis* cultivated in Egypt.

Constituents	January	April	July	October
Total	73.69	93.62	93.22	85.67
Hydrocarbon:				
Monoterpenoids	13.74	47.89	50.45	55.73
Sesquiterpenoids	59.95	45.73	42.77	29.94
Total Oxygenated:	26.16	6.02	6.59	12.13
Alcohols	10.42	5.77	5.64	10.17
Aldehydes	(-)	(-)	(-)	(-)
Ketones	(-)	(-)	(-)	(-)
Oxides	15.74	0.07	0.95	1.96
Esters	(-)	0.18	(-)	(-)
Ethers		(-)	(-)	(-)

*(-):Absent

*January, April, July, and October = oil samples hydrodistilled from leaves collected in winter, spring, summer, and autumn.

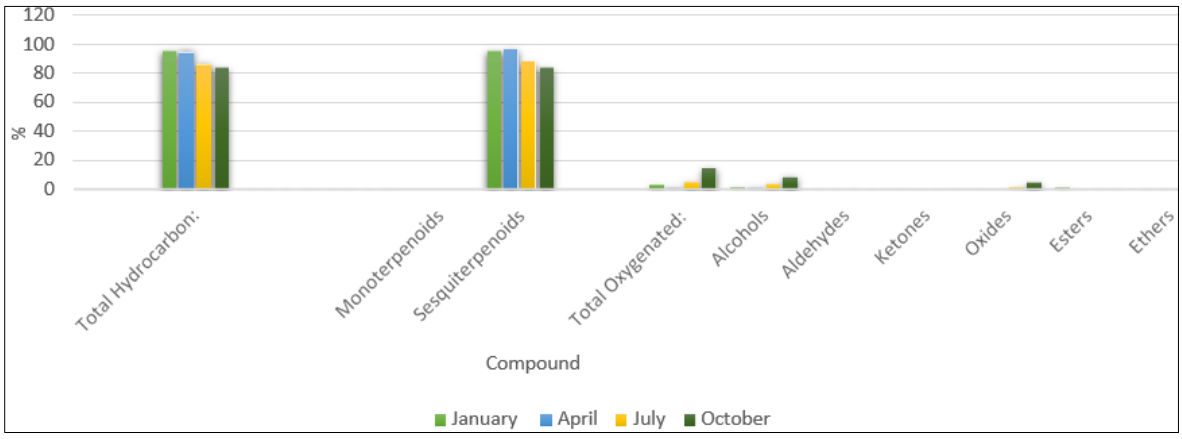


Fig 1: Histogram representing the effect of seasonal variation on the relative percentages of the different classes of constituents of the essential oil of the leaves of *Casimiroa edulis*

Table 3: Effect of seasonal variation on the relative percentages of the major constituents of the essential oil of the leaves of *Casimiroa edulis* cultivated in Egypt**.**

Compound	January	April	July	October
Caryophyllene	18.31	23.26	-	13.15
β-Bourbonene	15.1	3.01	3.51	1.37
Pseudolimonene	-	40.75	-	-
β-Caryophyllene	-	-	15.17	-
β-Pinene	12.58	-	35.2	42.73

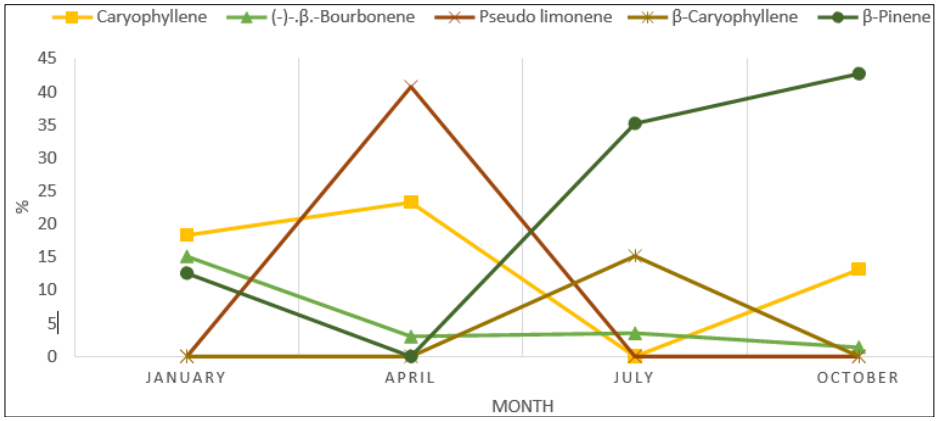


Fig 2: Line graph representing the effect of seasonal variation on the relative percentages of the major constituents of the essential oil of the leaves of *Casimiroa edulis* cultivated in Egypt.

Antimicrobial activity

Antimicrobial activities of the essential oil samples from ***Casimiroa edulis*** leaves are shown in Table 4. The applied oil displayed varying degrees of efficacy against different examined microbes. Autumn oil showed the highest activity, followed by winter oil, Summer oil, and spring oil respectively (Figure 3). *S. aureus* and *MRSA* were the most susceptible microorganisms. Generally, only the gram-positive bacteria were sensitive to the tested oil in all seasons except in winter oil also showed antibacterial activity against gram-negative *P. aeruginosa.*

Table 4: The antimicrobial activity of essential oils from *Casimiroa edulis* leaves collected during the four seasons

Tested Samples	Season	Diameters of zones of inhibition (mm)				
		Bacteria				
		Gram-positive		Gram-negative		Fungi
		S. aureus	MRSA	*E. coli*	*P. aeruginosa*	*C. albicans*
1	Summer	20	18	-	-	24
2	Winter	22	20	-	15	20
3	Autumn	22	24	-	-	10
4	Spring	20	12	-	-	18
Ofloxacin		20	22	30	30	-
Fluconazole		-	-	-	-	25

* DMSO shows a zone of inhibition of 7 mm for S aureus and no zone of inhibition for other isolates, DMSO was used as a control.

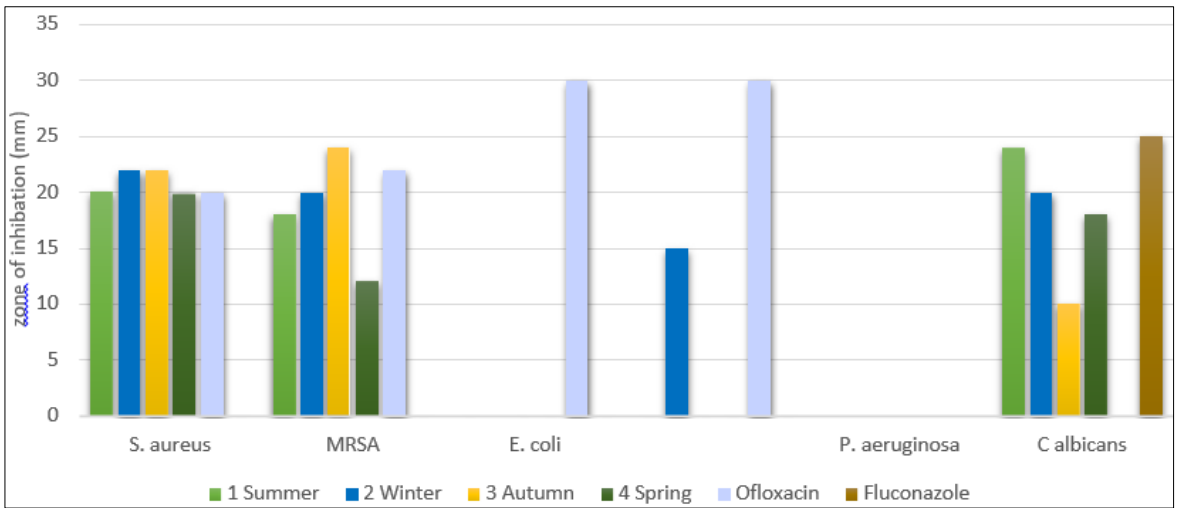


Fig 3: Seasonal effect on the antimicrobial activity of the essential oil samples

Discussion

Egypt's climate data represent four distinct seasons; Spring, Summer, Autumn, and Winter. Each season has its characteristic conditions (Temperature, humidity, and the presence or absence of rainfall) [13]. To investigate the effect of seasonal variation on the essential oils' composition and yield, the essential oils of ***Casimiroa edulis*** leaves over the period from January 2023 to October 2023 (four different seasons) were Collected and analyzed by GC-MS for identifying the oil components. The main chemical classes identified in the oil samples during different seasons were monoterpenoids hydrocarbons, sesquiterpene hydrocarbons, and oxygenated compounds. The most common class of compounds were the sesquiterpene hydrocarbons with the highest percentage (59.95%) in Winter followed by monoterpenoids hydrocarbons with the highest percentage (55.73%) in Autumn. Esters were represented as perilla alcohol angelate and perilla alcohol tiglate, methyl 10,11-tetradecadienoate which was identified only in the Spring sample with a percentage of 0.18% and not identified in the rest of the studied oils samples. The identified hydrocarbons (see Table 2 and Figure 1) were consistent in the Spring and Summer samples, ranging from 93.62% to 93.22%. However, a decrease was noted in the fall sample, totaling 85.67%, and a significant drop in the Winter sample, totaling 73.69%. The primary component in this group (see Table 3 and Figure 2) was β- Pinene, with the highest yield in the fall at 42.73% and the lowest in Winter at 12.58%. The Spring sample had no β-Pinene, but it reached 35.2% in the Summer sample. The greatest oil yield occurred in Spring and Autumn, suggesting the plant's good adaptation to these seasonal climates. In contrast, the lowest yields were recorded in Summer and Winter, likely due to the harsh environmental and climatic conditions typical of these seasons [14]. The amount of water is believed to play a key role in regulating sesquiterpene levels. Increased water content tends to enhance sesquiterpene production [15]. This observation may account for the high levels of sesquiterpene hydrocarbons found in the oil from Winter leaves (wet season) and the noticeable decrease in their percentage in the oils from Autumn, Summer, and Spring leaves (dry seasons). However, the accumulation of sesquiterpenes is influenced by both light and temperature, which might explain why oxygenated sesquiterpenes were present in the lowest percentages among the oils analyzed [16].The major chemical components of the samples among the seasonal cycles were β-pinene (42.73%), pseudolimonene (40.75%), caryophyllene (23.26%), and β-caryophyllene (15.17%). It is possible to conclude that these components represented the typical strong aroma of ***Casimiroa edulis*** oil. By referring to (Table 1), α-pinene, β-copaene and β-bourbonene were present in all oil samples. β-pinene, β-myrcene, and caryophyllene oxide were present in all oil samples except that of Spring. α -ylangene, β-ylangene, valencene, ylangene, trans- β- caryophyllene, junipene, β-guaiene, 4-epi-cubedol, ledene oxide-(II), trans- farnesol and spathulenol were only identified in Winter leaves while (5,5-dimethyl-1- vinylbicyclo[2.1.1]hexane, camphene,β -phellandrene, α -terpinolene,β -ocimene,γ -terpinene, aromadendrene, δ-guajene, linalool,2-cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-,trans-, trans-pinocarveole,terpinen-4-ol, myrtenol, nerol, α -terpineol, linalool oxide Cis, cis-farnesol, viridiflorol, cubenol, α -cadinol, aromadendrene oxide-(2) and 5-cyclodecen-1-**ol** were only identified in Autumn oil. γ-elemene, *cubedol,* and *isoaromadendrene epoxide* were only identified in Autumn and Winter samples. β-thujene, sabinene, β-caryophyllene, α-humulene, and α-bergamotene were only present in Summer oil.α- farnesene and isospathulenol were not identified in Autumn and Winter seasons. germacrene D, bicyclogermacrene, and nerolidol were only present in Summer and Autumn samples. Sabinene, pseudolimonene, bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl-,p-cymene, 7-propylidene- bicyclo[4.1.0]heptane,δ-eIemene, β-elemene, isogermacrene D, aromandendreneγ-cadinene, β-cadinene, β-germacrene, viridflorene, himachalene-1,4-diene, γ-Gurjunene, ω-decenol, cis-Sabinenhydrate, trans- linalool oxide, eudesma-4(15),7-dien-1- β -ol, (1R,7S, E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol, 14-hydroxy caryophyllene, β-costol, methyl 10,11-tetradecadienoate, perilla alcohol angelate, perilla alcohol tiglate only identified in Spring oil The study indicates that seasonal and climatic conditions in Egypt significantly impact the volatile oil chemical profile of ***Casimiroa edulis*** The findings show notable differences in the composition, proportions, and yield of the volatile oils across the four seasons, with some compounds appearing or disappearing and fluctuations in the percentages of identified compounds throughout the whole year. This demonstrates that the chemical profile of the oil is influenced by seasonal changes and climate conditions. This research is part of an ongoing effort to identify valuable and promising natural compounds from Egyptian plants. In this study, we found that the essential oils extracted from the leaves of *Casimiroa edulis* exhibit significant antimicrobial properties. Our findings showed that the oils extracted during winter and Autumn strongly inhibited the growth of gram-positive *MRSA*. They also demonstrated moderate activity against gram-positive *S. aureus* and minimal activity against the gram- negative *P. aeruginosa*. However, no antibacterial effect was observed against gram-negative *E. coli*. Notably, the oils exhibited the highest antifungal activity against *C. albicans* during the summer and winter seasons. As concerns, the autumn oil inhibited the growth of both S. *aureus* and *MRSA* with greater inhibition zones in the case of *MRSA*. Therefore, Volatile oils with antimicrobial properties can be applied in both pharmaceuticals and the food industry. In pharmaceuticals, they are added to topical treatments like creams and ointments to combat resistant strains such as *MRSA* and *Candida*, providing a natural alternative to traditional antibiotics. In the food industry, these oils serve as natural preservatives, preventing the growth of pathogens like *Staphylococcus* and *Candida*, and thus extending the shelf life of organic and clean-label products without synthetic chemicals [17].

Conclusions

The chemical composition and biological activities of *Casimiroa edulis* essential oils were affected by the season in which they were harvested. The seasonal variation data can help identifying the best time for optimal yield. The results of this study suggest that *Casimiroa edulis* essential oils grown in Egypt have strong antimicrobial properties. This is the first report identifying the ***Casimiroa edulis*** essential oils. Further *in vivo* and clinical studies are recommended to explore the potential medicinal applications of these essential oils.

Conflict of interest

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.

References

1. Awaad AS, El-Sayed NH, Maitland DJ, Mabry TJ. Phenolic antioxidants from *Casimiroa edulis* leaves. Pharm Biol. 2006;44(4):258-62.
2. Tun KNW, Aminah NS, Kristanti AN, Aung HT, Takaya Y. Natural products isolated from Casimiroa. Open Chem. 2020;18(1):778-97.
3. Abdelmajeed NA, Danial EN, Ayad HSJAD. The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. Arch Des Sci. 2013;66(4):100-20.
4. Dhifi W, *et al*. Essential oils' chemical characterization and investigation of some biological activities: A critical review. J Essent Oil Res. 2016;3(4):25.
5. Ghimire BK, *et al*. GC–MS analysis of volatile compounds of *Perilla frutescens* Britton var. Japonica accessions: Morphological and seasonal variability. J Essent Oil Res. 2017;10(7):643-51.
6. de Souza SJ, *et al*. Chemical composition and seasonality variability of the *Spiranthera odoratissima* volatile oils leaves. J Essent Oil Res. 2018;28:16-20.
7. Raposo JDA, *et al*. Seasonal and circadian study of the essential oil of *Myrcia sylvatica* (G. Mey) DC., a valuable aromatic species occurring in the Lower Amazon River region. J Essent Oil Res. 2018;79:21-29.
8. Verma RS, *et al*. Chemical composition variability of essential oil during ontogenesis of *Daucus carota* L. subsp. sativus (Hoffm.) Arcang. J Essent Oil Res. 2014;52:809-14.
9. Villa-Ruano N, *et al*. Volatiles and seasonal variation of the essential oil composition from the leaves of *Clinopodium macrostemum* var. laevigatum and its biological activities. J Essent Oil Res. 2015;77:741-7.
10. Zribi I, *et al*. GC-MS analysis of the volatile profile and the essential oil compositions of Tunisian *Borago officinalis* L.: Regional locality and organ dependency. J Essent Oil Res. 2019;129:290-8.
11. Barnes JJDS. Pharmacovigilance of herbal medicines. Pharmacovigilance. 2003;26(12):829-51.
12. Shubha HS, Hiremath RS. Evaluation of antimicrobial activity of Rasaka Bhasma. AYU (An Int Quart J Res Ayurveda). 2010;31(2):260.
13. Keichline J. The Climate of Egypt. JAMA. 1906;46(13):973.
14. Saqib F, *et al*. Validation of ethnopharmacological uses of Murraya paniculata in disorders of diarrhea, asthma, and hypertension. BMC Complement Altern Med. 2015;15(1):1-8.
15. Abdelmajeed NA, Danial EN, Ayad HS. The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. Arch Des Sci. 2013;66(4):100-20.
16. de Almeida LFR, *et al*. Non-oxygenated sesquiterpenes in the essential oil of *Copaifera langsdorffii* Desf. Increase during the day in the dry season. PLoS One. 2016, 11(2).
17. Jaradat N, Hawash M, Al-Maharik N, Qadi M, Issa L, Sobuh S. Characterization of volatile compounds and evaluation of antibacterial, antifungal, and cytotoxic properties of *Stachys palaestina* from Palestine. Arab J Sci Eng. 2024;1–8.