Wild chamomile essential oils bioactivity at different locations in sulaimani region, Iraq

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Abstract

This study was conducted to evaluate the bioactivity of wild chamomile (*Matricaria chamomile* L.), in response to different elevations flower samples at full blooming. The analysis of chamomile flower essential oils (EOs) through GC-MS identified the predominant abundance of terpenoids including Bisabolol oxides, farnesene, and Azulen, constituting 61.765% of the identified compounds across various locations. The highest concentration was 74.554% out of the total components observed at Kanipanka, while it was the minimum (16.414%) at the Qaradagh location. Oppositely the highest concentration of Farnasene was observed at the Qaradagh location, while the lowest concentration occurred with the essential oil of Kanipanka.α-Bisabolol oxide A% dominates, except in the Qara Dagh location. The best antioxidant concentration was found in Sharbazher essential oil. The essential oil in Sharbazher significantly shows the highest inhibition capacity (IC%). The stronger IC50% value was 64.645 µg mL^{-1}, recorded at the Qara Dagh location. The disc diffusion assay revealed that the activity of chamomile essential oil at various locations significantly affected the death of most bacterial strains (Gram-negative and Gram-positive). Chamomile EOs from the Kanipanka location recorded maximum antibacterial activity on *Pseudomonas aeruginosa*. Penjween EOs observed the maximum antibacterial activity against *Staphylococcus haemolyticus*, while a high level inhibition zone (IZ) against *Enterococcus faecalis* was observed in Bakrajo. The finding revealed that the highest MIC and MBC against *Pseudomonas aeruginosa* were achieved with the concentration of 2.5 µg mL^{-1} of Kanipanka EOs.

Keywords: *Matricaria chamomilla* L., altitudes, Bioactive constituent, Inhibition capacity IC%, IC50%, Inhibition Zone, MIC, and MBC.

Introduction

Historically, many people have utilized medicinal and aromatic plants to treat ailments including diabetes, infections, and skin issues. These plants are regarded are one of the world's most significant sources of medicine [1]. The Asteraceae family comprises many genera Chamomile (*Matricaria chamomilla* L.) is one of these genera and is considered a medicinal plant. Chamomile is an aromatic herb that is grown extensively in various countries and widely distributed around the world [2,3,4]. Chamomile is grown naturally in Iraqi locations and widely in Hawraman [5], South of Kirkuk [6], Erbil [7], and Baghdad [8]. EOs are the most diverse secondary metabolites; they are complex, volatile, and distinctively fragrant and are produced by plant organs [9,10]. Throughout human history EOs, have been widely employed in folk medicine. EOs are used for a variety of functions, including ritualistic, medicinal, sanitary, spiritual, antioxidant, anesthetic, and antiseptic effects [11]. Wide-ranging biological effects of essential oils (EOs) including antiviral, anticancer, antioxidant, and antibacterial activity were recorded [4,12]. Plant EOs are an abundant source of chemical bioactive constituents that have potential applications in medicine and are used as anti-inflammatory, and anti-cancer agents [13,14].

The main organ (site) for EO production in chamomile is the flower heads. Flowers of chamomile contain EOs with blue color ranging from 0.24–2.0% [2,15]. The most abundant chemical compounds in chamomile EOs include α-bisabolol oxide A and B, α and β-farnesene, chamazulene, azulenes, and spiroether, while minor bioactive compounds are limonene, isoborneol, phytol, germacrene-D, α- farnesol, n-hexanols, nerol, β-bourbonene, spatthulenol, (E)-nerolidol, element, and camphene [16]. These active compounds also possess antibacterial activities against several strains of bacteria and are used as a natural alternative to synthetic antibiotics [17]. EOs composition, optimum yield, and biological activity are influenced by various factors, these factors include the genetics of the plant, altitude, growing conditions, harvest time, topography, climate, and genotypes [18]. Altitude is the most important environmental factor that affects the chemical compound content of the plant EOs [19]. The flower head and leaves of the chamomile plant possess the highest antioxidant potential, this could be due to their chemically active compound contents, such as chamazulene, apigenin, and bisabolol [20].

This study investigates the diversity of bioactive compounds and antioxidant potential in flower head essential oils across multiple locations in the Sulaimani region. It aims to assess the MIC and MBC values for the most potent essential oils obtained from these locations.

Materials and Methods

Plant Samples Collection

The collection of wild Chamomile (*Matricaria chamomile* L.) plants occurred in April 2022 at six distinct locations within the Sulaimani region, specifically during the full

bloom stage (Table 1, Figure 1). Locations were selected based on the different Meters Above Sea Level (MASL), and the Global Positioning System (MGRS UTM GPS version 1.9.4) was used to record the information about these locations.

Table (1): Collection place information

Locations	Latitude (North)	Longitude (East)	Altitude (m)	Soil pH	Organic Matter %
Kanipanka	35° 22′ 55″	45° 43′6″	568	7.95	3.778
Sartak	34° 59′ 47″	45° 42′28″	652	7.85	4.469
Bakrajo	35° 33′ 36″	45° 21′46″	758	7.86	1.012
Qara Dagh	35° 20′ 1″	45° 23′50″	982	7.73	4.324
Penjwen	35° 37′ 7″	45° 57′ 33″	1259	7.71	5.033
Sharbazher	35° 52′ 44″	45° 35′ 8″	1446	7.86	2.600

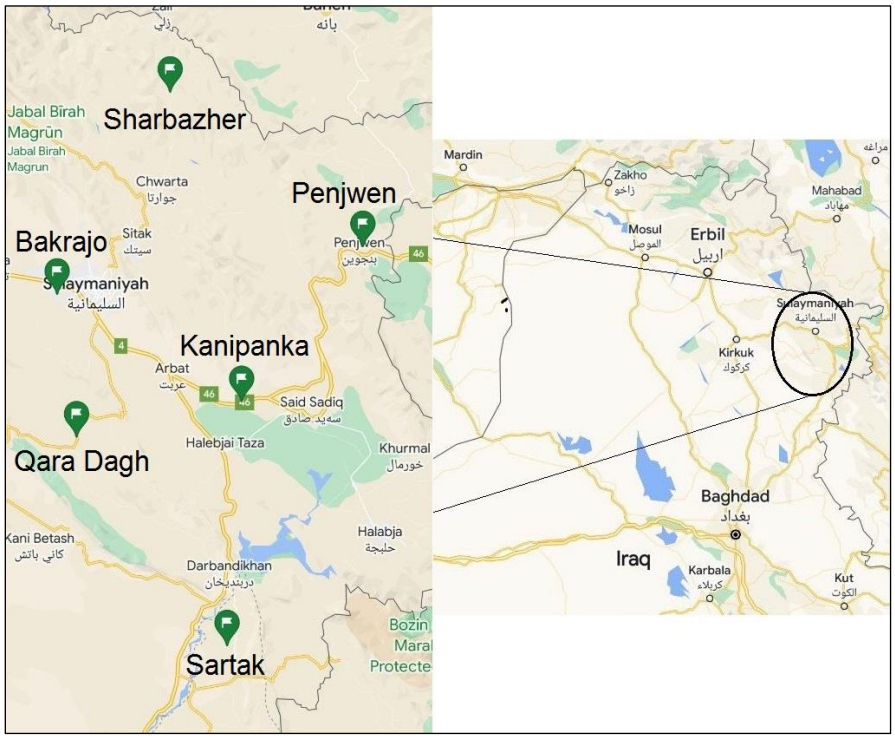

Figure (1): The geographical distribution of chamomile across the chosen locations.

Plant Samples Identification

Sample specimens were identified and deposited in the University of Sulaimani, College of Agricultural Engineering Science Herbarium (SUFA, acronym according to Thiers, 2021) for references and further investigation [21].

EOs Extraction

Chamomile-dried flowers were placed on a hydro-distillation system using Clevenger-type apparatus [22] to extract EOs. Each plant sample was extracted by adding 120g of dry flower heads, 5 g of spherical molecular sieves with 2-4 mm diameter, 5A grade, and 150 mL of distilled water into a conical flask (2000 mL) and then heated to boiling point for 5 hours. As the mixture boils, the essential oil vaporizes and passes through the condenser, which is then cooled and liquefied. EOs then accumulate in the graduated tube. A separatory funnel was used for the separation of the EOs and the water.

GC-MS Analysis of Bioactive Constituents

The analysis of chamomile EOs was done by Gas Chromatography-Mass Spectrometry (GC-Ms) with an Agilent Technologies 7890A gas chromatograph equipped at the University of Kurdistan, Sanandaj, Iran (Faculty of Basic Science - Chemistry Department) with a mass selective detector and an Agilent Technologies 5975C inert XL MSD mass spectrometer. The column of GC-MS was Agilent 190915-433:325°C (30m×250µm×0.25µm). The initial column temperature was 40 °C, with the temperature increasing every 10 °C per minute until it reached 280°C. The temperature of the injector port was 290°C with a flow rate of 1 ml min^{-1} of the helium carrier gas.

DPPH Radical Scavenging Capacity Assay

The antioxidant potential of the chamomile EOs from all locations was tested to evaluate the basis of their scavenging potential for the stable diphenyl-2-picrylhydrazyl (DPPH) free radical in the quantitative assay. Two mL (6×10-5M) of DPPH solution was mixed with a 20 µL sample. Methanol and a DPPH solution were used as controls, and 95% methanol was used as a blank. The samples and control were incubated in the dark at 35 °C for 25 min., and the absorbance was taken at 517 nm using a spectrophotometer (UV/Vis spectrophotometer UVM6100) [23,24]. Glass cuvettes (1 cm×1 cm×4.5 cm) were used for visible absorbance measurements. The inhibition capacity is calculated as follows:

$$\text{IC}\ (\%) = \frac{\text{A}\ sample - \text{A}\ blank}{\text{A}\ blank} \times 100 \qquad \text{Eq. 1}$$

Where,

A blank is the absorbance of the control (containing all reagents except the test compound)

A sample is the absorbance of the tested compound.

The third direction to determine the antioxidant concentration of the sample was calculated from the calibration curve of different concentrations of Trolox as a standard antioxidant, which was plotted as the X coordinate axis and the absorbance at 517 nm as the Y coordinate axis at 6 points (Figure 2). Trolox concentrations were 0.325, 0.650, 1.300, 1.950, 2.600, and 3.250 µg mL^{-1}, obtained from the calibration curve $\hat{y}$=0.0127+0.0074X, R^2=0.99, and each concentration calculated from the following equation. The calibration

curve was obtained, and each concentration was calculated from the following equation [25]:

$$Antioxidant\ Concentration\ (\mu gmL^{-1}) = \frac{A\ (sample - control\ ) - a}{b} \qquad \text{Eq. 2}$$

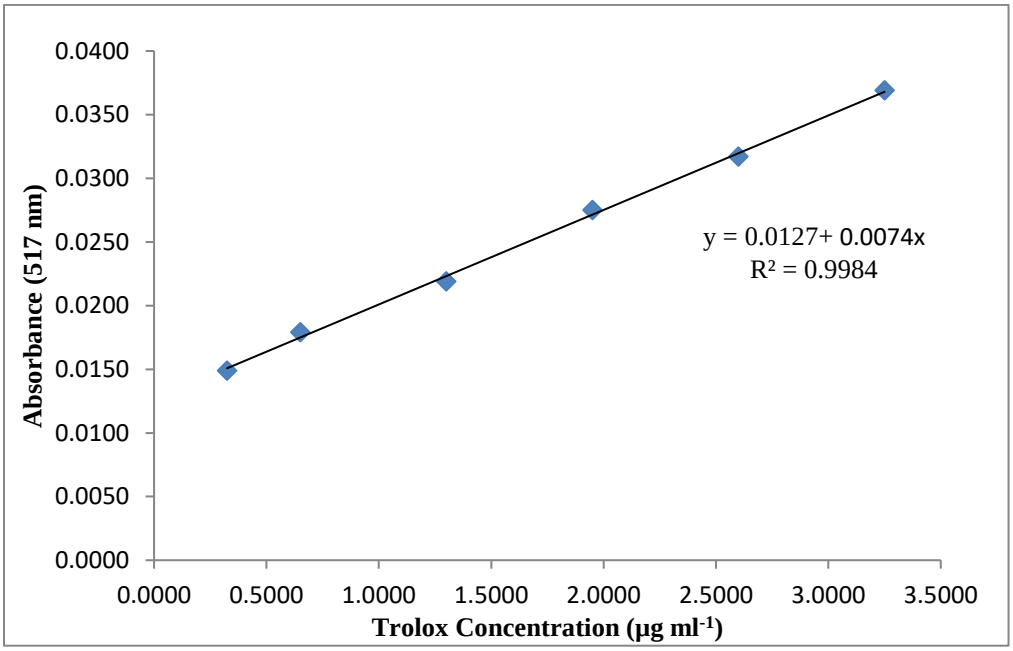

Figure (2): Different concentrations of Trolox calibration curve.

Where,
Y is the absorbance at ƛ 517 nm,
X is the sample concentration
A (control – Trolox) is the control absorbance minus the absorbance of the Trolox,
a is the value of absorbance when the concentration of the Trolox is zero,
b is the slope of the calibration curve (i.e., it signifies the average change in absorbance as the concentration increases by one unit).

Antibacterial Activity

Culture Media Preparation

To determine the chamomile essential oil antibacterial activity, HIMEDIA Muller Hinton Agar (MHA) and HIMEDIA Muller Hinton Broth (MHB) were used, and manufacturing instructions were used for preparing the all-culture media. The powder (38.1 g of MHA and 21 g of MHB) was dissolved into 1 L of distilled water by using a magnetic stirrer, then heated to boiling to dissolve the medium, and sterilized by autoclave at 15 lb. pressure and 121 °C for 15 min. to become cool and reach near 45–50 °C. It was then poured into sterilized (90 mm×15 mm) plastic Petri dishes. Each petri dish was

approximately filled with 25 ml of MHA-prepared media and left to cool and dry before use to avoid water condensation inside the plates, while the MHB was poured into sterilized tubes [26].

Bacterial Strains

Four species of bacterial strains (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*) gram-negative and (*Staphylococcus haemolyticus* and *Enterococcus faecalis*) gram-positive were used for susceptibility tests. Table 2 shows the places, specimens, and identification reports of bacterial isolations.

Table (2): Places and specimens of pathogenic bacteria isolations

Bacteria isolations	Locations	Specimen	Isolates susceptibility
P. aeruginosa	Burn and Plastic Surgery Hospital/Emergency	Clinical	MDR
A. baumannii		The patient's cabinet surface	MDR
S. haemolyticus	Sulaimani Teaching Hospital	Wound	MDR
E. faecalis		Urine	MDR

Preparation of Bacterial Inoculum

After the streaking technique, uniform and morphologically similar multi-single colonies of all studied bacteria were cultured in MHB and then incubated for 24 hours at 37°C to obtain a fresh suspension of bacterial inoculum.

Disc Diffusion Assay

To assess chamomile essential oil's antibacterial properties, a disc diffusion experiment was used [26, 27] Fresh overnight bacterial samples were titrated to 1.5 × 108 CFU mL^{-1} or 0.5 McFarland turbidity standards [28]. To prevent over-inoculation of the cotton swap with bacteria, the sterilized cotton swap was then submerged in an appropriate bacterial suspension. Any extra fluid was then removed by pressing and rotating the swab against the tube's interior. Swapping on the plate surface has been done in three different directions, to ensure evenly distributed bacterial inoculum, so no gap has been left between swap streaks. Filter paper discs (grade 393) with a 6 mm diameter were sunk into different chamomile essential oils, which were diluted with methanol 98.9% 1:1 [29]. Also, broth and methanol 1:1 were used as a positive control to determine the effect of methanol on bacterial growth. Discs with a ten µL absorption capacity were placed on a petri dish and incubated at 37°C for 24 hrs. All inhibition zone diameters around the discs were measured to determine the antibacterial effect of chamomile essential oil.

Chamomile EOs MIC and MBC Determination

The Macrobroth dilution assay [27] was employed to determine the MIC values for the two most sensitive bacterial strains, in conjunction with the most effective chamomile EOs identified through the disk diffusion assay. Bacterial strains were cultured in 15 mL of Muller Hinton Broth (MHB) and incubated for 18–20 hours at 37°C, then adjusted to 0.5 McFarland turbidity standards. Thirteen sterile cotton-capped tubes were prepared, one of them filled with one mL of MHB as a negative control and one filled with one mL of adjusted bacterial suspension. One tube was also filled with 950 µL of adjusted bacterial suspension and 50 µL of methanol as a positive control. The remaining ten tubes were filled with one mL solution of adjusted bacterial suspension and chamomile essential oils at concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, and 0.08 µg mL^{-1}. The chamomile essential oil from each concentration was diluted with 98.9% methanol at a 1:1 ratio for the disk diffusion assay. All tubes were well mixed to ensure even dispersion of the essential oils throughout the broth and then incubated at 37°C for 16 –20 hours. The tubes were then examined for optical density to determine MIC values. Following this, 5 µL of each concentration was cultured on (MHA) plates to evaluate bacterial growth, MIC, and MBC.

Statistical analysis

To determine the difference between locations, a one-way ANOVA was used. Results are presented as the mean value of five replications. LSD test was used to compare the means with $p \leq 0.01$ and $p \leq 0.05$ levels [30].

Results and Discussions

Bioactive Constituents Analysis by GC-MS

The findings showed that of the thirty-four chemicals found in chamomile flower EOs detected by GC-MS analysis, each location had a corresponding retention time, relative peak area, and each chemical class total area (Table 3). Terpenoids were the main components with 61.765% and distributed in a way that monoterpenes (9.254%), oxygenated monoterpene (28.571%), sesquiterpene (28.571%), and oxygenated sesquiterpene (33.333%), the identified compounds were relatively similar to those detected in all locations, with differences in their concentrations and structures. EOs' most abundant bioactive compounds were α-oxide B and A, α, and β- farnesene, bisabolol, and azulene. Among all the major constituents, α-Bisabolol oxide A% made up 59.417, 59.061, 54.456, 60.435, and 52.119% of the total area for all locations, respectively, except for Qara Dagh, which was not detected from the GC Mass analysis. High temperatures and sunshine had a negative impact on the EOs content, while the sunshine duration increased the content of α-bisabolol [31].

The variation in EO constituents is mostly affected by geographical locations. This allows for the choice of essential oils with preferred components to be used in food

industries, perfume, drugs, and pharmaceuticals [32]. Studies have revealed that plants belonging to the same species but growing in different environments have different concentrations of a specific secondary metabolite. This is because, to combat environmental stress, the plant must produce secondary metabolites in a specific quantity and quality. As a result, research on each environmental element is crucial to understanding plant availability and adaptability in a given area [33]. α-Bisabolol oxide A% was the most abundant constituent in essential oil composition. With the same results, Seidler-Lozykowska [31] discovered that the oil content ranged from 0.63% to 1.46% and the a-Bisabolol content from 0.82% to 64.85%. When the average daily temperature changed from 14.1°C to 19.2°C and the amount of rainfall varied from 22.1 mm to 75.0 mm, the content of chamazulene likewise varied from 3.11 to 23.81%.

DPPH Radical Scavenging Capacity

Table 4 demonstrates the significant impact of different locations on the antioxidant capacity of chamomile EOs. Sharbazher EOs exhibited a maximum antioxidant concentration of 110.176 µg mL^{-1} compared to other locations, which may be because of the highest value of EOs compound content like α- bisabolol A and B and chamazulene, while Qara Dagh showed a minimum antioxidant concentration of 89.950 µg mL^{-1}. A study presented by Sukkaew [34] observed that bisabolol and chamazulene were the most potent components in terms of antioxidant activity. Regarding the inhibition capacity (IC%), there was a significant direct relationship between antioxidant concentrations and IC%. The more antioxidant concentration, the higher the IC%; hence, essential oil at Sharbazher significantly shows 84.923% inhibition capacity compared to the lowest IC%, which was recorded at Qara Dagh (69.573%).

Table (3): Chamomile essential oil constituent, retention time, and relative peak area for all studied locations

Peak No.	Chemical constituents	Kanipanka		Sartak		Bakrajo		Qara Dagh		Penjwen		Sharbazher	
		Rt.	Area (%)	Rt.	Area (%)	Rt.	Area (%)	Rt.	Area (%)	Rt.	Area (%)	Rt.	Area (%)
1	Yomogi alcohol ($C_{10}H_{18}O$)	4.566	0.087	/	/	/	/	/	/	/	/	/	/
2	Artemisia ketone ($C_{10}H_{18}O$)	5.790	0.159	/	/	5.794	0.139	/	/	/	/	/	/
3	Artemesia alcohol ($C_{10}H_{16}O$)	5.942	0.193	/	/	5.963	0.043	/	/	/	/	/	/
4	Trans-2,7-Dimethyl-3,6-octadien-2-ol ($C_{10}H_{18}O$)	6.614	0.051	/	/	/	/	/	/	/	/	/	/
5	Chrysanthemyl alcohol ($C_{10}H_{18}O$)	7.303	0.158	/	/	/	/	/	/	7.338	0.052	/	/
6	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxy-decalin ($C_{13}H_{22}O_3$)	7.429	0.096	/	/	/	/	/	/	7.460	0.080	/	/
7	Borneol ($C_{10}H_{18}O$)	7.670	0.042	/	/	/	/	/	/		/	/	/
8	Butanoic acid, 2-methyl-, 3-hexenyl ester, (Z)- ($C_{11}H_{20}O_2$)	8.186	0.254	/	/	8.199	0.266	8.398	0.405	8.211	0.206	/	/
9	Butanoic acid, 3-methyl-, 2-hexenyl ester, (E)- ($C_{11}H_{20}O_2$)	8.363	0.295	8.378	0.327	8.375	0.374	/	/	8.387	0.255	/	/
10	Ethanone, 1-(3,3-dimethylbicyclo [2.2.1] hept-2-yl)-, exo- ($C_{11}H_{18}O$)	8.477	0.132	/	/	/	/	/	/	8.513	0.045	/	/
11	1-Methylverbenol ($C_{11}H_{18}O$)	8.609	0.750	8.628	0.351	8.627	0.234	8.645	0.721	8.638	0.369	8.627	0.482
12	Cyclopentane, 1-methyl-2-acetyl-3-(1-methylethenyl)- ($C_{11}H_{18}O$)	8.756	0.229	/	/	/	/	/	/	/	/	/	/
13	4,8-Decadienal, 5,9-dimethyl- ($C_{12}H_{20}O$)	/	/	/	/	9.034	0.114	/	/	9.058	2.033	/	/
14	Epoxy- α -terpenyl acetate ($C_{12}H_{20}O_3$)	/	/	9.041	0.131	/	/	9.058	0.278	/	/	9.040	0.170
15	4,8-Dimethyl-nona-3,8-dien-2-one ($C_{11}H_{18}O$)	9.162	3.315	9.174	1.646	9.172	1.233	9.197	3.992	9.194	2.033	9.180	2.701
16	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z, E)]- ($C_{15}H_{24}$)	/	/	10.150	0.295	/	/	/	/	/	/	/	/
17	Azulene ($C_{10}H_8$)	10.135	0.326	10.585	0.306	10.142	0.218	10.190	0.962	10.175	0.366	10.621	0.332
18	Cedrene ($C_{15}H_{24}$)	10.570	/	10.750	0.281	10.567	0.394	10.657	0.570	10.632	0.268	/	/
19	1-Naphthalenol ($C_{15}H_{26}O$)	/	0.105	/	/	10.744	0.078	/	/	/	/	/	/
20	β-Farnesene ($C_{15}H_{24}$)	11.015	13.823	11.060	19.591	11.072	17.300	11.145	61.793	11.186	20.418	11.075	23.874
21	(+)-Sativen ($C_{15}H_{24}$)	11.245	0.247	11.262	0.148	11.257	0.196	/	/	/	/	/	/
22	α-Bisabolol ($C_{15}H_{26}O$)	11.383	0.308	11.411	0.256	11.400	0.275	/	/	/	/	/	/
23	Naphthalene ($C_{10}H_8$)	11.639	0.475	11.668	0.081	11.663	0.822	11.748	6.588	11.734	0.611	11.691	3.211
24	α-Farnesene ($C_{15}H_{24}$)	11.756	0.390	11.802	0.757	11.783	0.824	/	/	11.920	0.709	/	/
25	Phenethyl isovalerate ($C_{13}H_{18}O_2$)	11.531	0.187	12.577	0.145	/	/	/	/	/	/	/	/
26	β-Sesquiphellandrene ($C_{15}H_{24}$)	/	/	/	/	12.131	1.220	/	/	/	/	/	/
27	7-Methyl-1-naphthol ($C_{11}H_{10}O$)	12.693	0.525	12.626	0.818	12.702	0.836	12.808	1.367	12.836	1.069	12.750	0.822
28	Nerolidol ($C_{15}H_{26}O$)	12.945	2.243	12.999	2.870	12.977	1.709	13.272	6.980	/	/	13.087	2.855
29	(+, -)-E-Nuciferol ($C_{15}H_{22}O$)	13.453	0.573	13.530	0.661	13.326	0.808	/	/	/	/	/	/
30	Lanceol, cis ($C_{15}H_{24}O$)	13.889	0.734	/	/	/	/	/	/	/	/	/	/
31	1-Naphthalenol, 4-methoxy- ($C_{11}H_{10}O_2$)	/	/	/	/	14.84	8.223	/	/	/	/	/	/
32	Formic acid, 3,7,11-trimethyl-1,6,10-dode-catrien-3-yl ester ($C_{16}H_{26}O_2$)	/	/	/	/	/	/	/	/	13.265	2.137	/	/
33	α-Bisabolol oxide B ($C_{15}H_{26}O_2$)	14.671	14.829	14.768	11.247	14.725	10.236	15.166	16.414	15.363	10.798	14.932	13.432
34	α-Bisabolol oxide A ($C_{15}H_{26}O2$)	17.627	59.417	20.029	59.061	20.484	54.456	/	/	20.142	60.435	20.664	52.119

Table 4 also shows the IC50% values. This explains the inhibition of 50% of free radicals by the antioxidant concentration in the samples, and the lower IC50% value represents stronger antioxidant activity. The relative correlation between antioxidant content and inhibitory capacity was used for calculating IC50%. The stronger IC50% value was 64.645 µg mL^{-1}, recorded at the Qara Dagh location, while the weakest IC50% value of 64.868 µg mL^{-1} occurred at the Sharbazher location. From the results obtained, the quality and type of antioxidant agents determine the antioxidant potential in a sample, not only the number of antioxidant agents. The results were in agreement with Asnaashari [35], they used the DPPH assay to investigate the antioxidant capacity of chamomile EOs collected at different locations in Iran. The findings showed the locations had a significant effect on the antioxidant capacity of chamomile EOs. Similarly, Sarikurkcu [36], showed that the different locations affected the antioxidant capacity of chamomile EOs in Turkey, using various assays, including DPPH, β-Carotene, and FRAP.

Table (4): The means of antioxidant concentration, inhibition capacity IC (%), and IC50% of chamomile essential oil at different locations.

Essential Oil Samples	Absorbance (517 nm)	Antioxidant Concentration (µg mL^{-1})	Inhibition Capacity IC (%)	IC50% (µg mL^{-1})
Control	0.975	-	-	-
Kanipanka	0.205	102.383	79.009	64.792
Sartak	0.179	105.806	81.607	64.827
Bakrajo	0.215	100.941	77.915	64.777
Qara Dagh	0.297	89.950	69.573	64.645
Penjween	0.202	102.743	79.282	64.796
Sharbazher	0.147	110.176	84.923	64.868
L.S.D ($p \leq 0.05$)		1.393	1.057	0.015
L.S.D ($p \leq 0.01$)		1.952	1.482	0.021

Antibacterial Activity

The activity of chamomile essential oil at different locations significantly affected the death of most bacteria strains using disc diffusion assays (Table 5). The inhibitory zone of bacterial strains was measured in millimeters mm (Figure 3). The highest antibacterial activity against *Pseudomonas aeruginosa* was observed in Kanipanka at an Inhibition Zone (IZ) of 11.067 mm. However, chamomile essential oil did not show any effect against *Pseudomonas aeruginosa* in Sartak, Bakrajo, and Penjween. Unexpectedly, the activity of chamomile essential oil at all different locations did not show any effect against *Acinetobacter baumannii*.

Table (5): The means of an inhibition zone IZ of chamomile essential oil as affected by different locations.

Locations	*Pseudomonas aeruginosa* (mm)	*Acinetobacter baumannii* (mm)	*Staphylococcus haemolyticus* (mm)	*Enterococcus faecalis* (mm)
Kanipanka	**11.067**	**0.000**	**12.100**	**8.667**
Sartak	**0.000**	**0.000**	**11.833**	**8.333**
Bakrajo	**0.000**	**0.000**	**14.333**	**11.500**
Qara Dagh	**9.167**	**0.000**	**12.267**	**9.667**
Penjween	**0.000**	**0.000**	**15.567**	**10.000**
Sharbazher	**9.333**	**0.000**	**12.667**	**9.667**
LSD $_{(p\leq0.05)}$	**0.913**	**0.000**	**2.064**	**1.646**
LSD $_{(p\leq0.01)}$	**1.280**	**0.000**	**2.894**	**2.228**

In terms of the *Staphylococcus haemolyticus* bacterial strain, the highest antibacterial activity was observed in Penjween (15.567 mm). The lowest value (11.833 mm) was also observed in Sartak. No significant effect of the essential oil against *Enterococcus faecalis* was obtained. However, the Bakrajo location exhibited the highest inhibition zone (IZ) level against *Enterococcus faecalis* (11.500 mm). In contrast, the lowest IZ was observed at the Sartak location (8.333 mm). Essential oil constituents possess potent antimicrobial properties against both the membrane and cytoplasm of microorganisms. Moreover, these components have been observed to induce morphological changes in plant cells under certain circumstances [37]. The presence of higher concentrations of chamomile bioactive chemicals, such as chamazulene, farnesene, and bisabolol oxide A and B, can easily inhibit the action of membrane enzymes and hinder bacterial strains from penetrating the wall [38]. Collectively, the findings show a notable influence exerted by EOs derived from *M. chamomilla* and its efficacy against diverse strains of pathogenic bacteria. The antibacterial attributes of chamomile essential oil delineate a discernible connection between varying altitudes and the distinct array of essential oil constituents, manifesting varying degrees of antibacterial potency, even within specific plant sources. The results are consistent with those of Sienkiewicz [39], who discovered that chamomile EOs have strong antibacterial abilities on *Staphylococcus aureus* and *Escherichia coli*. Similarly, *Pseudomonas aeruginosa* was among the studied bacterial strains gram positive and gram negative that chamomile EOs were shown to have strong antibacterial action against [40].

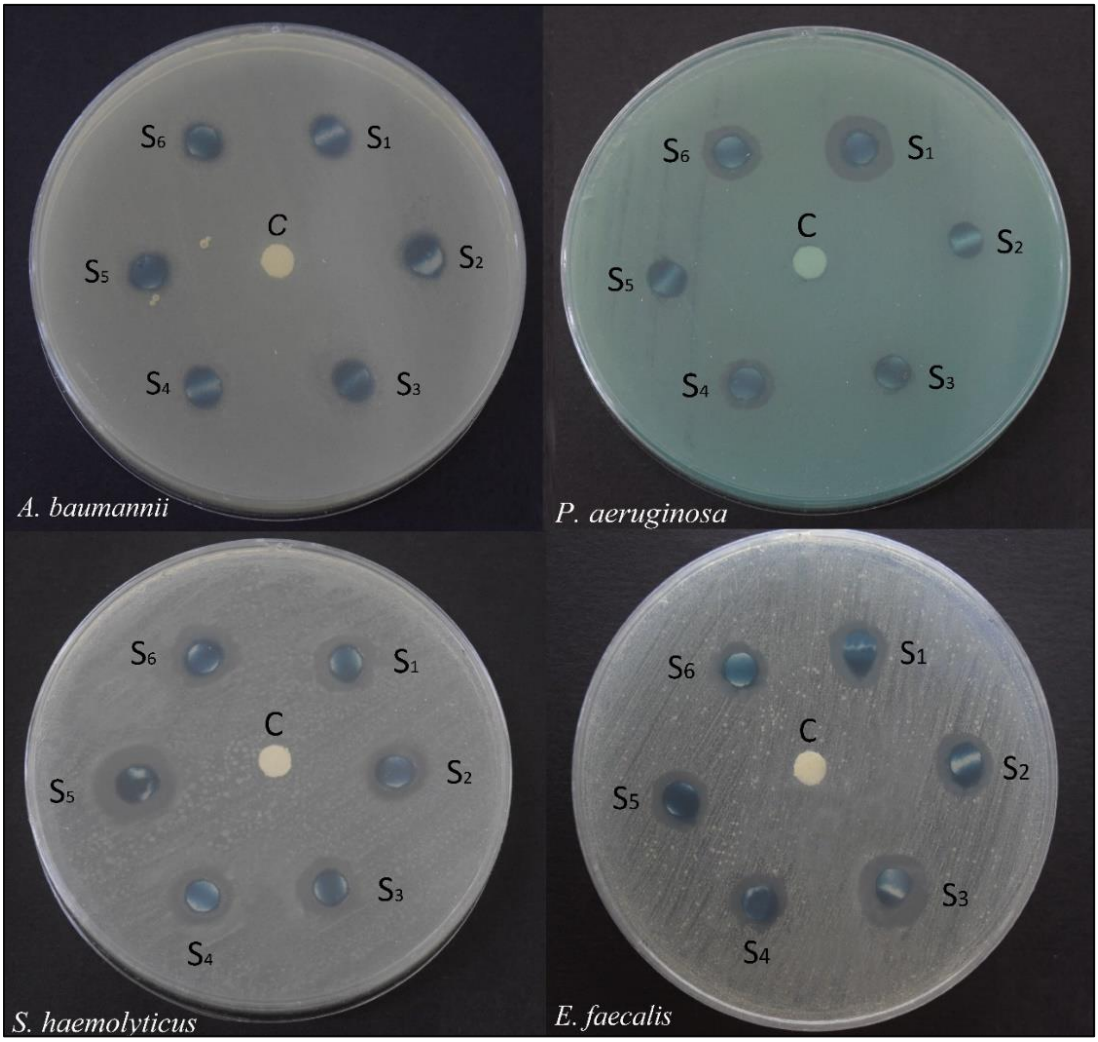

Figure (3): The inhibition zones for all studied bacterial stains, applied with different essential oils. S_1 Kanipanka, S_2 Sartak, S_3 Bakrajo, S_4 Qara Dagh, S_5 Penjween, S_6 Sharbazher, C Positive Control.

Furthermore, the chamomile EOs showed no antibacterial effect on *Acinetobacter baumannii* observed in this study, which is inconsistent with the findings of a study by Al-Mariri and Safi [41], they found that chamomile EOs have a significant antibacterial effect on *Acinetobacter baumannii*. Table 5 illustrates the diminished impact of EO exposure on gram-negative bacteria, specifically *A. baumannii,* and *P. aeruginosa*, in contrast to gram-positive bacteria. This discrepancy in response could be due to variances in the susceptibility of bacterial cell walls. Concerning the antibacterial efficacy of chamomile EOs, this discovery contrasts with the findings presented by Solidônio [42], who reported the absence of antibacterial effects in chamomile EOs on *P. aeruginosa*. Conversely, the present investigation demonstrates a

notable increase in the antibacterial effectiveness of the chamomile EOs on *P. aeruginosa* across three distinct geographical locations. This phenomenon is likely attributed to the existence of bioactive constituents within particular plant species that exhibit potent antibacterial properties. To support this observation, Aliheidari [43] posited that the heightened challenge in targeting gram-negative strains, such as *P. aeruginosa*, is due to their dual membrane composition, rendering attachment more intricate compared to gram-positive bacteria with a solitary membrane. The employment of specific bacterial strains and meticulous bioassay methodologies emerge as imperative prerequisites for the comprehensive investigation of antibacterial activity, particularly concerning the bioactive constituents housed within plant essential oils.

Chamomile EOs MIC and MBC

According to the data obtained from the previous section, the most sensitive bacteria from each strain were selected with the EOs from the location that gave the highest inhibition zone for further investigation to determine MIC and MBC (Tables 6 and 7). The chamomile EOs in Kanipanka gave maximum inhibition against *Pseudomonas aeruginosa,* with a 2.5 µg mL^{-1} MIC and MBC. Regarding the gram-positive bacteria, *Staphylococcus haemolyticus* gave a MIC and an MBC at a concentration of 0.16 and 0.63 µg mL^{-1} at the Penjween location, respectively. Tavakoli [44] reported that chamomile EOs exhibited a significant inhibitory effect against *Pseudomonas aeruginosa*, ranging from 0.5 to 1 µg mL^{-1} MIC. Similarly, another investigation by Guinoiseau (45) found that the MIC values ranged from 0.13 to 0.5 µg mL^{-1} of chamomile EOs against *Staphylococcus aureus*. Yoon [46] examined the antibacterial properties of chamomile EOs against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. They stated that the MIC ranged from 0.5 to 1.1 µg mL^{-1} concentration, and demonstrated a substantial inhibitory impact against both bacteria.

Table (6): MIC and MBC of *Pseudomonas aeruginosa* using Kanipanka location essential oil.

Concentrations	Essential oil concentration (µg mL^{-1})									
	40	20	10	5	2.5	1.25	0.63	0.32	0.16	0.08
MIC	-	-	-	-	-	+	+	+	+	+
MBC	-	-	-	-	-	+	+	+	+	+

+ refers to bacterial growth

- Refers to bacteriostatic or bactericidal effect

Table (7): MIC and MBC of *Staphylococcus haemolyticus* using Penjween location essential oil.

Concentrations	Essential oil concentration (µg mL^{-1})									
	40	20	10	5	2.5	1.25	0.63	0.32	0.16	0.08
MIC	-	-	-	-	-	-	-	-	-	+
MBC	-	-	-	-	-	-	-	+	+	+

+ refers to bacterial growth
- Refers to bacteriostatic or bactericidal effect

The chamomile essential oil's percentage and composition of active constituents are significantly influenced by its geographical location. The level of antioxidants found in chamomile flower essential oils differs considerably based on the geographical location of flower cultivation. The highest concentration of antioxidants was found in the essential oil extracted from the Sharbazher location. EOs of the *M. chamomilla* antibacterial efficacy were higher against gram-positive than gram-negative bacterial strains. The observed inhibitory effect is under previous investigations exploring the antimicrobial properties of chamomile essential oils.

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