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Tripleurospermum disciforme (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip, and *Achillea biebersteinii* Afan.: efficiency, chemical profile, and biological properties of essential oil

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Abstract

Background: *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip., and *Achillea biebersteinii* Afan. are the most important species of the Asteraceae family that are used in traditional medicine as antiseptics, analgesics, and anti-ulcers. This study aimed to evaluate and compare the yield, chemical profile, and antibacterial and antifungal properties of the essential oils of these three species for the first time. For this purpose, plant materials were collected in June 2019 from Javinan region (Kashan, Iran).

Results: Based on the ANOVA results the species had a significant effect on yield, chemical composition, and diameter of the inhibition zone of some microorganisms ($P \leq 0.01$). The highest yield belonged to *T. disciforme* essential oil (~1.433%). Analysis of essential oil compounds showed that in *T. disciforme*, anisole, *p*-1-cyclohexen-1-yl- (55.95%), modephene (10.00%), and *cis*- β -farnesene (11.94%), in *T. parthenium*, camphor (43.43%), camphene (9.40%), and bornyl acetate (6.76%), and in *A. biebersteinii* linalool (34.49%), *p*-cymene (15.31%), and α -terpineol (7.43%) were the main and predominant compounds. The highest inhibition zone diameter by the essential oil of *T. parthenium* and *A. biebersteinii* against *Aspergillus brasiliensis* (~13 mm) was observed. The strongest inhibitory and lethal activity was related to *T. disciforme* essential oil against *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Escherichia coli* (~8.50 mm), and *Candida albicans* (MIC and MBC = 62.5 μ g/mL), which were equivalent to rifampin and twice as potent as nystatin, respectively.

Conclusions: Therefore, the essential oil of the studied species of Asteraceae may be a promising and potential strategy for controlling some microorganisms and a possible natural alternative to some antibiotics.

Keywords: Asteraceae, Yield, Essential oil compounds, Antimicrobial properties, *T. disciforme*

Introduction

Despite major medical breakthroughs over the past three decades, infectious diseases are still one of the leading causes of death in the world [1]. The discovery of antibiotics increases human health and longevity. However,

increasing antibiotic resistance decreases the antibacterial activity of antibiotics [2]. Improper, excessive, and haphazard use of antibiotics to treat infectious diseases is one of the main reasons for the emergence of resistant microorganisms [3]. The global problem of antimicrobial resistance is of particular importance in developing countries, where bacterial and fungal infectious diseases are highly prevalent and cost constraints prevent the widespread use of newer and more expensive agents [4].

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These infections are caused by several bacterial strains, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [5]. Even after treatment, *Candida* strains cause high mortality, ranging from 40 to 60% [6]

Antimicrobial compounds of medicinal plants are one of the valuable resources in medicine and therefore, despite the spread of infectious diseases, identifying more of these plants and purifying their effective compounds can be useful in treating diseases [7, 8]. Essential oils have bioactivities including antimicrobial, antioxidant, anti-diabetic, and anti-cancer activities [9]. Their reputation as disinfectants stems from their use in traditional medicine around the world [10]. Essential oils with antibacterial activity can be a promising factor for this purpose. These compounds often affect the cell wall of microorganisms and due to their hydrophobic and oily nature, they penetrate the membrane structure [11]. Consequently, ions are released, resulting in changes in cell osmotic pressure and, finally, the death of microorganisms [12].

Asteraceae is the largest plant genus [13], and has been studied extensively due to many different genera and species, its global distribution, and the fact that it is endowed with many beneficial plants [14]. Previous studies on the antimicrobial properties of Asteraceae plants show their moderate-to-strong antimicrobial activity [15, 16].

Tripleurospermum disciforme (C.A.Mey.) Sch.Bip. is known as the “Plain Chamomile” of the Asteraceae family [17]. This plant has been cultivated in many parts of Iran and has been used in Iranian herbal medicine as a sedative and anti-inflammatory, and for relaxation, muscle pain, fatigue, and also in stress therapy [18]. In Iran, it has had many traditional and folk uses [19], but there were few reports of its antimicrobial effects [20]. According to recent studies, the essential oil of this species has anti-inflammatory [21–23], antispasmodic and antiseptic [17, 24], antifungal [25, 26], antibacterial [20, 27], and antioxidant effects [28, 29]. So far, little research has been done on the chemical composition of *T. disciforme* essential oil. The main constituents of this essential oil are *p*-methoxy- β -cyclopropylstyrene, (E)- β -farnesene, β -sesquiphellandrene, and *cis*-calamenene [27, 29–34].

Tanacetum parthenium (L.) Sch.Bip. is a plant with a long history of use in traditional medicine [35]. All parts of the plant have a foul odor, especially after rubbing [36]. It is popularly known as “Feverfew” [37]. It is a well-known drug for treating a variety of ailments, including osteoarthritis, fever, dizziness, migraine, menstrual disorders, stomach pain, toothache, insect bites, and psoriasis [38]. According to recent studies, the essential oil of this plant has anti-inflammatory [39, 40], anti-cancer [41], antibacterial [15, 42–45], antifungal [46, 47],

antiviral [48], and insecticidal effects [49]. The biological activity of this plant’s essential oil is due to terpenoid components and the content of phenolic compounds including phenolic acids and flavonoids [40, 49]. The essential oils of *Tanacetum* species contain mainly sesquiterpenoids and flavonoids, while other terpenoids and phenolic compounds are rarely found in these plants [50]. In most cases, camphor and chrysanthenyl acetate are the main components in *T. parthenium* essential oil along with various secondary components [51–53]. While camphene, *p*-cymene, and (E)-Chrysanthenole were found in other studies in addition to previous superiority [15, 54, 55]. Some studies have shown that there are large amounts of sesquiterpene, lactone, parthenolide, and flavonoids in this plant, indicating its strong antibacterial activity [40, 55–57].

Due to the synthesis of significant amounts of secondary metabolites, especially essential oils, *Achilles* is one of the most important and valuable medicinal plants in the world and has wide applications in the pharmaceutical, cosmetic, and health industries of plant essential oils [58]. *Achillea biebersteinii* Afan. is one of the medicinal species of *Achillea* that has been used as an anti-flatulence and carminative medicine in Jordanian traditional medicine for a long time, while in Turkey it is used as a medicine to relieve abdominal pain and heal wounds [59–62]. Various biological activities such as antifungal [63, 64], antibacterial [64], antioxidant [64–67] and insecticidal effects [68, 69] have been found in the essential oil of *A. biebersteinii*. According to previous studies, the main components of *A. biebersteinii* essential oil are oxygen monoterpenes. In this plant, piperitone, camphor, borneol, 8,1-cinnamol, para-cement, and ascaridole have been identified as the main constituents of essential oil [62, 64, 68–74].

A review of studies shows that there are few reports of antimicrobial activity in the essential oils of these three species from some regions of Iran and the world against some microorganisms. On the other hand, so far no comprehensive and simultaneous study has been done on these species using different strains of microorganisms. Therefore, this research aimed to study and compare the chemical composition and antimicrobial activity of the essential oils of these three species against 11 strains.

Materials and methods

Plant species collection and identification

In order to sample the plants under study during their full flowering time, flowering branches of *A. biebersteinii* and flowers of *T. disciforme* and *T. parthenium* were randomly collected from three points and different rootstocks (100 rootstocks per region) in Javanan region, located in Kashan, Iran (longitude: 51° 26′ 48″ E; latitude:

33° 41' 22" N) in June 2019. After harvesting, the samples were transferred to the laboratory. Also, a complete plant sample of the species was collected and after identification in the herbarium of the Faculty of Natural Resources and Earth Sciences, University of Kashan, Kashan, was coded and stored. The plant was identified by Mansureh Ghavam and recorded with Code number 1410, 1411, and 1412.

Extraction of essential oils by hydrodistillation (Clevenger apparatus)

After complete drying, the samples were reduced to fine particles by a small electric mill. Then 100 g of each plant sample was weighed and its essential oil was extracted by distillation with water and using a Clevenger for 5 h. The weight of essential oil collected after dehydration with sodium sulfate was accurately calculated and the yield of essential oil of three replications (points) was reported as the mean \pm standard deviation. The essential oils were then stored in dark bottles at 4 °C until use in the next step.

Analysis of essential oil compounds

Gas chromatography–mass spectrometry

The composition of the obtained essential oil samples was determined by GC–MS. Chromatograph model 6890 coupled with Agilent mass spectrometer model N-5973 with capillary column HP-5MS with a static phase of 5% methylphenyl siloxane (length 30 m, inner diameter 0.25 mm, and thickness of static layer 0.25 μ m) and ionization energy of 70 eV were used to qualitatively identify the components. Temperature programming for analysis was first set at 60 °C and then increased by 3 °C/min to reach 246 °C. The temperature of the injector and the detector was 250 °C, the volume of the injected sample was 1 μ L with a split mode of 1.50, and helium gas with a flow rate of 1.5 mL/min.

Identification of chemical constituents of essential oils

Chemical components of essential oils were identified based on chromatogram analysis of each essential oil sample in relation to inhibition indices (RI), *n*-alkane (C8–C20) mixed standards, and mass spectral data of each peak using a computer library (spectral library Wiley-14 and NIST-14) and comparing the results with the results in the literature [75].

Determination of antibacterial and antifungal activities

Microbial strains and growth conditions

Standard microbial strains include four Gram-positive bacteria *Staphylococcus epidermidis* (CIP 81.55), *Staphylococcus aureus* (ATCC 29737), *Streptococcus pyogenes* (ATCC 19615), and *Bacillus subtilis* (ATCC 6633), five

Gram-negative bacteria *Klebsiella pneumonia* (ATCC 10031), *Escherichia coli* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi-A serotype* (ATCC 5702), and *Shigella dysenteriae* (PTCC 1188) and three fungal strains of *Aspergillus brasiliensis* (ATCC 16404), *Aspergillus niger* (ATCC 9029), and *Candida albicans* (ATCC 16404), which were procured from the Iranian Scientific and Technological Research Organization (IROST). Bacterial strains were cultured in nutrient agar medium and fungi were cultured in Sabouraud dextrose agar medium, incubated overnight at 37 °C and 30 °C, respectively.

Agar well diffusion (WD) assay

Agar well diffusion method was performed according to the Institute of Clinical and Laboratory Standards [76]. Here, 100 μ L of microbial suspensions with turbidity equivalent to half McFarland were cultured under uniform conditions in the culture medium (Müller–Hinton agar medium for bacteria and sabouraud dextrose agar for fungi). The essential oil was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 300 μ g/mL. Wells with a diameter of 6 mm and a thickness of 4 mm were made in culture media and 10 μ L of the essential oil was added to each well. Plates inoculated with bacterial strains were heated at 37 °C for 24 h and those inoculated with fungi and yeast strains at 30 °C for 72 and 48 h, respectively. Inhibition zone diameter was measured using an antibiogram ruler (in millimeters). The antibiotics gentamicin (10 μ g/disc) and rifampin (5 μ g/disc) for bacteria and nystatin (100,000 unit/mL) for fungi were used as standard drugs for positive control under the same conditions. Dimethyl-sulfoxide was used as negative control. For each essential oil, the experiment was repeated three times and the inhibition zone diameter was reported in terms of mean \pm standard deviation.

Minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MFC) assay

To determine the minimum inhibitory concentration (MIC) of bacterial and yeast strains, a 96-well microtiter plate sterile and broth microdilution method were used according to the CLSI agenda [76]. For fungal strains, the agar dilution assay method was used according to the agenda of Gul et al. [77]. At first, various dilutions of essential oils were prepared. In this way, a certain amount of essential oil sample was weighed and a suitable ratio of culture medium and solvent of dimethyl sulfoxide was used to prepare the initial stock, so that the initial concentration of 4000 μ g/mL was selected. Then, 2000, 500, 250, 125, 62.5, 31.25, and 15.63 μ g/mL concentrations were prepared from the initial concentration. Each microplate well received 200 μ L of a solution containing

95 μL brain heart infusion (BHI) broth for bacteria, Sabouraud dextrose broth for yeast, and Sabouraud dextrose agar broth for fungi, 5 μL bacterial suspension with 0.5 McFarland dilution, and 100 μL of one of the different essential oil concentrations. Plates with bacterial strains were inoculated at 37 $^{\circ}\text{C}$ for 24 h and those with yeast and fungi at 30 $^{\circ}\text{C}$ for 48 and 72 h. The resulting color change in each of the microplate wells was determined through MIC. In wells related to the negative control, the culture medium was used instead of essential oil. For positive control, gentamicin and rifampin antibiotic powder for bacteria and nystatin antibiotic powder for yeast and fungi were used instead of essential oil. The experiment was repeated three times for each essential oil sample and reported as MIC. To determine the minimum bactericidal/fungicidal concentration (MBC/MFC), 5 μL of each microplate well with no growth (clear well) was inoculated into nutrient agar/Sabouraud dextrose agar medium and incubated at 37 $^{\circ}\text{C}$ for 24 h for bacterial strains, and for 48 h and 72 h at 30 $^{\circ}\text{C}$ for fungal strains.

Statistical analysis

The statistical analysis was performed using SPSS 22. Data normality was checked using the Kolmogorov–Smirnov test. After ensuring the normality of the data, the variance of the data (essential oil and antimicrobial activity) was analyzed using one-way analysis of variance (ANOVA) and univariate. All the data were analyzed in triplicates and expressed as mean \pm SD with Duncan test at $\alpha=0.01$.

Results

Essential oil yield

Based on the ANOVA results, the essential oil yield of the three species was significantly different (Table 1). The highest yield belonged to *T. disciforme* essential oil (1.433 \pm 0.006%) with saffron yellow color. *A. biebersteinii* essential oil with pale yellow color and a yield of 0.717 \pm 0.006% was ranked second in terms of yield. The essential oil of *T. parthenium* was blue and its yield was 0.650 \pm 0.010%, which was the lowest yield.

GC–MS analysis of essential oils

Based on the results of essential oil analysis by GC–MS, 109 different compounds (98.78–99.55%) were identified in the studied species (Table 2) whose profiles are shown in Figs. 1, 2 and 3. Oxygenated monoterpenes were the largest group of compounds in *A. biebersteinii* (51.76%). In *T. parthenium*, oxygenated monoterpenes with 47.21% contained the highest percentage of compounds. However, in *T. disciforme*, nonterpenoids with 61.95% were the most common compounds.

Based on the findings, only the composition of Caryophyllene was observed in the essential oils of all three species. ANOVA results showed that there was a significant difference between the means of combination of different species ($P\leq 0.01$) (Tables 1 and 2). The amount of this compound was very small and its highest amount was observed in the essential oil of *T. disciforme* (1.66%). Anisole, *p*-1-cyclohexen-1-yl- (55.95%), and modephene (10.00%) were the most important predominant compounds in *T. disciforme* essential oil. Meanwhile, *cis*- β -farnesene (11.94%) was the second predominant component of *T. disciforme* essential oil.

β -Sesquiphellandrene with 6.58% was another major compound in *T. disciforme* consistent with the findings of Cavar Zeljkovic et al. [29] (9.29%), Chehregani et al. [27] (17.85%), and Javidnia et al. [30] (4.15%). However, Nazar Alipoor and Sefidkon [78] reported a small amount (0.22%), which contradicts the present results.

Camphor with 43.43% was the predominant composition of *T. parthenium* essential oil, which was also observed in *T. disciforme* essential oil (0.15%). The second dominant compound in *T. parthenium* essential oil was camphene (9.40%). Bornyl acetate with 6.76% was another major component of *T. parthenium* essential oil.

In *A. biebersteinii* essential oil, linalool (34.49%), *p*-cymene (15.31%), α -terpineol (7.43%), terpinen-4-ol (5.04%), and linalool acetate (4.35%) were the predominant compounds.

Analysis of antimicrobial properties of essential oils

ANOVA results showed that the species had a significant effect on the inhibition zone diameter of essential oils of different species due to some microorganisms ($P\leq 0.01$) (Table 3). The evaluation of the antimicrobial activity of essential oils in the studied species by agar well diffusion method against different strains is shown in Table 4. The highest inhibition zone diameter by the essential oil of *T. parthenium* and *A. biebersteinii* against *Aspergillus brasiliensis* (~13 mm) was observed here, which showed relatively good antifungal activity in comparison with the antibiotic nystatin (~30 mm). *T. disciforme* essential oil has also affected this fungus with less power (~12 mm). Similarly, the inhibition zone diameter of essential oil of *T. parthenium* and *A. biebersteinii* against Gram-positive *B. subtilis* (~13 mm) had relatively good activity compared to rifampin (~19 mm) and relatively good activity compared to gentamicin (~30 mm). The potency of this activity was reduced in the essential oil of *T. disciforme* (~9 mm). The findings also indicated that the MIC and MFC values of the essential oils of all three species against *A. brasiliensis* were 2000 $\mu\text{g}/\text{mL}$, which performed poorly compared to rifampin (31.2 $\mu\text{g}/\text{mL}$) (Tables 5 and 6). On the other hand, *T. disciforme*

Table 1 ANOVA of the effect of species on yield and caryophyllene of essential oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan

Source of variation	Df	MS	
		Yield of essential oil	Caryophyllene
Species	2	0.566**	1.743**
Error	10	0.000056	0.000015

**1% level of probability is significant

essential oil with MIC value equal to 125 µg/mL against *B. subtilis* had better inhibitory power compared to the other two essential oils (2000 µg/mL).

Another notable activity of *T. disciforme* essential oil is establishing the diameter of the inhibition zone against Gram-negative *K. pneumoniae* (~8.50 mm), which has a strong activity equal to rifampin (~8 mm) and has a relatively good activity compared to gentamicin (~17 mm). The findings also indicate that the MIC and MBC values of this essential oil against this bacterium were 125 and 250 µg/mL, respectively, which are relatively significant concentrations compared to rifampin (15.36 µg/mL) and gentamicin (3.90 µg/mL). Similarly, this essential oil fights against Gram-negative bacteria *Sh. dysenteriae* and *E. coli* (~8 mm) with a strong activity compared to rifampin (~9 and ~11 mm) and relatively good activity compared to gentamicin (~17 and ~20 mm). Although this essential oil's inhibitory effect (MIC = 125 µg/mL) and lethality (MBC = 250 µg/mL) against *Sh. dysenteriae* and *E. coli* were relatively strong, it had poorer performance compared to control antibiotics.

The strongest inhibitory and lethal activity of *T. disciforme* essential oil was against *Candida albicans* (MIC and MBC < 62.50 µg/mL), which is very significant (twice as strong) compared to nystatin (125 µg/mL). The effect of this essential oil in creating the inhibition zone diameter (~10 mm) was less potent than that of nystatin (~33 mm) against *C. albicans*.

Although *T. disciforme* essential oil did not affect the inhibition zone diameter against other bacterial strains, with different concentrations, it affected their inhibition and lethality. One of the significant inhibitory and lethal activities of false *T. disciforme* essential oil was against Gram-negative bacteria *P. aeruginosa* (MIC and MBC > 62.50 µg/mL), which has a strong activity in comparison with rifampin (31.25 µg/mL) and relatively good activity in comparison with gentamicin (7.81 µg/mL), but this inhibitory power (2000 µg/mL) was severely reduced in the other two essential oils.

T. parthenium essential oil had a relatively good activity compared to rifampin and gentamicin (~21 mm and

~27 mm) by creating a growth inhibition zone diameter of about 10 mm against Gram-positive *S. aureus* bacteria, which is consistent with the antibacterial activity against this bacterium in Hamedan (~28 mm). However, the inhibitory and lethal power of the essential oil against this bacterium (MIC = 2000 µg/mL and MBC = 4000 µg/mL) was very weak.

Discussion

The effect of species on essential oil yield was significant ($P \leq 0.01$) (Table 1). Similar results were obtained by Golkar et al. [79] for species of the genus *Thymus* and *Zataria*, Silva et al. [80] for species of the Myrtaceae family, and Ngahang Kamte et al. [81] for species of the Apiaceae family. Researchers have shown that the essential oil yield of species varies depending on the species, solvent, and extraction method and is sometimes affected by ecological stresses in the region [82].

The highest yield belonged to *T. disciforme* essential oil (1.433 + 0.006%) with saffron yellow color which was higher than the study by Nazar Alipoor and Sefidkon [78] in Taleghan (0.43%), Javidnia et al. [30] in Shiraz (0.16%), Chehregani et al. [27] in Hamedan (0.92%), and Özturk et al. [34] in Turkey (0.04%). However, in the study of Alizadeh et al. [83], the highest yield was recorded in Ardabil (13.3%), which is higher than the present study. The difference in essential oil yield was consistent with the theory of Golparvar and Ghasemi Pirbalouti [84], reporting that essential oil is a quantitative and complex trait influenced by a variety of factors including population genetics, crop density and arrangement, irrigation schedule, fertilization, history of planting, temperature, and light. When the plant can make the most of these factors, it will also produce the most quantitative and qualitative yield. The essential oil of *T. parthenium* was blue and its yield was 0.650 + 0.010%, which was consistent with the results of Shakeri et al. [85] in Irene (0.7%) and Polatoglu et al. [15] in Turkey (0.7%). However, it was not consistent with Mohsenzadeh et al. [43] in Iran (1.02%), Shafaghat et al. [86] in Iran (0.8%), Maxia et al. [46] in Italy (0.4%), and Akpulat et al. [53] in Turkey (0.43%). The highest yield of essential oil of this species was recorded in Masuleh hills of Iran with a value of 3.5% [51].

The lowest number of compounds belonged to the essential oil of *T. disciforme* (26 compounds), which was significantly different from the number of compounds in *T. parthenium* and *A. biebersteinii* species (44 and 55 compounds). Similarly, Chehregani et al. [27] recorded 21 compounds in *T. disciforme*. According to chemical studies, the main components of *A. biebersteinii* essential oil are oxygenated monoterpenes [67]. On the contrary, in the studies by Özturk et al. [34] and Javidnia et al. [30], sesquiterpene hydrocarbons were the major constituents

of the essential oil of this plant. Changes in the chemical composition of essential oils are due to physiological changes, environmental conditions, geographical changes, genetic factors, evolution, as well as the amount of plant material [87].

The amount of Caryophyllene was very small and its highest amount was observed in the essential oil of *T. disciforme* (1.66%). Anisole, *p*-1-cyclohexen-1-yl- and modephene have not been reported before in *T. disciforme*, and are most likely due to chemotypic differences induced by environmental conditions and climate of the studied habitats [88–91], indicating the unique characteristics of this plant in this area. Meanwhile, *cis*- β -Farnesene recorded by Nazar Alipoor and Sefidkon [78] with 12.54% and Özturk et al. [34] with 18.2% in *T. disciforme* essential oil. It has four geometric isomers as reported by Javidnia et al. [30], with the isomer (E)- β -Farnesene as the dominant compound in this plant (15.6%). Farnesene is a component of many aromatic essential oils. It is a fragrant perfume that has many applications and is a very useful substance in the production of quality perfumes [78].

Similarly, in previous studies, camphor was 56.9% in Sivas, Turkey [53], 48.90% in Tehran [44], 60.8% in Savat, Turkey [15], 45.01% in Hamedan [45], 52.86% in Shahrekord [85], 45.5% in Khalkhal-Asalem road, Ardabil province, [86], and 43.97% in Masuleh hills [51], recording the dominant composition of *T. parthenium* essential oil. Camphor has many applications in traditional and modern medicine. It is also important as an antiseptic compound with very effective antibiotic effects [92].

Also camphene reported by Shakeri et al. [85] at 9.1%, Polatoglu et al. [15] at 6.8%, Akpulat et al. [53] at 12.7%, and Shafaghat et al. [86] at 6.95%. This was, however, inconsistent with Rezaei et al. [51]. Some of camphene medicinal effects are acting as a sedative, antidepressant, anti-diarrheal, analgesic and it has also shown anti-abortion effects in pregnant mice. Bornyl acetate has been reported as the third dominant compound in various studies with 8.63% [85], 2.9% [15], and 4.6% [53]. This is inconsistent with the results of Rezaei et al. [51], Mohsenzadeh et al. [43], and Saharkhiz et al. [44]. Bornyl acetate is used as a food additive and for flavoring and fragrance [93].

Linalool in *A. biebersteinii* essential oil is mainly either not reported or identified in very small amounts of 1.4% [72], 0.45% [64], 2.3% [69], and 0.2% [67], which is inconsistent with the present results. Changes in the chemical properties of essential oils in different regions related to factors affecting the chemical composition of essential oils (i.e., genetic, climatic, seasonal, and geographical conditions as well as changes in secondary metabolism, the effect of planting time, growth stage, and biological stresses such as drought or salinity). In

addition, extraction techniques and storage conditions can also affect the composition of essential oils [94].

Linalool has important therapeutic effects, especially antimicrobial activity [89–91, 95]. Linalool is found only in large quantities in coriander seeds [96] and its presence in significant amounts in the studied *A. biebersteinii* essential oil can be a reason that this species is a chemotype in this region, rich in valuable chemical compounds. Moreover, *p*-cymene has been reported as one of the predominant essential oil compounds of this plant in other studies with varying amounts, including 20.8% [97], 16.9–34.8% [67], 19.2% [74], and 4.6% [69], which is consistent with the present findings. Antibacterial effects have also been observed in this compound [98]. The compounds α -terpineol and terpinen-4-ol in this essential oil were either mostly not present or in small amounts in previous studies, for instance, 3.2% and 2.7% [73], 2.2% and 0.9% [67], and 1.2% and 2.7% [74], which does not correspond to the present study. Linalool acetate has not been reported in any of the previous studies on this plant. These differences can be due to the effect of different ecological, additive, and climatic factors on the composition of essential oils of different populations of the same species that are distributed and grown in different geographical areas [88]. Linalool acetate and Linalool are the main components of lavender essential oil. This suggests that the studied *A. biebersteinii*, like lavender, could be a potential source for extracting these compounds.

The effect of plant essential oils on Gram-positive bacteria was greater than their effect on Gram-negative bacteria [99]. Caryophyllene was the only common compound in all three essential oils, the largest amount of this compound belonged to *T. disciforme*, which can be one of the contributing factors. Similarly, Shafaghat et al. [86] and Polatoglu et al. [15] reported the relatively good effect of *T. parthenium* essential oil on *B. subtilis* (17.9 \pm 0.2 mm and 125 μ g/mL), which is consistent with the present findings. Furthermore, in Baris et al. [64], no inhibition zone diameter from *A. biebersteinii* essential oil against *B. subtilis* was observed, which contradicts the present results. It should be noted that the antifungal activity against *A. brasiliensis* by the studied essential oils has not been recorded in any region so far and this is the present study is the first to identify this antifungal activity. The antimicrobial activity of different essential oils depends on their chemical profile [89–91, 100]. It seems that the similarity of the antimicrobial activity of *A. biebersteinii* and *T. parthenium* essential oils can be due to the similarity of their chemical profile, especially in terms of monoterpenes such as α -pinene, camphene, sabinene, δ -carene, *p*-cymene, and γ -terpinene. Terpenes are a group of organic materials found abundantly in nature [101]. Terpenes with high hydrophobic properties

Table 2 Compounds identified by GC–MS of the essential oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan

No.	Component	RI Exp	RI Lit	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch.Bip	<i>Achillea biebersteinii</i> Afan	Molecular formula
1	1,3-Cyclopentadiene, 5-(1,1-dimethylethyl)-	800.000	788		1.72 ± 0.00 ^a		C ₉ H ₁₄
2	1-Butanol, 2-methyl-	800.200	762			0.13 ± 0.02 ^a	C ₅ H ₁₂ O
3	Isobutyric acid, isobutyl ester	855.172	908			0.07 ± 0.01 ^a	C ₈ H ₁₆ O ₂
4	Tricyclene	870.443	921		0.57 ± 0.02 ^a		C ₁₀ H ₁₆
5	α-Thujene	871.921	924			0.40 ± 0.00 ^a	C ₁₀ H ₁₆
6	α-Pinene	880.788	932		0.48 ± 0.02 ^b	1.03 ± 0.02 ^a	C ₁₀ H ₁₆
7	Camphene	902.317	953		9.40 ± 0.04 ^a	0.19 ± 0.00 ^b	C ₁₀ H ₁₆
8	Sabinene	917.880	969		0.12 ± 0.01 ^b	2.17 ± 0.01 ^a	C ₁₀ H ₁₆
9	β-Pinene	921.192	974		0.33 ± 0.00 ^a		C ₁₀ H ₁₆
10	β-Myrcene	932.119	988			1.31 ± 0.00 ^a	C ₁₀ H ₁₆
11	Furan, 2-pentyl-	932.119	996	0.20 ± 0.00 ^a	0.19 ± 0.01 ^a		C ₉ H ₁₄ O
12	α-Phellandrene	944.701	1002			1.39 ± 0.01 ^a	C ₁₀ H ₁₆
13	δ-Carene	952.980	1008		0.22 ± 0.02 ^b	1.32 ± 0.01 ^a	C ₁₀ H ₁₆
14	p-Cymene	963.245	1020		3.27 ± 0.00 ^b	15.31 ± 0.03 ^a	C ₁₀ H ₁₄
15	β-Ocimene	980.463	1032			0.96 ± 0.01 ^a	C ₁₀ H ₁₆
16	γ-Terpinene	990.066	1054		1.36 ± 0.01 ^b	2.54 ± 0.00 ^a	C ₁₀ H ₁₆
17	Filifolone	1023.809	1082		0.27 ± 0.02 ^a		C ₁₀ H ₁₄ O
18	α-Terpinolene	1012.698	1086			1.15 ± 0.00 ^a	C ₁₀ H ₁₆
19	Linalool oxide	1005.820	1093			0.39 ± 0.00 ^a	C ₁₀ H ₁₈ O ₂
20	Linalool	1049.735	1095			34.49 ± 0.02 ^a	C ₁₀ H ₁₈ O
21	2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-	1026.984	1109		0.99 ± 0.05 ^a		C ₁₀ H ₁₆ O ₂
22	Chrysanthenone	1041.798	1124		0.47 ± 0.01 ^a		C ₁₀ H ₁₄ O
23	Camphor	1056.084	1141	0.15 ± 0.01 ^b	43.43 ± 0.03 ^a		C ₁₀ H ₁₆ O
24	(+)-Camphor	1076.984	1142		0.38 ± 0.01 ^a		C ₁₀ H ₁₆ O
25	Borneol	1081.460	1165		0.95 ± 0.01 ^a		C ₁₀ H ₁₈ O
26	Albene	1061.111	1166	0.13 ± 0.00 ^a			C ₁₂ H ₁₈
27	Lilac aldehyde D	1062.169	1169			0.15 ± 0.00 ^a	C ₁₀ H ₁₆ O ₂
28	Nerol oxide	1067.724	1154			0.83 ± 0.00 ^a	C ₁₀ H ₁₆ O
30	β-Citronellene	1078.190	1088			0.14 ± 0.01 ^a	C ₁₀ H ₁₈
31	Biisobutenyl	1079.894	1032			0.28 ± 0.00 ^a	C ₈ H ₁₄
32	(-)-Terpinen-4-ol	1086.243	1175		1.38 ± 0.04 ^a		C ₁₀ H ₁₈ O
33	Terpinen-4-ol	1093.650	1177			5.04 ± 0.02 ^a	C ₁₀ H ₁₈ O
34	α-Terpineol	1108.413	1186			7.43 ± 0.01 ^a	C ₁₀ H ₁₈ O
35	Cyclofenchene	1099.206	882		0.45 ± 0.03 ^a		C ₁₀ H ₁₆
36	Sabinol	1113.701	1137		0.33 ± 0.00 ^a		C ₁₀ H ₁₆ O
37	Captan	1113.461	1141			0.70 ± 0.00 ^a	C ₉ H ₈ Cl ₃ NO ₂ S
38	cis-Geraniol	1125.240	1231			0.75 ± 0.00 ^a	C ₁₀ H ₁₈ O
39	Linalool acetate	1131.971	1253			4.35 ± 0.02 ^a	C ₁₂ H ₂₀ O ₂
40	Geraniol	1141.346	1249			2.70 ± 0.00 ^a	C ₁₀ H ₁₈ O
41	Bornyl acetate	1149.759	1287		6.76 ± 0.03 ^a		C ₁₂ H ₂₀ O ₂
42	(-)-Bornyl acetate	1151.201	1289			0.39 ± 0.01 ^a	C ₁₂ H ₂₀ O ₂
43	Silphiperfol-5-ene	1172.355	1330	0.16 ± 0.01 ^a			C ₁₅ H ₂₄
44	α-Guaiene	1186.778	1437	0.90 ± 0.00 ^a			C ₁₅ H ₂₄

Table 2 (continued)

No.	Component	RI Exp	RI Lit	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch.Bip	<i>Achillea biebersteinii</i> Afan	Molecular formula
45	α -Terpineol acetate	1191.586	1367			1.22 \pm 0.03 ^a	C ₁₂ H ₂₀ O ₂
46	Nerol acetate	1197.836	1365			1.05 \pm 0.00 ^a	C ₁₂ H ₂₀ O ₂
47	γ -Patchoulene	1199.519	1502		0.15 \pm 0.00 ^a		C ₁₅ H ₂₄
48	α -Cubebene	1204.028	1381		0.56 \pm 0.01 ^a		C ₁₅ H ₂₄
49	Geraniol acetate	1210.663	1386			2.15 \pm 0.02 ^a	C ₁₂ H ₂₀ O ₂
50	Modephene	1213.033	1392	10.00 \pm 0.01 ^a	0.37 \pm 0.02 ^b		C ₁₅ H ₂₄
51	<i>cis</i> -Jasmone	1221.090	1396			1.35 \pm 0.01 ^a	C ₁₁ H ₁₆ O
52	Caryophyllene	1233.886	1418	1.66 \pm 0.00 ^a	0.25 \pm 0.01 ^c	0.46 \pm 0.02 ^b	C ₁₅ H ₂₄
53	Propanoic acid, 2-methyl-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, <i>exo</i> -	1226.540	1419		0.49 \pm 0.00 ^a		C ₁₄ H ₂₄ O ₂
54	Selina-5,11-diene	1227.962	1447	0.79 \pm 0.01 ^a			C ₁₅ H ₂₄
55	<i>trans</i> - β -Farnesene	1250.473	1461			0.10 \pm 0.00 ^a	C ₁₅ H ₂₄
56	<i>cis</i> - β -Farnesene	1255.450	1454	11.94 \pm 0.01 ^a	1.29 \pm 0.03 ^a		C ₁₅ H ₂₄
57	γ -Selinene	1266.113	1492			0.16 \pm 0.02 ^a	C ₁₅ H ₂₄
58	Germacrene D	1271.327	1484		2.43 \pm 0.03 ^a	0.22 \pm 0.01 ^b	C ₁₅ H ₂₄
59	Bornyl isovalerate	1281.279	1512		2.81 \pm 0.01 ^a		C ₁₅ H ₂₆ O ₂
60	α -Amorphene	1271.563	1483	1.17 \pm 0.04 ^a			C ₁₅ H ₂₄
61	Aromandendrene	1275.592	1439			0.05 \pm 0.00 ^a	C ₁₅ H ₂₄
62	β -Bisabolene	1283.649	1506	0.70 \pm 0.01 ^a			C ₁₅ H ₂₄
63	β -Sesquiphellandrene	1296.445	1525	6.58 \pm 0.02 ^a	0.64 \pm 0.00 ^b		C ₁₅ H ₂₄
64	3H-Pyrazol-3-one, 2,4-dihydro-2-methyl-5-phenyl-	1304.600	1540			0.17 \pm 0.00 ^a	C ₁₀ H ₁₀ N ₂ O
65	Anisole, <i>p</i> -1-cyclohexen-1-yl-	1317.191	1560	55.95 \pm 0.03 ^a	4.99 \pm 0.01 ^b		C ₁₃ H ₁₆ O
66	<i>trans</i> -Nerolidol	1318.886	1562			0.10 \pm 0.01 ^a	C ₁₅ H ₂₆ O
67	Bornyl tiglate	1319.128	1615		0.44 \pm 0.00 ^a		C ₁₅ H ₂₄ O ₂
68	Spathulenol	1323.002	1571		1.52 \pm 0.01 ^a		C ₁₅ H ₂₄ O
69	Dendrasaline	1324.697	1579	1.14 \pm 0.03 ^a			C ₁₅ H ₂₂ O
70	Benzoic acid, hexyl ester	1329.297	1580			0.09 \pm 0.00 ^a	C ₁₃ H ₁₈ O ₂
71	Caryophyllene oxide	1334.382	1582			0.65 \pm 0.00 ^a	C ₁₅ H ₂₄ O
72	Mintketone=Salvia-4(14)-en-1-one	1339.467	1599		1.05 \pm 0.01 ^a		C ₁₅ H ₂₄ O
73	9-Oxatetracyclo[5.4.0.0(3,10).0(4,8)]undeca-5-en-2-one	1347.215				0.83 \pm 0.01 ^a	C ₁₀ H ₁₀ O ₂
74	δ -Cadinene	1351.815	1537		0.51 \pm 0.03 ^a		C ₁₅ H ₂₄
75	2-methyl-2-vinyl-5-isopropyltetrahydrofuran	1353.510	1074			0.20 \pm 0.00 ^a	C ₁₀ H ₁₈ O
76	<i>cis</i> -Farnesol	1356.416	1697		2.79 \pm 0.00 ^a		C ₁₅ H ₂₆ O
77	γ -Eudesmol	1363.680	1630		0.37 \pm 0.00 ^b	0.47 \pm 0.02 ^a	C ₁₅ H ₂₆ O
78	Valencene	1369.007	1496		0.83 \pm 0.00 ^a		C ₁₅ H ₂₄
79	Methyl jasmonate	1370.944	1655			0.47 \pm 0.01 ^a	C ₁₃ H ₂₀ O ₃
80	τ -Cadinol	1372.397	1639	0.81 \pm 0.00 ^a			C ₁₅ H ₂₆ O
81	6-Hydroxy caryophyllene	1376.513	1649	0.42 \pm 0.01 ^a			C ₁₅ H ₂₄ O
82	Neointermedeol	1379.176	1662		1.75 \pm 0.03 ^a		C ₁₅ H ₂₆ O

Table 2 (continued)

No.	Component	RI Exp	RI Lit	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch.Bip	<i>Achillea biebersteinii</i> Afan	Molecular formula
83	β -Eudesmol	1378.934	1649			1.18 \pm 0.01 ^a	C ₁₅ H ₂₆ O
84	2-Norcaranone, 3-methyl-	1387.167	1657	0.54 \pm 0.00 ^a			C ₉ H ₁₂ O
85	α -Bisabolol	1391.283	1685			0.52 \pm 0.01 ^a	C ₁₅ H ₂₆ O
86	4-(1,5-Dimethylhex-4-enyl) cyclohex-2-enone	1395.883	1697	1.54 \pm 0.01 ^a			C ₁₄ H ₂₂ O
87	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclo-dec-5-enol	1395.883	1694		0.55 \pm 0.03 ^a		C ₁₅ H ₂₄ O
88	γ -Costol	1428.967	1745			0.08 \pm 0.02 ^a	C ₁₅ H ₂₄ O
89	Caparratriene	1439.042	1692		0.57 \pm 0.02 ^a		C ₁₅ H ₂₆
90	15-nor-Prezizaan-7-one	1436.750	1572	0.34 \pm 0.04 ^a			C ₁₄ H ₂₂ O
91	Ascabiol	1443.073	1753			0.16 \pm 0.00 ^a	C ₁₄ H ₁₂ O ₂
92	(4R,5S)-4-Methyl-5-phenyl-2-oxazolidinone	1444.332	1754	0.83 \pm 0.03 ^a			C ₁₀ H ₁₁ NO ₂
93	Farnesol	1451.385	1747	0.28 \pm 0.02 ^a			C ₁₅ H ₂₆ O
94	(Z)-8-decen-4,6-diyn-1-yl 3-methylbutanoate	1465.491	1832	0.73 \pm 0.01 ^a			C ₁₅ H ₂₀ O ₂
95	Phthalic acid, diisobutyl ester	1485.138	1868	0.16 \pm 0.05 ^a			C ₁₆ H ₂₂ O ₄
96	(Z)-Tonghaosu	1501.842	1891		1.18 \pm 0.00 ^a		C ₁₃ H ₁₂ O ₂
97	Gerany- <i>p</i> -cymene	1524.736	1980			0.29 \pm 0.01 ^a	C ₂₀ H ₃₀
98	9-Octadecenal, (Z)-	1544.210	1995			0.19 \pm 0.00 ^a	C ₁₈ H ₃₄ O
99	(E)-Tibetin spiroether	1537.368	1888		0.22 \pm 0.00 ^a		C ₁₄ H ₁₄ O ₂
100	Hexadecanoic acid	1546.052	1959	0.75 \pm 0.02 ^a	0.63 \pm 0.02 ^b		C ₁₆ H ₃₂ O ₂
101	<i>cis</i> -1-Chloro-9-octadecene	1547.631	2241			0.11 \pm 0.03 ^a	C ₁₈ H ₃₅ Cl
102	1,15-Hexadecadiene	1580.000	1581			0.71 \pm 0.00 ^a	C ₁₆ H ₃₀
104	Behenic alcohol	1590.263	2470			0.26 \pm 0.01 ^a	C ₂₂ H ₄₆ O
105	Z-9-Octadecen-1-ol acetate	1635.734	1635			0.49 \pm 0.01 ^a	C ₂₀ H ₃₈ O ₂
106	Octadecane	1677.811	1789			0.08 \pm 0.03 ^a	C ₁₈ H ₃₈
107	Tricosane	1686.980	2300		0.54 \pm 0.01 ^a		C ₂₃ H ₄₈
108	Linoleic acid	1634.626	2134	0.53 \pm 0.02 ^a			C ₁₈ H ₃₂ O ₂
109	Phytan	1688.919	1811	0.38 \pm 0.01 ^a			C ₂₀ H ₄₂
	Total			98.78	99.55	99.94	
	Monoterpenes hydrocarbons			0.00	16.2	27.91	
	Oxygenated monoterpenes			0.15	47.21	51.76	
	Sesquiterpenes hydrocarbons			34.03	7.15	0.99	
	Oxygenated sesquiterpenes			2.65	8.03	3	
	Others			61.95	20.96	16.28	

Compounds are listed in order of their retention time from a HP-5 column. RI Exp., linear retention indices on HP-5 column, experimentally determined using homologue series of *n*-alkanes (C8–C20). RI Lit., linear retention index taken from Adams [75], or NIST 14 (2014) and literature. Values with different letters are statistically different (Duncan, $P \leq 0.01$), mean (%) \pm SD of three cultures were reported

can isolate lipids from the cell wall of bacteria and fungi, thereby increasing cell membrane permeability. Disturbances in the function of the cell membrane lead to the release of ions and disturbance of the electron balance of the membrane, making the passage of substances difficult and ultimately leading to cell death. Several sources introduce terpenes and monoterpenes as effective factors

in the antibacterial and antifungal activity of essential oils [102, 103].

In addition, due to the same antimicrobial properties of *A. biebersteinii* and *T. parthenium* essential oils, it can be inferred that different ratios of chemical compounds in different essential oils have balanced their antimicrobial activity [90, 104]. In *A. biebersteinii* essential oil,

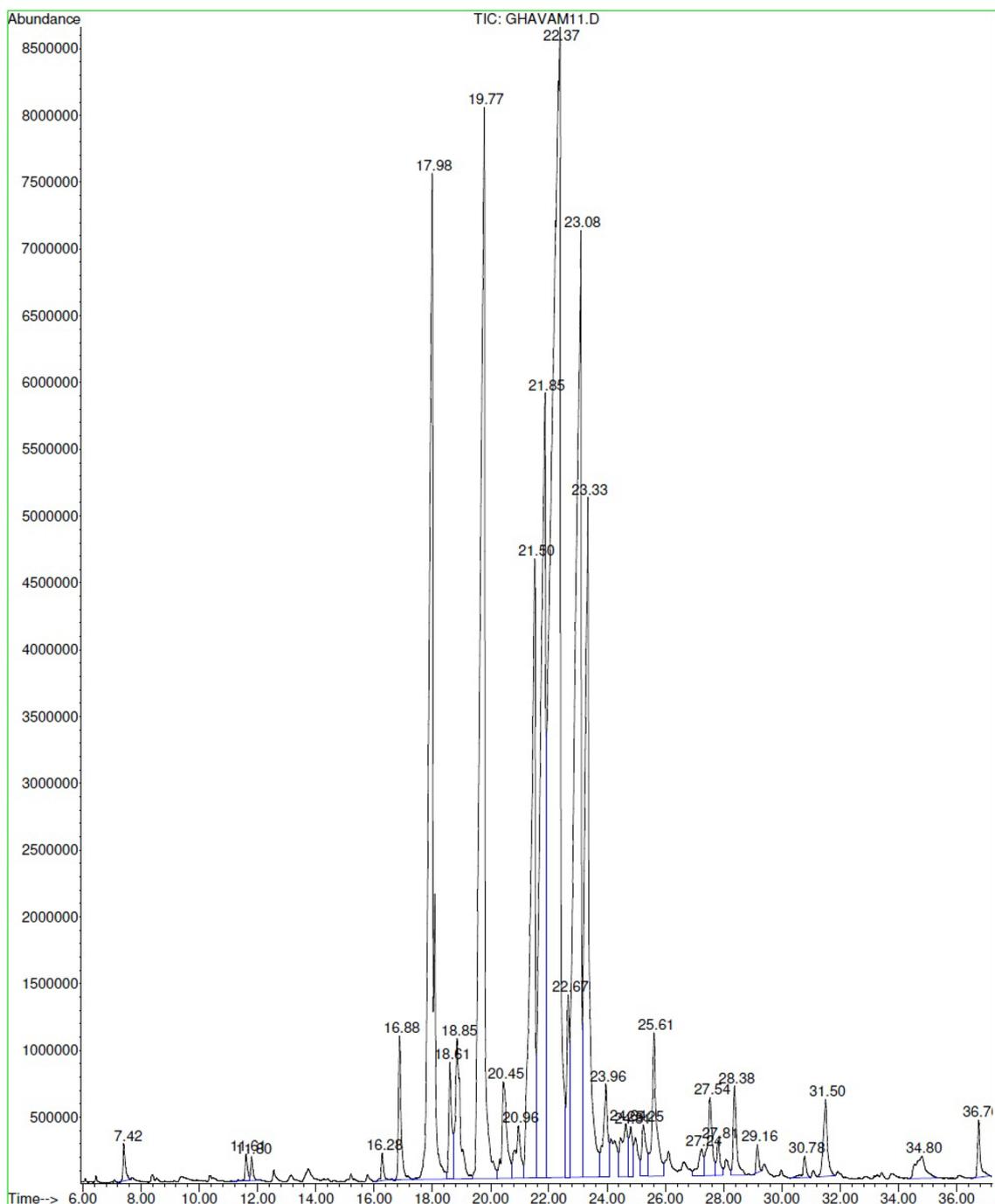


Fig. 1 Chemical profile of essential oil of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip.

the predominance of *p*-cymene, linalool, α -terpineol, terpinen-4-ol, and linalool acetate and in *T. parthenium* essential oil, the predominance of camphene, camphor, and bornyl acetate can be the most effective factors on this activity of essential oils. Many studies have confirmed the antimicrobial activity of these compounds against different strains [56, 105–110].

Findings of Tofighi et al. [20] proved that *T. disciforme* essential oil was not inactive against *E. coli*, which is consistent with the present results. Differences in the antimicrobial activities of the essential oil of a plant species in different regions can be due to

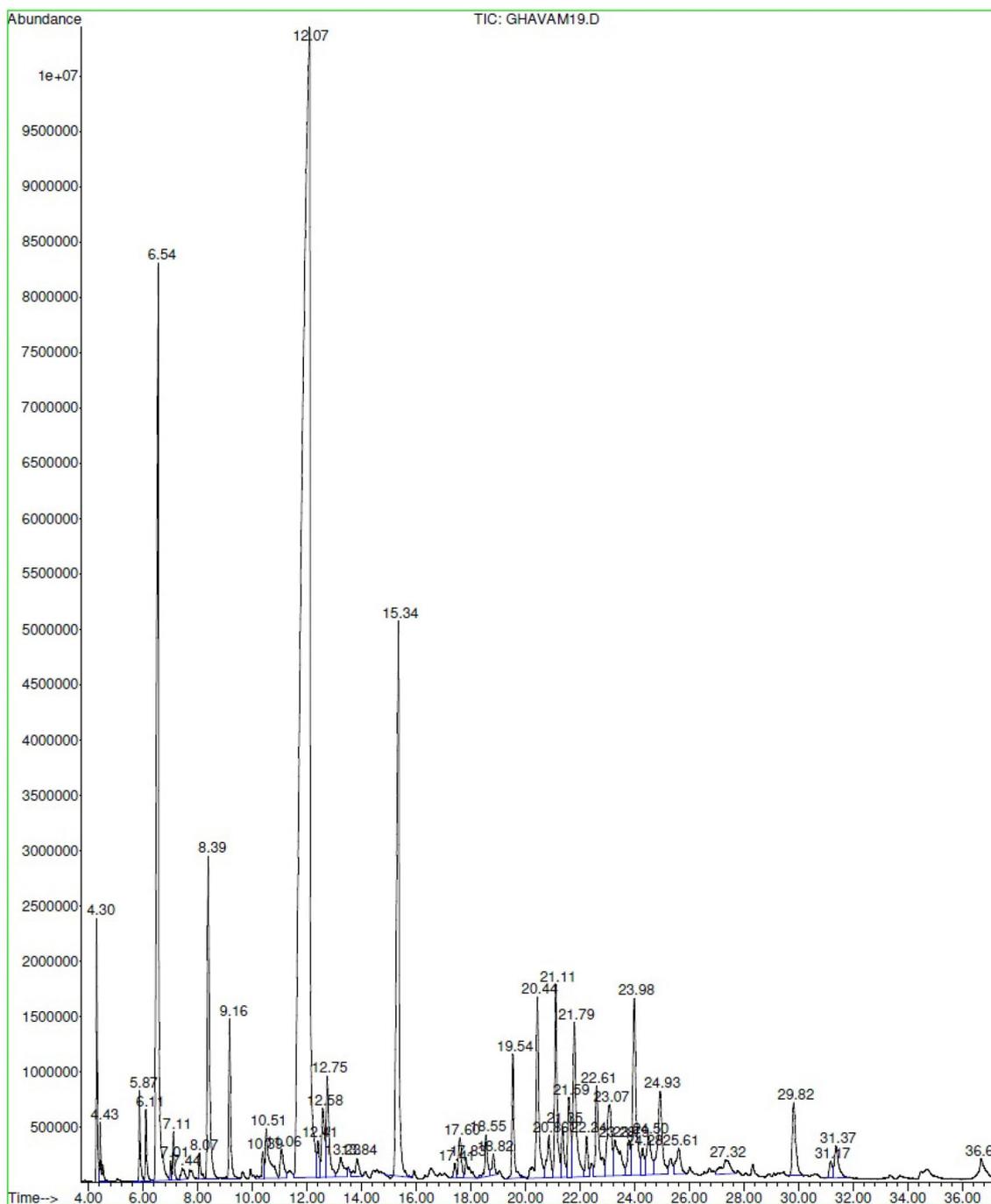


Fig. 2 Chemical profile of essential oil of *Tanacetum parthenium* (L.) Sch.Bip.

differences in its predominant compounds or the presence of different chemical compounds in it [111].

The predominance of modephene, *cis*- β -farnesene, β -sesquiphellandrene, and anisole, *p*-1-cyclohexen-1-yl in *T. disciforme* essential oil can be the cause of strong antibacterial activity. On the other hand,

the antimicrobial activity of essential oils does not depend only on their predominant compounds, and minor compounds may have a synergistic effect with other compounds. Enough attention must be given to these synergistic effects due to the diversity between the main and minor compounds of essential oils in

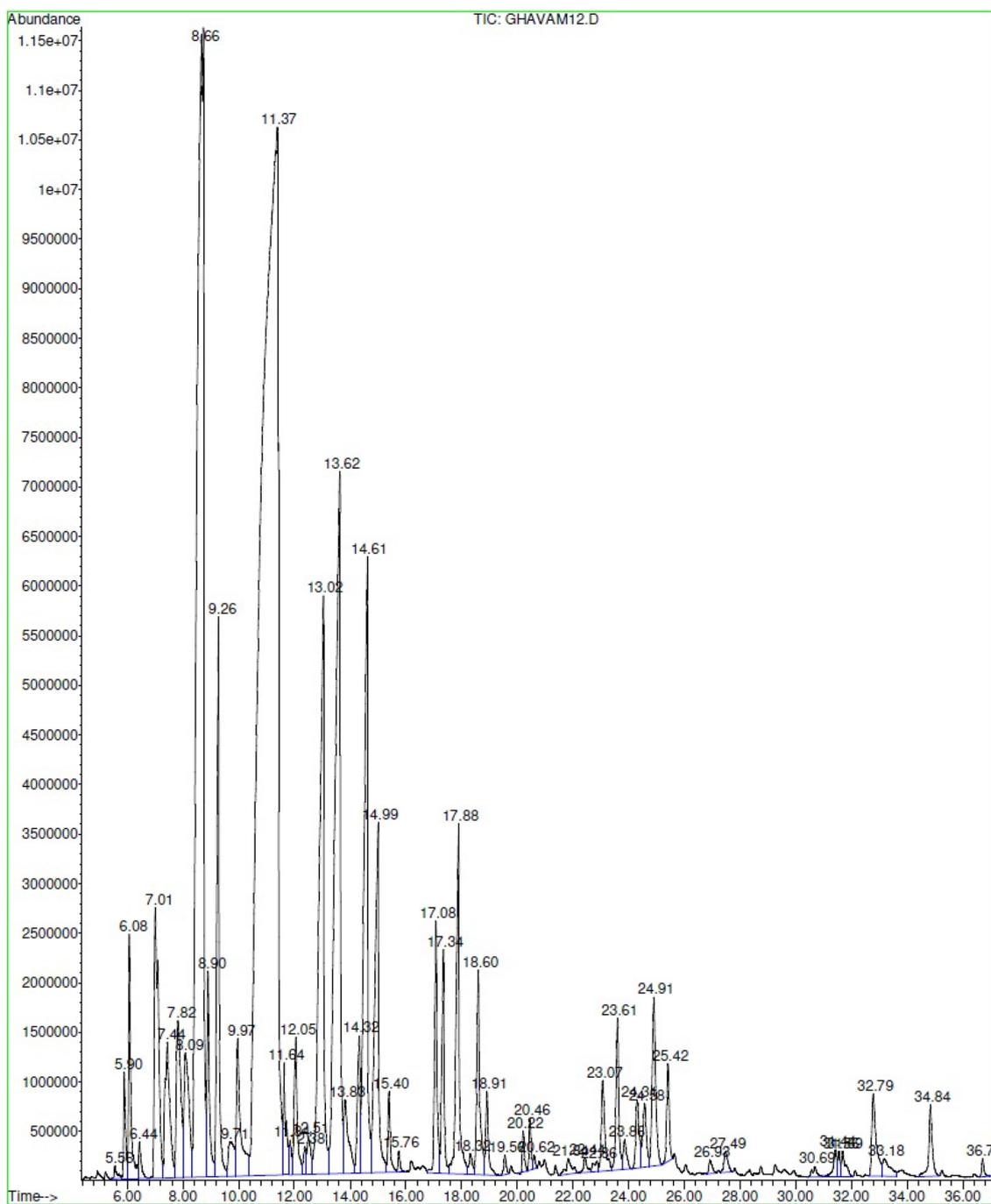


Fig. 3 Chemical profile of essential oil of *Achillea biebersteinii* Afan

their antimicrobial activity [89–91, 112, 113]. Therefore, a higher amount of caryophyllene compared to the other two essential oils and also the small and exclusive presence of some sesquiterpene such as α -guaiene, α -amorphene, β -bisabolene, τ -cadinol, and farnesol, can be other possible factors affecting

this antibacterial activity. The effect of sesquiterpene on different strains of bacteria has been documented [114]. The presence of low fatty acid linoleic acid can also be another factor since the antibacterial effect of fatty acids against *E. coli* has been proven [115].

Table 3 ANOVA of the effect of species on inhibition zone of essential oil of oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan. for some tested strains

Source of variation	df	MS					
		<i>A. brasiliensis</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>K. pneumoniae/E. coli</i>	<i>Sh. dysenteriae</i>	<i>S. aureus</i>
Species	2	1.444 ^{ns}	16.083**	18.778**	6.250**	5.444**	20.250**
Error	6	0.444	0.472	0.111	0.083	0.11	0.083

ns not significant

**1% level of probability is significant

Table 4 WD (mm) of essential oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan. against tested strains

Strains	WD (mm)					
	Essential oils			Antibiotics		
	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch.Bip	<i>Achillea biebersteinii</i> Afan	Rifampin	Gentamicin	Nystatin
<i>A. brasiliensis</i>	12.00 ± 0.50 ^b	13.00 ± 0.50 ^a	13.00 ± 0.50 ^a	NA	NA	30
<i>A. niger</i>	ND	ND	ND	NA	NA	27
<i>C. albicans</i>	10.33 ± 0.58 ^a	ND	ND	NA	NA	33
<i>B. subtilis</i>	9.17 ± 0.29 ^b	13.00 ± 0.50 ^a	13.00 ± 0.50 ^a	19	30	NA
<i>E. coli</i>	8.50 ± 0.50 ^a	ND	ND	11	20	NA
<i>K. pneumoniae</i>	8.50 ± 0.50 ^a	ND	ND	8	17	NA
<i>P. aeruginosa</i>	ND	ND	ND	ND	20	NA
<i>S. paratyphi-A</i>	ND	ND	ND	8	18	NA
<i>Sh. dysenteriae</i>	8.33 ± 0.58 ^a	ND	ND	9	17	NA
<i>S. aureus</i>	ND	10.50 ± 0.50 ^a	ND	21	27	NA
<i>S. epidermidis</i>	ND	ND	ND	27	45	NA
<i>S. pyogenes</i>	ND	ND	ND	21	32	NA

Values with different letters are statistically different (Duncan, $P \leq 0.01$); Mean (%) ± SD of three cultures were reported

WD The diameters of the inhibition zones includes the diameters of wells (6 mm), ND not determined, NA no activity

Anti-yeast activity may be due to the presence of farnesol in *T. disciforme* essential oil compared to other essential oils studied. Sesquiterpenols have 15 carbon atoms and have a variety of therapeutic effects and activity against *Candida* [116]. Tofighi et al. [20] did not establish the diameter of the inhibition zone of *T. disciforme* essential oil against *P. aeruginosa*. Similarly, the findings indicate that the MIC and MBC values of *T. disciforme* essential oil against *S. pyogenes* were < 62.50 µg/mL. Although it was 6 times weaker compared with rifampin and gentamicin (0.975 µg/mL), it had a good performance compared to the essential oils of the other two species (MIC and MBC = 2000 µg/mL).

Studies have shown that the essential oil as a whole has stronger antiseptic effects than each of its main components [117]. It has also been shown that many compounds in essential oils (even in small amounts)

have good antimicrobial effects and even synergistic effects on other compounds [118]. Camphor too has strong antimicrobial effects [119]. The pure antimicrobial properties of α-pinene against *S. aureus* have been proven [120]. β-Pinene has antibiotic properties against *Escherichia* and *Staphylococcus* bacteria [89, 121, 122]. Spathulenol is an alcoholic sesquiterpene with proven antibacterial and antifungal properties [123].

Conclusion

The present study showed that the essential oils of different species were significantly different in terms of yield, chemical properties and antimicrobial properties. The compounds of anisole, *p*-1-cyclohexane-1-yl-, camphor and linalool were the predominant and significant amounts in the essential oils of three plants, *T. disciforme*, *T. parthenium* and *A. biebersteinii*, respectively.

Table 5 MIC values of essential oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan. against tested strains

Strains	MIC					
	Essential oils			Antibiotics		
	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch. Bip	<i>Achillea biebersteinii</i> Afan	Rifampin	Gentamicin	Nystatin
<i>A. brasiliensis</i>	2000	2000	2000	NA	NA	31.2
<i>A. niger</i>	2000	2000	2000	NA	NA	31.2
<i>C. albicans</i>	> 62.50	2000	2000	NA	NA	125
<i>B. subtilis</i>	125	2000	2000	31.25	3.90	NA
<i>E. coli</i>	125	2000	2000	3.90	3.90	NA
<i>K. pneumoniae</i>	125	2000	2000	15.36	3.90	NA
<i>P. aeruginosa</i>	> 62.50	2000	2000	31.25	7.81	NA
<i>S. paratyphi-A</i>	125	2000	2000	15.36	3.90	NA
<i>Sh. dysenteriae</i>	125	2000	2000	15.36	3.90	NA
<i>S. aureus</i>	250	2000	2000	31.25	1.95	NA
<i>S. epidermidis</i>	125	2000	2000	1.95	1.95	NA
<i>S. pyogenes</i>	> 62.50	2000	2000	0.975	0.975	NA

Table 6 MFC/MBC values of essential oils of *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip. and *Achillea biebersteinii* Afan. against tested strains

Strains	MBC		
	Essential oils		
	<i>Tripleurospermum disciforme</i> (C.A.Mey.) Sch.Bip	<i>Tanacetum parthenium</i> (L.) Sch.Bip	<i>Achillea biebersteinii</i> Afan
<i>A. brasiliensis</i>	2000	2000	2000
<i>A. niger</i>	2000	2000	2000
<i>C. albicans</i>	> 62.50	2000	2000
<i>B. subtilis</i>	1000	4000	2000
<i>E. coli</i>	250	2000	2000
<i>K. pneumo-niae</i>	250	2000	2000
<i>P. aeruginosa</i>	250	2000	2000
<i>S. paratyphi-A</i>	250	2000	2000
<i>Sh. dysenteriae</i>	250	2000	2000
<i>S. aureus</i>	2000	4000	4000
<i>S. epidermidis</i>	125	2000	2000
<i>S. pyogenes</i>	> 62.50	2000	2000

Differences in the predominant and partial compositions of essential oils were caused different antimicrobial properties against different strains of microorganisms. The highest inhibition zone by essential oil of *T. parthenium* and *A. biebersteinii* against *A. brasiliensis* was observed. The strongest inhibitory and lethal effect was against *K. pneumoniae*, *Sh. Dysenteriae*, *E. coli*, and *C. albicans* by

the essential oil of *T. disciforme*. These essential oils can be a natural candidate for the treatment of some infectious diseases, but further clinical studies should be performed in the future.

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Authors' contributions

MG was the supervisor, designer of the hypotheses, and responsible and functor for all the steps (plant collection, laboratory, statistical analysis, data analysis, etc.) and wrote the text of the article. The author read and approved the final manuscript.

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