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ESSENTIAL OIL COMPOSITION OF YARROW SPECIES (*ACHILLEA MILLEFOLIUM* L. AND *ACHILLEA WILHELMSII* L.): ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OILS

Bunyamin YILDIRIM[a], Kamil EKICI[b], Mehmet Zeki KOCAK[c,*]

ABSTRACT. Of the medicinal plants' cosmos, yarrow species (*A. millefolium* L. and *A. wilhelmsii* L.) are of the reputed species due to their phytochemical composition and thereby antioxidant and antibacterial activities. Owing to the high diversity in chemical composition and production of essential oil, the species deserves to be investigated more. In this context, wild yarrow plants were collected in Eastern Anatolia region (Van, Türkiye) and then the dried samples of the plants were subjected to hyd-rodistillation for essential oil extraction. In addition, the essential oils were assayed for their potential antioxidant and antibacterial activities. Gas Chromatography Mass Spectrometry (GC-MS) analysis revealed the presence of 1,8-cineole (75.19%), α-phellandrene (5.53%), P-eugenol (5.53%), camphor (5.45%), α-terpineol (2.09%), β-pinene (1.66%), camphene (1.20%), α-pinene(1.02%) from *A. millefolium* L. However, *A. wilhelmsii* was characterized with menthoglycol (35.84%), 1,8-cineole (34.04%), endo-borneol (9.93%), chrysanthenil acetate (4.76%), thymine (3.66%), terpinene-4-ol (2.33%), camphene (1.66%), and verbenole (1.53%). Regarding scavenging activities of the species, *A. wilhelmsii* exhibited better activity than *A. millefolium*, with a value of 6.5 mM and 4.2 mM Trolox equivalents (TEAC) respectively. With respect to the antibacterial activity against three gram-negative and three gram-positive bacteria, essential oils of both species were compared with standard antibiotic discs (ampicillin and ofloxacin).

Keywords: *Achillea millefolium, Achillea wilhelmsii, Yarrow, Essential oils, Biological activity, GC-MS analysis.*

[a] *Igdir University, Faculty of Agriculture, Department of Field Crops, Igdir, Turkey*
[b] *Yuzuncu Yil University, Faculty of Veterinary Medicine, Food Hygiene and Technology, Van, Turkey*
[c] *Igdir University, Vocational School of Technical Sciences, Department of Herbal and Animal Production, Igdir, Turkey*
[*] *Corresponding author: mehmetzekikocak@gmail.com*

INTRODUCTION

Medicinal and aromatic plants have been used for centuries, and are rich valuable natural source of biologically active compounds, being used in traditional medicine for the treatments of various ailments as the possess antioxidant, antimicrobial, anti-inflammatory and antispasmodic activities [1-2]. In addition, the plants were also evaluated in pharmaceutical, sanitary, cosmetics, fragrances, plant protection, agricultural and food industries [2-4]. The genus Achillea has a wide range of distribution, and comprises more than 115 species. Most of the species (N=85) are distributed in the British Isles, southern Europe, Asia, Australia and North America [5]. Of those species, yarrow species (*A. millefolium* L. and *A. wilhelmsii* L.) is a widespread rhizomous herbaceous perennial medicinal plant belonging to the Asteraceae family confined to the Northern hemisphere [6-7]. Regarding the species, Turkey (Türkiye) is estimated to be quite rich in Achillea species; in addition to, about forty Achillea species show a wide distribution in Turkey. The yarrow species is locally named as “civanperçemi, pireotu, yılançiçeği, ormaderen, buyucan, kılıçotu and çoban kirpiği” [8].

Approximately, in the last three decades, a quite number of studies linked to the essential oils from aerial parts or flowers of Achillea species have been carried out [6-9-10-11]. Five most abundant monoterpene compounds were 1,8-cineole, camphor, borneol, α- and β-pinenes in the essential oils of Achillea species [12]. A study in Turkey revealed that 1,8-cineole was a major component and followed by camphor and borneol in Achillea species [13]. The most common essential oils extracted from *A. millefolium* populations were α- and β-pinenes, P-menthane, thujane, pinane, chamazulene, β-caryophyllene, eudesmol, ascaridole and β-oxide [14]. However, *A. wilhelmsi* is rich in sesquiterpenes, lactones, flavonoids and monoterpenoids, which have antimicrobial and antioxidant activities [15-16].

Yarrow is widely used for gastrointestinal disorders in Iranian traditional medicine, and an appetizer, wound healer, diuretic, carminative or menstrual regulator, anti-hemorrhoids and decreasing cholesterol in Turkey [8]. They have a wide range of biological activities including antispasmodic, antiulcer, antioxidant, antibacterial, antifungal, antimicrobial, antihypertensive, anti-hyperlipidemic, vagolytic and antitumoral properties [6-17], and treatment of pain, inflammation, headache, and spasmodic diseases [18]. The hygiene industry utilizes the essential oils of these plants to make skin tender and to treat skin inflammations using cream formulations [17]. Turkey has a rich medicinal and aromatic plant flora that is widely available throughout the country. In this regard, a plethora of reports on those species is available. However, the reports on the species from Eastern Anatolia region are rare.

Due to great differences in the climatic conditions of Eastern Anatolia region rather than other regions of Turkey, we hypothesized that the species of this region would have distinct chemical composition in relation to the species of other regions. The plausible variations in chemical composition would be manifested in their antioxidant and antibacterial activities. To test the hypothesis, we collected yarrow species from the relevant region and extracted the essential oil. Finally, both chemical composition and essential oil content were determined.

RESULTS AND DISCUSSION

Essential oil constituents of *A. millefolium* L. and *A. wilhelmsii* L.

The essential oil compounds identified by Gas Chromatography Mass Spectrometry (GC-MS) analysis of the species were listed in Table 1 along with retention time and percentage of composition. Fifteen compounds from *A. millefolium* and eighteen compounds from *A. wilhelmsii* were identified representing 100% of the total essential oil in the both species. Major components of *A. millefolium* were 1,8-cineole (75.19%), α-phellandrene (5.53%), P-eugenol (5.53%), camphor (5.45%), α-terpineol (2.09%), β-pinene (1.66%), camphene (1.20%) and α-pinene (1.02%). However, main components of *A. wilhelmsii* were menthoglycol (35.84%), 1,8-cineole (34.04%), endo-borneol (9.93%), chrysanthenil acetate (4.76%), thymine (3.66%), terpinene-4-ol (2.33%), camphene (1.66%), and verbenole (1.53%) (Table 1; Fig 1 and 2).

The relative of results, P-eugenol is the main constituent of clove (*Achillea millefolium*). The antimicrobial activity of eugenol is mainly attributed to its phenolic structure and to the hydrophobic character of this compound, facilitating its interaction with the microbial cell envelope. In addition, interfere with microbial virulence, reducing biofilm formation and modulating the expression of target virulence genes in gram-negative pathogens.

As a result of the literature studies, it has been observed that there are great differences in the chemical composition and essential oil components of the species found in different countries. Because of their genetically and chemically polymorphic structure, the essential oil content Achillea species could be affected by various factors such as environmental, genotypic, seasonal, soil conditions, developmental stage of plant, harvest time and storage conditions [18]. The main essential oil constituents of *A. millefolium* varied depending on the environmental conditions, locations and genotypes: 1,8-cineole, camphor, borneol, terpinolene, γ-terpinene, thujone, sabinene, cis-chrysanthenol or germacrene D, α-copaene, β-pinene, chamuzulene, p-cymene and methyl eugenol in Iran [19], D-cardinene, limonene, alloaromadendrene in Turkey [1],

1,8-cineole and germacrene-D in Serbia [20], 1,8-cineole, borneol, camphor, chamazulene, β-pinene, nerolidol in Lithuania, 1,8-cineole, β-pinene, β-caryophyllene, chamazulene, sabinene, (E)-nerolidol, guaiol in Estonia [21], camphor, 1,8-cineole, β-pinene, sabinene in Kazakhstan [12].

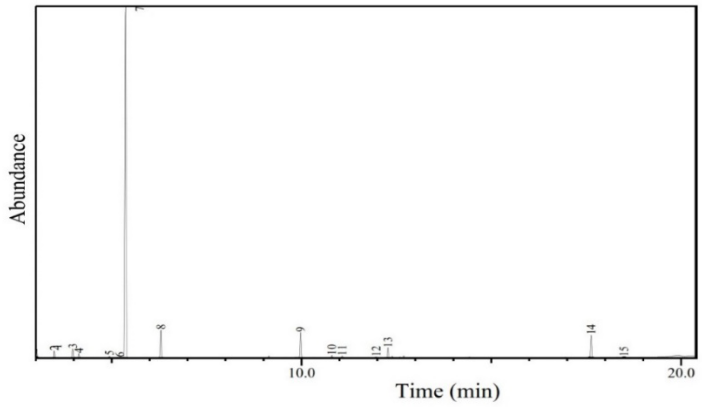

Figure 1. Gas-chromatogram of *Achillea millefolium* essential oils. The peaks correspond to identified compounds: (1) α-Pinene, (2) Camphene, (3) β-Pinene, (4) β-Phellandrene, (5) Terpinolene, (6) Paradiprene, (7) 1,8-cineole, (8) α-Phellandrene, (9) Camphor, (10) Endobornyl acetate, (11) Terpinene-4-ol, (12) δ-Terpineol, (13) α-Terpineol, (14) P-Eugenol, (15) Silaethane.

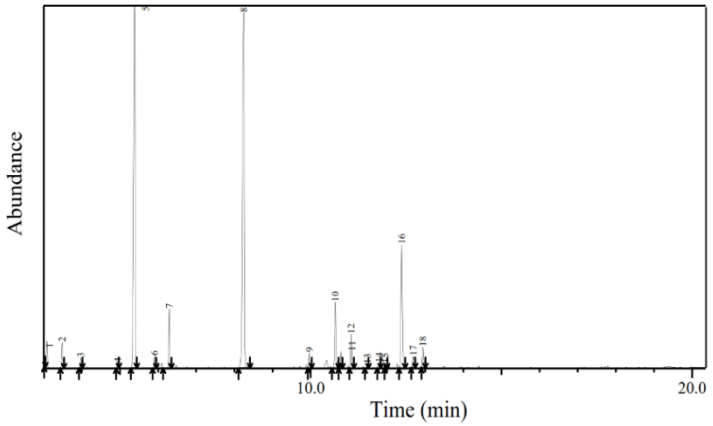

Figure 2. Gas-chromatogram of *Achillea wilhelmsii* essential oils. The peaks correspond to identified compounds: (1) α-Pinene, (2) Camphene, (3) β-Pinene, (4) α- Terpinolene, (5) 1,8-cineole, (6) γ- Terpinene, (7) Thymene, (8) Menthoglycol, (9) Camphor, (10) Chrysanthenil acetate, (11) α-Fenchyl acetate, (12) Terpinene-4-ol, (13) Trans limonene oxide, (14) Isopinocarvol, (15) α-Terpineol, (16) Ascaridole, (17) Endo-borneol, (18) Verbenole.

Table 1. Percentage and retention time of chemical components of essential oils of *Achillea millefolium* L. and *Achillea wilhelmsii* L.

Achillea millefolium				***Achillea wilhelmsii***			
Peak	**Components**	**Retention Index**	**Rate (%)**	**Peak**	**Components**	**Retention Index**	**Rate (%)**
1	**α-Pinene**	868	1.02	**1**	**α-Pinene**	868	0.23
2	**Camphene**	881	1.20	**2**	**Camphene**	881	1.66
3	**β-Pinene**	893	1.66	**3**	**β-Pinene**	893	0.65
4	**β-Phellandrene**	898	0.82	**4**	**α-Terpinolene**	919	0.32
5	**Terpinolene**	919	0.29	**5**	**1,8-Cineole**	931	34.04
6	**Paradiprene**	927	0.13	**6**	**γ-Terpinene**	946	0.71
7	**1,8-Cineole**	931	75.19	**7**	**Thymene**	957	3.66
8	**α-Phellandrene**	957	5.53	**8**	**Menthoglycol**	1008	35.84
9	**Camphor**	1045	5.45	**9**	**Camphor**	1045	1.09
10	**Endobornyl acetate**	1066	0.48	**10**	**Chrysanthenil acetate**	1059	4.76
11	**Terpinene-4-ol**	1068	0.36	**11**	**α-Fenchyl acetate**	1062	1.22
12	**δ-Terpineol**	1087	0.20	**12**	**Terpinene-4-ol**	1068	2.33
13	**α-Terpineol**	1094	2.09	**13**	**Trans limonene oxide**	1077	0.29
14	**P-Eugenol**	1209	5.53	**14**	**Isopinocarvol**	1084	0.41
15	**Silaethane**	1228	0.05	**15**	**α-Terpineol**	1094	0.37
16	**–**	–	–	**16**	**Ascaridole**	1103	0.95
17	**–**	–	–	**17**	**Endo-borneol**	1106	9.93
18	**–**	–	–	**18**	**Verbenole**	1108	1.53
Total			**100.0**				**100.0**

The essential oil composition of *A. wilhelmsii* were determined as following: 1,8-cineole, camphor, borneol, linalool, carvacrol, α-pinene, camphene, α-thujene, α-terpineol, terpinen-4-ol, thymol, P-cymene, artemisia alcohol, methyleugenol, sabinene, caryophyllene oxide, chrysanthenyl acetate, E-nerolidol, dihydrocarvone [6-15].

After comparing all detected results with values from previous studies, we can confirm the idea that geographic origin has a significant influence on the chemical composition of Achillea species. However, it has been declared that climatic and environmental conditions have little effect on essential oils of Achillea species [22].

The major aromatic compounds from *A. millefolium* L. and *A. wilhelmsii* was 1,8-cineole (75.19% and 34.04%, respectively) in the present study. The

results indicated 1,8-cineole suppressed human colorectal cancer proliferation by inducing apoptosis suggesting 1,8-cineole would be an effective strategy to treat colorectal cancer [23]. The study of demonstrated antispasmodic and antisecretory activities of 1,8-cineole, and rationalized the traditional use of the plant containing various levels of this terpene in the treatment of gastrointestinal complains such as diarrhea [24].

Antioxidant activity of *A. millefolium* and *A. wilhelmsii* essential oils

Natural antioxidants obtained mainly from herbal materials are effective agents in the prevention of adverse effects of free radicals. Antioxidative effectiveness in natural sources was reported to be mostly due to phenolic compounds, and these compounds have an important role in inhibiting auto-oxidation of the essential oils [8-17]. Trolox equivalent antioxidant capacity (TEAC) or ABTS+ method relies on the reduction of the blue-green cation radical of ABTS. The extent of decolorization, expressed as percentage inhibition of ABTS+, is determined as a function of the concentration and the time, and it is calibrating against Trolox as the reference standard [25]. The concentration of antioxidants that produce the same effect as 1 mM Trolox and ABTS absorbance differences is considered TEAC. In addition, ABTS+ radicals are commonly used as "indicator compounds" for testing hydrogen donating potential and thus antioxidant activity. The indicated method is the most widely used method for the determination of antioxidant activity of plant extracts and Table 2 shows the inhibition of ABTS+ radical by essential oils of yarrow plant species.

The findings of the present study revealed that yarrow essential oils exhibited antioxidant activities but to be distinct according to the species. Although *Achillea millefolium* samples demonstrated slightly well radical scavenging activity against ABTS radicals, *Achillea wilhelmsii* showed good radical scavenging activity against ABTS radicals: *Achillea millefolium* showed 4.2 mM Trolox equivalents activity; however, (Table 2) *Achillea wilhelmsii* has 6.5 mM Trolox equivalents activity [18]. Suggested that essential oils of yarrow species have significant antioxidative effect and scavenging effects are related to camphor and borneol of volatile substances.

Table 2. Radical scavenging activities of essential oils *Achillea millefolium* L. and *Achillea wilhelmsii* L. against ABTS+ radical

Sample	TEAC, mM Trolox (±SE, Standard Error)
Achillea millefolium	4.2 ± 1.4
Achillea wilhelmsii	6.5 ± 1.6

Antimicrobial activity of *A. millefolium* and *A. wilhelmsii* essential oils

Extracts from aromatic plants, particularly essential oils, are a rich source of biologically active compounds showing antimicrobial properties. Therefore, it is logical to expect a variety of plant constituents in these oils with specific as well as general antimicrobial activity or antibiotic potential [26]. In this study, the potential antimicrobial activity of essential oils from yarrow on the growth of bacteria was investigated. In order to evaluate the antibacterial potential the essential oils from yarrow were screened for activity against three gram-negative (*Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Escherichia coli*) and three gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Enterecoccus faecalis*). The essential oil showed different degrees of inhibitory effect on the growth of tested bacterial strains. The comparison of the antibacterial activity of essential oils of yarrow and two antibiotics (ampicillin and ofloxacin) are presented in Table 3 and Fig 3. The essential oils of yarrow showed lower antimicrobial activities in comparison to the antibiotics according to the inhibition zone.

Table 3. Comparison of antimicrobial activity of *Achillea millefolium* and *Achillea wilhelmsii* essential oils (in µL) and two antibiotics (ampicillin and ofloxacin) against some pathogens (diameter zones of inhibition, mm)

Pathogens	Antibiotics and Essential oil			
	Ampicillin	**Ofloxacin**	***A. millefolium***	***A. wilhelmssi***
***Staphylococcus aureus* ATCC 12600**	20 – 22	24 – 26	8 – 10	10 – 12
***Bacillus subtilis* ATCC 6051**	25 – 25	30 – 30	10 – 12	11 – 12
***Pseudomonas aeruginosa* ATCC 10145**	22 – 23	26 – 28	10 – 12	11 – 12
***Enterecoccus faecalis* ATCC 29212**	26 – 28	20 – 22	12 – 14	14 – 14
***Salmonella typhimurium* ATCC 25241**	22 – 24	24 – 26	8 – 10	12 – 12
***Escherichia coli* ATCC 11775**	26 – 28	26 – 28	8 – 8	9 – 10

Insensitive (-) : diameter of inhibition zones is smaller than 8 mm; Sensitive (+) : diameter of inhibition zones is between 9-14 mm; very sensitive (+ +): diameter of inhibition zones is between 15-19 mm; Extremely sensitive (+ + +) : diameter of inhibition zones is larger than 20 mm.

Although previous studies indicated that gram-positive bacteria were to be more sensitive to the action of many natural extracts [23], current results revealed there were no significant changes between these two bacteria groups. However, some studies have reported that gram-negative bacteria

are more resistant than gram-positives, due to restricted diffusion of the hydrophobic compounds through the hydrophilic cell wall structure, such as lipo-polysaccharides [27]. Although demonstrated that gram-positive bacterial strains were more susceptible to volatile oils of yarrow plant extracts [6], illustated gram-negative bacteria more sensitive to *A. wilhelmsii* essential oils. The results of revealed that the essential oils of yarrow species were effective for controlling of certain important gram-positive bacteria, which produces many infectious diseases [27].

Oxygenated monoterpenes like camphor, 1,8-cineole, linaool,-terpinol, 1-terpinen-4-ol, and borneol, which are major compounds in a few essential oils examined were reported to show antimicrobial activity. The oils of the yarrow plant, rich in camphor and 1,8-cineol content, have previously been shown to have effective antimicrobial activities in vitro [28]. It has been shown in another study that thymol and carvacrol essential oils inhibited *Eschericia coli, Salmonella enteritidis, Salmonella choleraesuis* and *Salmonella typhimurium* bacterial strains [29]. The results of revealed that essential oils of yarrow have a pronounced antioxidative activity [8], but low antimicrobial activity in vitro [27] illustrated that the essential oils of yarrow species were effective for controlling of certain important gram-positive bacteria, which produces many infectious diseases. Essential oils of *A. wilhelmsii* were effective on human pathogens such as *Proteus mirabilis, Staphylococcus aureus*, *Serratia marcescens* [30]. The suggested that essential oils of yarrow species have significant antibacterial effect and the presence of chamazulene increased the antibacterial activity of volatile substances [18].

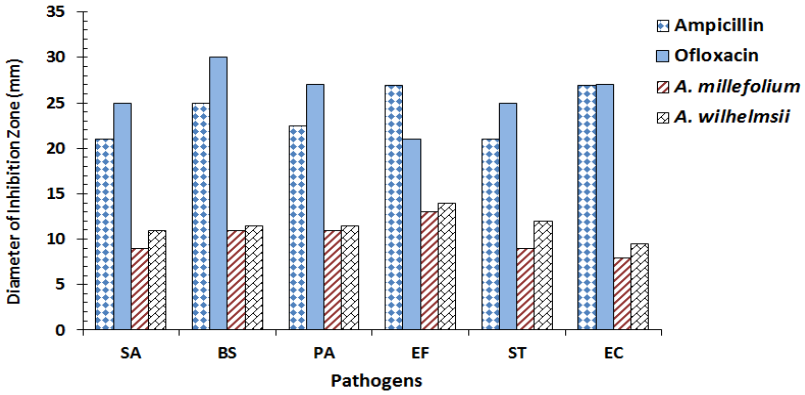

Figure 3. The comparison antimicrobial activity of essential oils of *Achillea millefolium* and *Achillea wilhelmsii* leaves and two antibiotics (ampicillin and ofloxacin) against six bacterial pathogen strains (SA: *Staphylococcus aureus* ATCC 12600, PS: *Bacillus subtilis* ATCC 605, PA: *Pseudomonas aeruginosa* ATCC 10145, EF: *Enterecoccus faecalis* ATCC 29212, ST: *Salmonella typhimurium* ATCC 25241, EC: *Escherichia coli* ATCC 11775).

Previous researches revealed that whole essential oils have a greater antibacterial activity than the major components mixed, which suggests that the minor components have a critical function to the antimicrobial activity and may have a synergistic effect [31]. However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Possible synergistic and antagonistic effect of compounds in the oil should also be taken into consideration as often-stronger antibacterial effect can be observed with complete essential oils in comparison to single oil components.

CONCLUSIONS

Besides the identified nutritional value, yarrow species can be an interesting source of bioactive compounds. The prominent essential oils content and antibacterial activity of the plant suggest that it may be of important for human health. According to the results, 1,8-cineole, α-phellandrene, P-eugenol, camphor, and α-terpineol were the main compounds of *A. millefolium*, and menthoglycol, 1,8-cineole, endo-borneol, chrysanthenil acetate and thymine were the main compounds of *A. wilhelmsii.* The context, natural essential oils as biological active compounds may also effective, selective, biodegradable and less toxic to environment. This study establishes a relationship between the composition of the oils of the respective plant and their corresponding therapeutic properties. As a result of presented findings, it can be concluded that the essential oils obtained from *A. millefolium* and *A. wilhelmsii* are interesting from a medicinal standpoint because of their antioxidant and antibacterial activities. The results suggest a basis for selection of the plant for further phytochemical and pharmacological investigations. The improving of natural antimicrobials will help to reduce the negative effects of synthetic antibiotics. Finally, further studies are warranted to confirm these results and elucidate the effect of these plants on other biological activities by conducting additional antioxidant and antimicrobial activity assays.

EXPERIMENTAL SECTION

Collection and preparation of plants

The fresh aerial parts of *A. millefolium* L. and *A. wilhelmsii* L. plants were collected from the naturally growing plants on the plateaus and rangelands of Van, Turkey in August 2010. The relative plants collected from located between 38°23'00" N and 43°14'36" E GPS. The taxonomic description of the plant samples collected in the distribution area was made by a plant taxonomist

from the Department of Biology at Yuzuncu Yıl University, Van, Turkey. The collected plant materials were air-dried under shade, ground into small pieces, and kept for further analysis.

Chemicals

ABTS, helium tryptic soy broth, 2,2-azinobis (3 ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) and potassium persulfate were obtained from Sigma-Aldrich. In addition, all other chemicals were of analytical grade.

Antibiotics

For the comparison of the antibacterial activity of essential oils, two antibiotics (ampicillin and ofloxacin; BBL®) were used. Disks are 1/4" in diameter; 50 disks per vial.

Extraction of essential oil

The shade-dried for both plant samples (100g) were subjected to hydro distillation for 3 h using an Clevenger-type apparatus. The both plants oils were extracted with tap water and stored under N_2 atmosphere in a sealed vial until use at 20°C. The yields were based on dry materials of plant samples. After decanting, the obtained essential oils were dried over anhydrous sodium sulfate and after filtration stored in refrigerator at +4°C until tested and analyzed.

Identification of essential oil components

The aerial parts of two plants were shaken sequentially in percolation with N-hexane-ether (1:1,v/v) and methanol for 16 h at room temperature. Samples were sonicated separately for 15 min twice, and then solvents were removed subsequently under reduced pressure by rotary evaporator apparatus. The extracts were weighed and stored in refrigerator at +4°C until the analysis. The essential oils were transferred to the solvent (n-hexane) and after dilution; the desired components were identified by GC-MS. In the preliminary study of the plant species collected beforehand, essential oil components were identified. The best temperature program of essential oils was determined later in GC-MS analysis and the same program was used for all samples.

Gas Chromatography-Mass Spectrometry Analysis

The relevant analyses were carried out on Shimadzu QP2010 brand model gas chromatography quadrupole-mass spectrometry system appropriated with a TRB-WAX column (30 m×0.25 mm film with 0.25 μm thickness). Carrier

gas was helium with a linear velocity of 36.25 cm s^{-1}; split ratio was 1:50 at a flow rate of 1 mL min^{-1}. The first oven temperature 60°C for 2 min, after that programmed to increase from 60 to 240°C at 10°C min^{-1}, and finally held isothermally for 5 min at 240°C. The whole time was 25 min. The injection and ion source temperatures were 240°C. The injection volume was 1 µL in the splitless mode. Probably the ionization was with 70 eV. The mass range was from 40 to 300 m/z. The components of essential oils were determined by matching relative retention times and mass spectra with authentic samples from essential oil library data (Nist 27, Wiley, 7 and Nist 147) and by comparing relative retention indices (RRI) with published data [32].

Determination of antioxidant activity

Total antioxidant activity values of *A. millefolium* and *A. wilhelmsii* species were determined as described by [25]. ABTS radical cation (ABTS+) was obtained by reacting ABTS+ stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to keep waiting in the dark for 12–16h before use. The radical was stable in this form for more than two days when stored in dark at room temperature. Essential oils of yarrow species were used for antioxidant activity measurement. The ABTS solution was diluted with distilled water to an absorbance of 0.70±0.02 at 734 nm and equilibrated at 30°C. After mixing 2.95 mL of diluted ABTS+ solution into 5 mL of antioxidant compounds or Trolox standards in ethanol, the absorbance reading was taken at exactly 30°C 6 minutes after initial mixing. Solvent blanks were run for each assay. Percent inhibition of absorbance at 734 nm was calculated and plotted as a function of antioxidants and Trolox concentration for standard reference data. Total antioxidant activity was expressed as mM Trolox equivalents antioxidant activity (TEAC).

Determination of antibacterial activity

In vitro antibacterial studies were carried out against six bacteria strains: *Staphylococcus aureus* ATCC 12600, *Bacillus subtilis* ATCC 6051, *Pseudomonas aeruginosa* ATCC 10145, *Enterecoccus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 25241, *Escherichia coli* ATCC 11775). The microorganisms were obtained from the Department of Clinical Microbiology, Faculty of Medicine, and Van, Turkey Yuzuncu Yıl University. The antibacterial activity of essential oils was tested using the disc diffusion method [33]. Briefly, filter paper disks, 6mm in diameter, were imprenated with 5 µL of the essential oils (directly). The bacteria strains were inoculated on tryptic soy agar (Oxoid) for 3–4 h. The density of the cultures was adjusted according to the McFarland 0.5 tube turbidity. Activated microorganisms were spread

on the surface of predetermined Mueller-Hinton Agar (Merck) plates using a sterile swap, and incubated for half an hour. Paper discs (6 mm diameter, Whatman 2017-006) were impregnated with essential oils and transferred onto the Mueller-Hinton agar plates, whose surface had been spread with 0.5 mL of bacterial suspension. Ampicilline and ofloxacine were used as control agents.

The available microorganism species were incubated in the oven at the recommended temperature and time. After the colonies formed around the obtained zone diameter, the inhibition was measured in millimeters with the help of the zone scale. The sensitivity of the bacteria to essential oils and the size of the inhibitory areas were expressed by comparing those [34]. The results were evaluated as follows: zones that were smaller than 8 mm were classified as insensitive, zones 9–14 mm were sensitive, zones 15–19 mm were very sensitive, and those larger than 20 mm were extremely sensitive [35]. All tests were done in duplicate/triplicate and repeated 2/3 times. In addition, the results were expressed as average values.

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