



# Chemical Composition and Antimicrobial Activity of Essential Oils Obtained from *Inula germanica* L.

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**Abstract:** The genus *Inula* (Asteraceae), consisting of over 100 species predominantly found in Asia, Europe, and Africa, is recognized for its antimicrobial properties. This study explores the chemical composition and antimicrobial activity of essential oils obtained from the aerial parts of *Inula germanica* L. through conventional (T) and modified (M1, M2) hydrodistillation methods. Using gas chromatography and gas chromatography-mass spectrometry, the conventional method (T) yielded  $\alpha$ -bisabolol (30.1%) and 12-carboxydeudesma-3,11(13)-diene (14.9%) as major compounds. The modified method fraction M1 enriched monoterpenes such as *trans*-verbenol (9.5%), 1,8-cineole (9.5%), and *cis*-chrysanthenyl acetate (9.3%), whereas M2 was characterized by higher levels of sesquiterpenes like 12-carboxydeudesma-3,11(13)-diene (24.3%) and  $\alpha$ -bisabolol (11.7%). The essential oils exhibited moderate to weak antimicrobial activity against tested bacterial and *Candida* strains, with minimum inhibitory concentrations (MICs) ranging from 125 to 2000  $\mu$ g/mL. Among all studied samples, M1 showed the best antimicrobial activity against *Candida* strains in the MIC range of 125-500  $\mu$ g/mL.

**Keywords:** *Inula*; essential oils; hydrodistillation methods. © 2024 ACG Publications. All rights reserved.

## 1. Introduction

The genus *Inula* (Asteraceae), comprising over 100 species primarily distributed across Europe, Asia, and Africa, is characterized by its distinct morphology, featuring erect or ascending, often branched stems, and typically serrated leaves arranged along the stem [1, 2]. Employed in conventional medicine across various cultures, *Inula* species are valued for their diverse biological activities. *Inula helenium* L. has been utilized in Europe and Asia for its expectorant, antitussive, diaphoretic, and bactericidal properties, aiding in the treatment of bronchitis, tuberculosis, and enterogastritis. *Inula britannica* L. and *Inula japonica* Thunb. are recognized in Traditional Chinese Medicine for their efficacy in treating bacterial and viral infections, inflammation, and tumors [3, 4]. In Turkey, *I. helenium*, referred to as Andızotu, is traditionally employed for its dried roots, which have been reported to demonstrate expectorant, antitussive, tonic, and anthelmintic properties. Aerial

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parts of *Inula heterolepis* Boiss. are used in decoctions for the treatment of colds, bronchitis, and gastrointestinal disorders [5, 6].

The *Inula* species are rich in a variety of bioactive compounds, including terpenoids, flavonoids, and glycolipids, with sesquiterpenes particularly recognized for their antiproliferative and cytotoxic properties. These compounds collectively exhibit significant bioactivity, underscoring their potential for therapeutic applications [7-9]. The essential oil compositions of *Inula* species predominantly comprise monoterpenes (e.g., borneol and its derivatives [10] or monoterpene hydrocarbons, such as  $\alpha$ -phellandrene,  $\beta$ -phellandrene, and *p*-cymene [11, 12]), sesquiterpenes (e.g., alantolactone and diplophyllin [13, 14]). These constituents are especially notable for their antioxidant, antimicrobial, and anti-inflammatory activities, further highlighting the pharmacological significance of this genus.

*Inula germanica* L. is a species that has been studied to a limited extent. Related available literature research has studied the chemical composition of *I. germanica* in different locations, revealing significant variances in their volatile profiles and primary constituents caused by both ecological and genetic factors [15, 16]. A study carried out with *I. germanica* from Bulgaria revealed a composition dominated by oxygenated monoterpenes. The most abundant components were *cis*-carvyl acetate (20.7%) and 1,8-cineole (14.6%) [16]. In contrast, a Turkish study that worked on a sample collected from Erzincan, the eastern region of the country, found a sesquiterpene-rich profile. The primary components were  $\beta$ -caryophyllene (20.4%), germacrene D (11.4%), and borneol (11.3%) [15]. Our research examines a sample that was collected in Ödemiş/ İzmir, a region located in the western region of Türkiye.

This study was conducted on a sample of *Inula germanica* collected from a different geographical location than those previously reported in the literature. Alternative extraction techniques were used together with conventional hydrodistillation methods, and two additional essential oil fractions with unique compositions were obtained. This approach not only reveals the differences in the phytochemical profiles of *I. germanica* essential oil grown in different regions but also contributes to the originality and scientific value of this research by highlighting the differences in the essential oil profiles of various methodologies.

## 2. Materials and Methods

### 2.1. Plant Material

The botanical specimens were gathered from Ödemiş, İzmir in June 2015. The voucher specimens have been confirmed and deposited in the Herbarium of the Faculty of Pharmacy at Istanbul University, Türkiye (ISTE 109572). The scientific names of all identified taxa and families were verified using the World Flora Online Plant List (<https://wfoplantlist.org/>). To ensure clarity, the synonyms were also provided in brackets.

### 2.2. Isolation of the Essential Oil

Essential oils were extracted from freshly air-dried *Inula germanica* plant materials using a conventional hydrodistillation method (T) [17, 18] and a modified two-stage hydrodistillation method, which produced two fractions (M1 and M2) [19]. The oils were dried using anhydrous sodium sulfate and subsequently stored at +4°C in the dark until testing and analysis.

### 2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis was conducted using an Agilent 5975 GC-MSD system for GC-MS. The Innowax FSC column (60 m x 0.25 mm, 0.25  $\mu$ m film thickness) was employed with helium as the carrier gas at a flow rate of 0.8 ml/min. The GC oven temperature was set at 60 °C for 10 minutes, subsequently raised to 220 °C at a rate of 4 °C/min and held constant at 220 °C for an additional 10 minutes, before being programmed at 240 °C at a rate of 1 °C/min. The split ratio was altered to 40:1.

## Essential oil of *Inula germanica* L.

The injector temperature has been set at 250 °C. Mass spectra were obtained at 70 eV. The mass range extended from m/z 35 to 450.

### 2.4. Gas Chromatography (GC) Analysis

The Agilent 6890N GC system was employed to perform the GC analysis. The temperature of the FID detector was 300°C. Simultaneous auto-injection was carried out on a duplicate of the same column under identical operational conditions to achieve the same elution order with GC-MS. The FID chromatograms were used to determine the relative percentage quantities of the separated compounds.

The essential oil components were identified by comparing their relative retention times to those of authentic samples or by comparing their relative retention index (RRI) with a series of n-alkanes. The identification was carried out by computer matching against commercial (Wiley GC/MS Library, Mass Finder 3 Library) [20, 21] and in-house "Başer Library of Essential Oil Constituents" [22, 23], which consists of genuine components and compounds of known oils, as well as MS literature data.

### 2.3. Antimicrobial Assay

Antibacterial and anticandidal effects of the samples were evaluated by using partly modified CLSI (formerly NCCLS) microdilution broth methods M7-A7 and M27-A2 respectively [24, 25, 26]. Chloramphenicol (Merck), Ampicillin (Merck), Amphotericin-B (Sigma-Aldrich) and Ketoconazol (Sigma-Aldrich) were used as standard antimicrobial agents. *Candida albicans* ATCC 10231, *Candida utilis* NRRL Y-900, *Candida glabrata* ATCC 2001, *Candida tropicalis* ATCC 1369, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-4378, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 10145 and *S. epidermidis* ATCC 14990 were used as test microorganisms.

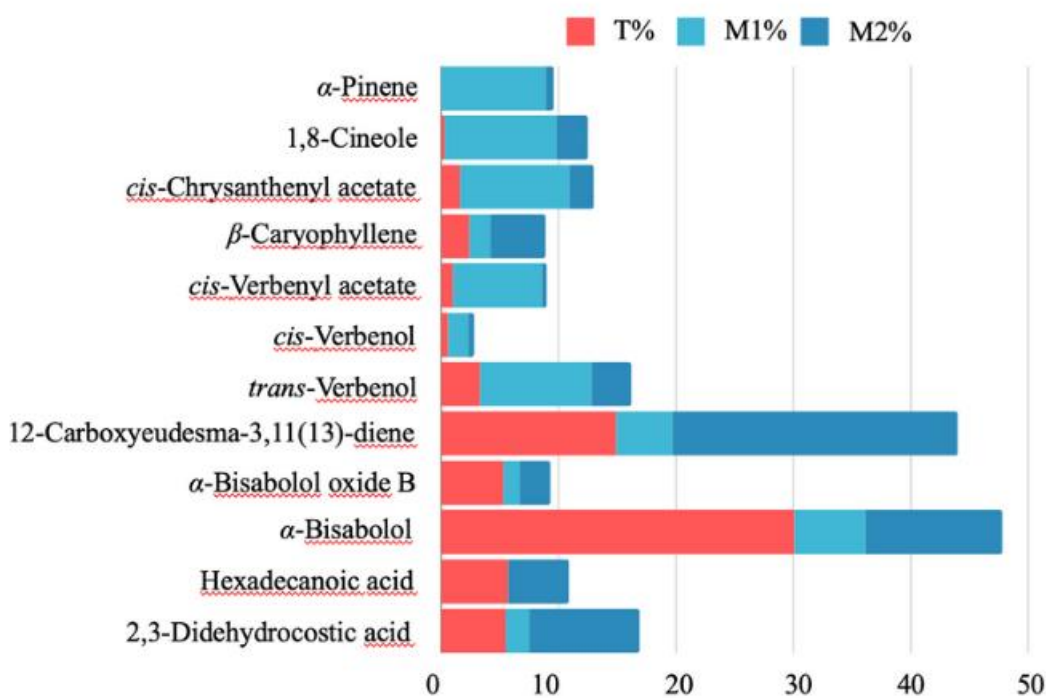
*Candida* cultures stocked at -85°C were inoculated onto Potato Dextrose Agar (Fluka) while bacteria were inoculated onto Mueller Hinton agar (Fluka) to check purity of cultures. All tests were performed by 96 multiwell microdilution plates. Unlike the standard protocol, stock solutions of the essential oils were prepared at a concentration of 4 mg/mL and diluted serially in micro-wells. Antibacterial test plates incubated at 35±2 °C for 16-20h while the anticandidal tests plates incubated at 35°C for 24h. After incubation period, a considerable decrease in turbidity in the rows was accepted as growth inhibition. Furthermore, according to M27-A2 method, *C. krusei* (ATCC ® 6258) and *C. parapsilosis* (ATCC ® 22019) were used as QC strains. All the experiments were performed in duplicate.

## 3. Results and Discussion

### 3.1. Essential Oil Composition

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were employed to analyze the essential oils from *Inula germanica* aerial parts, which were obtained using conventional (T) and modified hydrodistillation (M1, M2) methods. Conventional hydrodistillation (T) involved exposing the plant material directly to boiling water. To address the potential degradation of thermally sensitive compounds caused by direct exposure, a two-stage hydrodistillation process was designed. This method allowed for the separation of two distinct fractions (M1 and M2), ensuring the preservation of volatile components while minimizing thermal damage. The results of the analysis reveal that the chemical composition varies significantly depending on the extraction method. The conventional hydrodistillation (T) method primarily produced high levels of sesquiterpenes, including  $\alpha$ -bisabolol (30.1%) and 12-carboxydeudesma-3,11(13)-diene (14.9%), which are recognized for their biological properties. In contrast, M1 exhibited an increase in monoterpene content, with a particular emphasis on  $\alpha$ -pinene (8.9%) and 1,8-cineole (9.5%). This is likely a result of the procedural

modifications that favor the extraction of lighter, more volatile compounds. M2 improved the concentration of specific sesquiterpenes, including 12-carboxyeudesma-3,11(13)-diene (24.3%), while maintaining a moderate level of  $\alpha$ -bisabolol (11.7%). This resulted in a balanced chemical profile between M1 and T (Table 1, Figure 1, Figure S1-S3). In a nutshell, T generates a sesquiterpene-rich oil, M1 enriches monoterpenes, and M2 enhances specific sesquiterpenes.



**Figure 1.** Distribution of main components in essential oils from *Inula germanica*

In comparison to prior research on *Inula germanica* from Bulgaria and Turkey, our study also investigates the influence of different extraction methods on the oil composition [15, 16]. According to present literature, Turkish samples of the plant comprise a high amount of  $\beta$ -caryophyllene among other sesquiterpenes, while Bulgarian *I. germanica* primarily contained oxygenated monoterpenes, with *cis*-carvyl acetate (20.7%) and 1,8-cineole (14.6%) as main components. The comparative evaluation suggests that Bulgarian sample and Turkish sample are similar in terms of oxygenated monoterpenes, particularly 1,8-cineole, while Turkish sample is more aligned with T due to its high  $\beta$ -caryophyllene content. It is important to note that the composition of the Turkish sample also contains germacrene D (11.4%) and borneol (11.3%), which were not present in the essential oils used in this study. The Bulgarian sample of *I. germanica*, exhibiting a high 1,8-sineole content of 14.6%, demonstrates the closest similarity to M1, which has a content of 9.5%, indicating a shared profile in oxygenated monoterpenes. The essential oil composition is considerably influenced by the extraction method and collection area, which results in the development of unique chemotypes that may be suitable for a variety of biological applications.

Essential oil of *Inula germanica* L.**Table 1.** Chemical composition of the essential oil of the *Inula germanica*

No	RRI	RRI*	Compound	T (%)	M1 (%)	M2 (%)	IM
1	1032	1021	$\alpha$ -Pinene		<b>8.9</b>	0.7	$t_R$ , MS
2	1118	1107	$\beta$ -Pinene		2.2		$t_R$ , MS
3	1135	1128	Thuja-2,4(10)-diene		1.6		MS
4	1174	1156	Myrcene		0.5		$t_R$ , MS
5	1188	1176	$\alpha$ -Terpinene		0.5		$t_R$ , MS
6	1203	1196	Limonene		1.5		$t_R$ , MS
7	1213	1211	1,8-Cineole	0.3	<b>9.5</b>	2.7	$t_R$ , MS
8	1255	1239	$\gamma$ -Terpinene		1.1	0.4	$t_R$ , MS
9	1280	1430	<i>p</i> -Cymene		1.0	0.3	$t_R$ , MS
10	1474	1466	<i>trans</i> -Sabinene hydrate		0.6		MS
11	1505	1505	Dihydroedulane II			0.8	MS
12	1532	1508	Camphor			0.9	$t_R$ , MS
13	1556	1560	<i>cis</i> -Sabinene hydrate		0.3		MS
14	1572	1412	$\alpha$ -Bergamotene	0.2			MS
15	1583	1568	<i>cis</i> -Chrysanthenyl acetate	1.6	<b>9.3</b>	2.1	MS
16	1611	1590	Terpinen-4-ol			0.1	$t_R$ , MS
17	1612	1588	$\beta$ -Caryophyllene	2.4	1.8	4.7	$t_R$ , MS
18	1645	1280	<i>cis</i> -Verbenyl acetate	1.0	7.6	0.4	MS
19	1663	1143	<i>cis</i> -Verbenol	0.6	1.7	0.5	MS
20	1683	1680	<i>trans</i> -Verbenol	3.3	<b>9.5</b>	3.4	MS
21	1687	1658	$\alpha$ -Humulene	0.3		0.4	$t_R$ , MS
22	1709	1695	$\alpha$ -Terpinyl acetate	0.6	2.1	2.1	$t_R$ , MS
23	1744	1744	$\alpha$ -Selinene	0.4			MS
24	1764	1764	<i>cis</i> -Chrysanthenol		0.9	0.1	MS
25	1773	1747	$\delta$ -Cadinene	0.1			MS
26	1776	1750	$\gamma$ -Cadinene	tr			MS
27	1786	1786	<i>ar</i> -Curcumene	0.4		0.6	MS
28	1804	1804	Myrtenol		0.5	0.7	MS
29	1981	1922	12-Carboxyeudesma-3,11(13)-diene	<b>14.9</b>	4.8	<b>24.3</b>	MS
30	2008	2000	Caryophyllene oxide	1.4	0.9	2.2	$t_R$ , MS
31	2050	2032	( <i>E</i> )-Nerolidol	0.8		0.8	$t_R$ , MS
32	2056	1653	13-Tetradecanolide	0.6		0.9	MS
33	2071	2071	Humulene epoxide-II	0.5		0.5	MS
34	2073	1930	2,3-Didehydrocostic acid	5.5	2.0	9.4	MS
35	2131	2125	Hexahydrofarnesyl acetone	3.7		1.4	MS
36	2144	2136	Spathulenol			0.4	MS
37	2148	1570	( <i>Z</i> )-3-Hexen-1-yl benzoate	0.4		0.2	MS
38	2156	2156	$\alpha$ -Bisabolol oxide B	5.3	1.4	2.6	MS
39	2232	1685	$\alpha$ -Bisabolol	<b>30.1</b>	6.0	<b>11.7</b>	$t_R$ , MS
40	2260	2260	15-Hexadecanolide	1.6		0.9	MS
41	2255	2227	$\alpha$ -Cadinol			0.6	MS
42	2298	2263	Decanoic acid			0.3	$t_R$ , MS
43	2316	2316	Caryophylladienol I			0.4	MS
44	2324	2324	Caryophylladienol II	1.3		1.0	MS

45	2376	2247	(Z)- <i>trans</i> - $\alpha$ -Bergamotol	1.1		$t_R$ , MS
46	2389	2353	Caryophyllenol I	1.1	0.7	MS
47	2392	2389	Caryophyllenol II	0.6	0.9	MS
48	2438	2200	$\alpha$ -Bisabol oloxide A		1.4	MS
50	2500	2500	Pentacosane	1.6	1.2	$t_R$ , MS
51	2545	2545	(Z)-Nuciferol	1.6	0.9	MS
52	2622	2619	Phytol	2.9	1.4	MS
53	2670	2670	Tetradecanoic acid	1.6	1.1	$t_R$ , MS
54	2700	2700	Heptacosane		0.2	$t_R$ , MS
55	2822	2822	Pentadecanoic acid		0.2	$t_R$ , MS
56	2931	1961	Hexadecanoic acid	5.7	5.2	$t_R$ , MS

RRI Relative retention indices calculated against *n*-alkanes

% calculated from FID data

t. Trace (< 0.1 %)

RRI\* Retention index reported in the literature. Reference [27] for compounds 1-2, 4-6, 8-9, 12, 16-17, 21, 25-26, 31, 41, 50; reference [16] for compounds 7, 10-11, 13, 15, 20, 24, 30, 33, 35-36, 40, 42-44, 46-47, 52-55; reference [28] for compound 14; reference [29] for the compounds 27-28, 38-39, 48; reference [30] for the compound 3; reference [31] for the compound 23; reference [32] for the compounds 18, 22, 56; reference [33] for the compounds 19, 37; reference [34] for the compounds 45, 51; reference [35] for the compounds 29, 34 and reference [36] for the compound 32.

IM  $t_R$ , Identification method based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

### 3.2. Chemotaxonomic Evaluation

The chemical content of essential oils from various *Inula* species exhibits strong variability, influenced by geographic location, plant part, and extraction method. This variability reflects the metabolic diversity of the genus and its potential chemotaxonomic and pharmaceutical importance.

Depending on its geographic origin, the essential oil of *Inula oculus-christi* L. demonstrates different profiles. In contrast to the Iranian samples, which contained large concentrations of pentacosane (13.7%), palmitic acid (13.6%), dill apiole (11.4%), and methyl eugenol (9.6%), the Serbian samples were primarily composed of caryophyllene oxide (9.8%), *trans*-longipinocarveol (9.2%), and 1,8-cineol (7.3%) [33, 37]. A similar situation is observed for *Inula crithmoides* L. (*Limbarda crithmoides* (L.) Dumort.). Essential oils from this species, collected in Sicily [38], Central Italy [39], Tunisia [40], Spain, Malta, and Greece [11], are dominated by monoterpene hydrocarbons (32.1–87.4%), such as  $\alpha$ -phellandrene (2.2–26.2%) and *p*-cymene (trace–53.8%). Notably,  $\beta$ -phellandrene was found only in Greek (30.7%) and Sicilian (8.5%) samples, while  $\beta$ -myrcene, typically a key monoterpene, was nearly absent in all analyzed oils. Most samples, except those from Sicily and Malta, lacked sesquiterpene derivatives. Unique to Sicilian samples was the presence of thymol (2.1%) and the absence of compounds like scopoletin and (12Z)-abienol [11, 39].

The dominant components of the essential oil of *Inula germanica* are  $\alpha$ -bisabolol and 12-carboxyeudesma-3,11(13)-diene, which are oxygenated monoterpenes. These results are consistent with one prior research, which also emphasized the environmental variability in chemical profiles of *Inula germanica* [15, 16]. *Inula graveolens* (L.) Desf. (syn. *Ditrichia graveolens* (L.) Greuter) and *Inula viscosa* (L.) Aiton (syn. *Ditrichia viscosa* (L.) Greuter) are also characterized by oxygenated monoterpenes, with bornyl derivatives, including borneol and bornyl acetate, being the primary constituents [16, 41, 42]. The absence of monoterpene hydrocarbons is a common characteristic observed in several *Inula* species, including *I. aschersoniana* Janka [43], *I. verbascifolia* Hausskn. [38, 44], *I. oculus-christi* [33, 45, 37], *I. britannica* M.Bieb [37], and *I. cuspidata* (DC.) C.B. Clarke [28].

The essential oil of *Inula helenium* Asso is primarily composed of sesquiterpene lactones, with alantolactone, isoalantolactone, and diplophyllin being the predominant constituents [3, 13, 14, 46]. *Inula britannica* contains significant levels of sesquiterpenoids, notably viridiflorol (8.2%) and himachalol (8.71%), as well as lactones like 13-tetradecanolide (4.87%) [36].

Essential oil of *Inula germanica* L.

Fatty acids represent a crucial component in species like *Inula ensifolia* L. and *Inula oculus-christi*. In *Inula ensifolia*, hexadecanoic acid (12.4%) and 1,8-cineole (9.1%) were the most significant components, along with sesquiterpenoids such as  $\beta$ -bourbonene and intermedeol. The predominance of fatty acids contrasts with the profiles rich in sesquiterpenes and monoterpenes found in other *Inula* species [31]. Rare compounds, including  $\gamma$ -palmitolactone, dihydroactinidiolide, and macrolides, have been identified in *Inula britannica* from Bulgaria [36]. Additionally, theaspiranes and dihydroedulane are reported in *Inula viscosa* and *Inula thapsoides* [42, 47]. Caryophylladienol and caryophyllenols are present in species like *Inula graveolens* and *Inula viscosa*, enhancing the chemotaxonomic diversity of the genus [41].

The *Inula* genus exhibits diverse chemical profiles, prominently featuring sesquiterpenoids, oxygenated monoterpenes, and fatty acids as the primary classes of compounds. Geographic and environmental factors, as well as genetic variability, are essential in shaping these profiles. The existence of distinctive and bioactive compounds highlights the genus's potential for pharmaceutical and chemotaxonomic uses. Future research on underexplored species and regional variations will improve our understanding of the metabolic diversity within this genus.

## 3.3. Antimicrobial Assay

The antimicrobial efficacy of *Inula germanica* essential oils, extracted through conventional (T) and modified hydrodistillation (M1, M2) methods, exhibits significant variations against various fungal and bacterial strains. Comparisons are drawn with conventional antifungal agents (Ketoconazole, Amphotericin-B) and antibacterial agents (Ampicillin, chloramphenicol). The minimum inhibitory concentration (MIC) values of *Inula germanica* essential oils are summarized in Table 2.

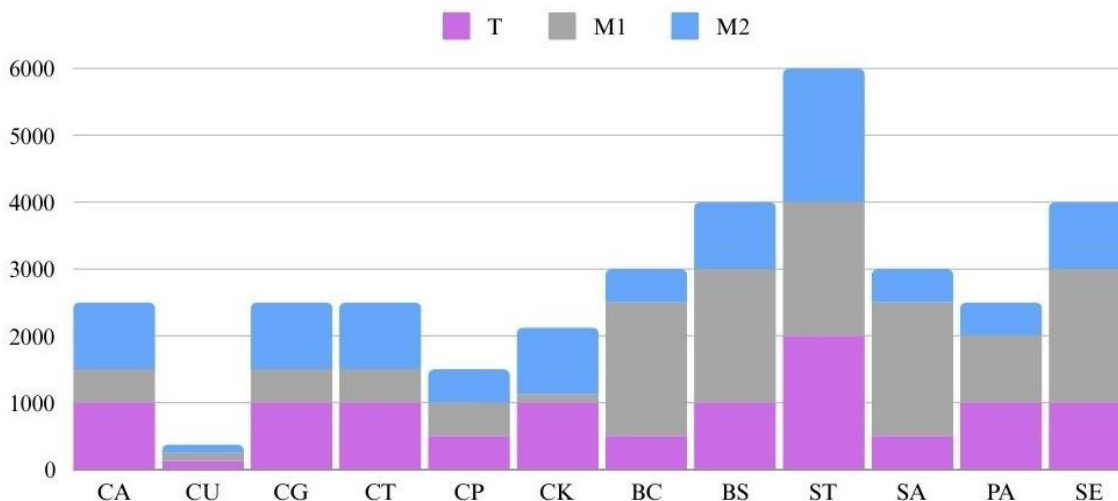
**Table 2.** Antimicrobial Activity results of the *I. germanica* essential oil (MIC  $\mu\text{g/mL}$ )

Microorganisms	Source	T	M1	M2	St-1	St-2
<i>Candida albicans</i>	ATCC 10231	1000	500	1000	1	0.06
<i>Candida utilis</i>	NRRL Y-900	125	125	125	1	0.12
<i>Candida glabrata</i>	ATCC 2001	1000	500	1000	1	0.06
<i>Candida tropicalis</i>	ATCC 1369	1000	500	1000	1	0.06
<i>Candida parapsilosis</i>	ATCC 22019	500	500	500	0.5	0.06
<i>Candida krusei</i>	ATCC 6258	1000	125	1000	1	0.25
					<b>St-3</b>	<b>St-4</b>
<i>Bacillus cereus</i>	NRRL B-3711	500	2000	500	1	4
<i>Bacillus subtilis</i>	NRRL B-4378	1000	2000	1000	1	1
<i>Salmonella typhimurium</i>	ATCC 14028	2000	2000	2000	0.5	2
<i>Staphylococcus aureus</i>	ATCC 43300	500	2000	500	16	1
<i>Pseudomonas aeruginosa</i>	ATCC 10145	1000	1000	500	32	64
<i>S. epidermidis</i>	ATCC 14990	1000	2000	1000	0.5	2

T: Essential oil of T, M1: Essential oil of M1, M2: Essential oil of M2, St-1: Amphotericin-B, St-2: Ketoconazole, St-3: Ampicillin, St-4: Chloramphenicol

Essential oil M1 demonstrated better inhibitory activity against fungal strains than bacteria, exhibiting a minimum inhibitory concentration (MIC) of 125  $\mu\text{g/mL}$  for *Candida utilis* and *Candida krusei*. This activity exceeded that of conventional oil T and modified oil M2, led to MICs of 1000  $\mu\text{g/mL}$  for the same strains. M1 demonstrated a minimum inhibitory concentration (MIC) of 500  $\mu\text{g/mL}$  against *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*, showing slightly improved antifungal activity compared to T and M2 (Figure 2). Nonetheless, these values are considerably greater than those of Amphotericin-B (MIC= 1  $\mu\text{g/mL}$ ) and Ketoconazole (MIC as low as 0.06  $\mu\text{g/mL}$ ), suggesting that, although M1 exhibits some enhanced activity, it remains strongly less effective than standard antifungals.

M2 exhibited superior antibacterial efficacy against *Pseudomonas aeruginosa*, with a minimum inhibitory concentration (MIC) of 500 µg/mL, whereas T and M1 showed higher MICs of 1000 µg/mL each. M1 and T demonstrated efficacy against *Bacillus cereus*, exhibiting a MIC of 500 µg/mL. However, M1's activity was significantly reduced against other bacterial strains, with MICs reaching 2000 µg/ml for *Bacillus subtilis* and *Salmonella typhimurium*. M2 also demonstrated comparable performance to T against *Staphylococcus aureus* and *Bacillus cereus*; however, its MICs were significantly higher than those of the standard antibiotics, ampicillin, and chloramphenicol.



**Figure 2.** Antifungal and antibacterial effects of the obtained essential oils (T: Essential oil of T, M1: Essential oil of M1, M2: Essential oil of M2, CA: *Candida albicans*, CU: *C. utilis*, CG: *C. glabrata*, CT: *C. tropicalis*, CP: *C. parapsilosis*, CK: *C. krusei*, BC: *Bacillus cereus*, BS: *B. subtilis*, ST: *Salmonella typhimurium*, SA: *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, SE: *S. epidermidis*)

In conclusion, M1 and M2 demonstrate improvements over T regarding antimicrobial efficacy against certain strains; however, they remain considerably less effective than standard antimicrobial agents, especially in clinical applications. This indicates that *Inula germanica* essential oils may possess limited efficacy as standalone antimicrobial agents; however, the increased activity of modified extractions requires further exploration for potential synergistic applications.

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