

Essential Oil Composition and Antimicrobial Activity of *Achillea biebersteinii* Afan. (Asteraceae) from Erzincan Region, Turkey

Emre SEVİNDİK^{1*}, Sinem AYDIN², Elif EREN APAYDIN³,
Mustafa SÜRMEN⁴

¹Adnan Menderes University, Faculty of Agriculture, Department of Agricultural Biotechnology, South Campus, Çakmar, Aydın, Turkey; ph.d-emre@hotmail.com (*corresponding author)

²Giresun University, Faculty of Science and Arts, Department of Biology, Giresun, Turkey

³Giresun University, Center Research Laboratory Application and Research Center, Giresun, Turkey

⁴Adnan Menderes University, Faculty of Agriculture, Department of Field Crops, Aydın, Turkey

Abstract

In the present study, chemical composition determination and *in vitro* antimicrobial effects of essential oil of *Achillea biebersteinii* plant grown under Erzincan ecological conditions were evaluated. Extractions were carried out with Clevenger apparatus and essential oil composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS). Microorganisms used for the antimicrobial studies were *Salmonella enterica* serovar. *typhimurium* ATCC 14028, *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Yersinia pseudotuberculosis* ATCC 911, *Bacillus cereus* 702 ROMA, *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22 and *Proteus vulgaris* FMC 1. As a result, a total of 29 components were detected in *Achillea biebersteinii*. Among them, 1,8-cineole (20.36%), cyclohexanone (8.39%), 2-cyclohexen-1-one (5.38%) and spathulenol (4.19%) were found as the major components. For the *in vitro* antimicrobial activity determination of essential oil, disc diffusion method was used in our study. Furthermore, 12-14 mm zone diameters were detected in antimicrobial activity assay. The highest resistance zone was detected against *B. subtilis* with 14 mm diameter while the least resistance zone was detected against *Y. pseudotuberculosis*, *E. aerogenes* and *P. vulgaris* with 12 mm diameter. Consequently, it was concluded that the essential oil extracted from the *A. biebersteinii* grown under Erzincan ecological conditions had an inhibitory effects on the pathogenic microorganisms in used method.

Keywords: *Achillea biebersteinii*; antimicrobial activity; Erzincan; essential oil

Introduction

Autoecological studies on economically important plants in Anatolia, which has a rich vegetation cover thanks to its geographical location and climate, are of great significance in order to understand the growth conditions and effective use of these plants (Çelik *et al.*, 2004; Paksoy *et al.*, 2006). The Asteraceae family, which is known to have the greatest number of species in the world, consists of approximately 23000 species and 1535 genera (Öztürk and Çetin, 2013; Nylinder and Anderberg, 2015). It has been reported that a large number of species of the Asteraceae

family, expanding widely over the world and in Anatolia, show pharmacological activities. The plants in this family include sesquiterpene lactone metabolites having numerous biological activities such as antibacterial, antifungal, antihelminthic, anti-inflammatory, insecticide and antitumor, in addition to diterpenes and flavonoids (Picman, 1986; Shing *et al.*, 2002; Ertürk, 2003; Bağcı *et al.*, 2008). *Achillea* L. is a large genus belonging to the family Asteraceae. The genus *Achillea* L. includes 59 taxa divided into 6 sections. Among them, 31 taxa are endemic to Turkey (53%) (Arabaci, 2012; Aytac *et al.*, 2016; Tabanca *et al.*, 2016). The *Achillea* genus has a wide expansion range (Çelik and Akpulat, 2008). Differences in oil composition can be due to different environmental factors such as seasonality and developmental stage in addition to plant genetic type, especially in chemically polymorphic and

perennial plants (Bezic *et al.*, 2003). Terpenoids are the principle components of *Achillea* essential oils (Si *et al.*, 2006; Nemeth and Bernath, 2008; Motavalizadehkakhky *et al.*, 2013). *Achillea biebersteinii* is called “kılıçotu” (swordfish) and “sarı ot” (yellow weed) in Turkish and it is traditionally used to treat bleeding, infertility, asthma, stomach ache and cancer (Yıldırım *et al.*, 2015). The aim of the present study was to determine the essential oil contents of *A. biebersteinii* specimens grown under Erzincan ecological conditions, and to investigate their antimicrobial activity against various pathogenic bacteria.

Materials and Methods

Plant material and GC-MS analysis

Achillea biebersteinii samples of the plants were collected as study materials in July 2016 from Erzincan/Turkey surroundings (approximately 1,200 m altitude). Extractions were carried out with Clevenger apparatus (Basaran cam, Turkey and Misung Scientific Co., Korea) using water distillation (Balnova and Dyakov, 1974) and essential oil compositions were determined by Gas Chromatography-Mass Spectrometry (GC-MS). Characterization of essential oil components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.

Test bacteria and antimicrobial activity

Seven bacterial strains were utilized to detect antimicrobial action of the essential oil. *Salmonella enterica* serovar *typhimurium* ATCC 14028 and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 were obtained from Giresun Province Control Laboratory, *Yersinia pseudotuberculosis* ATCC 911 and *Bacillus cereus* 702 ROMA were obtained from Molecular Biology Department of Rize University. *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22 and *Proteus vulgaris* FMC 1 were obtained from Department of Biology, Firat University. Bacterial strains were maintained on nutrient agar at 4 °C. Examination of essential oil for antimicrobial efficiency was performed by the disc diffusion method. It was applied using a 24 h culture at 37 °C in Mueller Hinton Broth. The turbidity of bacterial suspensions was adjusted to 108 cfu/mL (turbidity = McFarland standard 0.5). The oil was dissolved in DMSO (dimethyl sulfoxide) 1:2 (v/v) to give stock solution after which they were mixed for total solubilization at 180 rpm for 10 minutes. Dissolved extracts was sterilized by using 0.45 µm pore sized filter (Dobre *et al.*, 2011). Inhibition zones of the essential oil were compared with standard antibiotics (tetracycline and gentamycin). Sterilized nutrient agar medium was poured in petri dishes and was allowed to solidify. The bacterial suspension inoculated into Mueller Hinton agar plates. Sterile discs were put (5 mm diameter) on the agar and 20 µL *A. biebersteinii* essential oil, and 20 µL DMSO were put on the discs, respectively. The inoculated plates were left in refrigerator for one hour then plates were incubated at 37 °C overnight (Murray *et al.*, 1995; Saric *et al.*, 2009). Diameter of inhibition zones was measured in millimetres.

Results and Discussion

Chemical composition of the essential oil

Essential oils are complex mixtures obtained from the leaves, fruits, shells and roots of plants through distillation or pressing (Grassmann and Elstner, 2003; Wallace, 2004; Oussalah *et al.*, 2006). Essential or volatile oils are natural products which are liquid at room temperature, easily crystallizable, usually colorless or pale yellow, volatile and strongly aromatic. Since they do not mix with water, they are different from oils although they are defined to be so (Grassmann and Elstner, 2003; Bicer *et al.*, 2003; Kılıç, 2008; Evren and Tekgüler, 2011). In the hereby study, 29 components were isolated from *A. biebersteinii*, which formed 60.26% of the total essential oil amount. Based on the results of the gas chromatography-mass spectrometry analysis, the most commonly found substances were detected as; 1, 8-cineole (20.36%), cyclohexanone (8.39%), 2-cyclohexen-1-one (5.38%) and spathulenol (4.19%) (Table 1). There are many previous studies relating to the chemical composition of the essential oils obtained from *A. biebersteinii*. Bader *et al.* (2003) analyzed the essential oil composition of *A. biebersteinii* collected from Jordan. In their study, the concentrations of some essential oils from highest to lowest were as follows: cis-ascaridol (36.2%), *p*-cymene (31.6%), carvenone oxide (6.4%) and camphor (4.7%). Barış *et al.* (2006) investigated the essential oil and biological activities of *A. biebersteinii* collected from Erzurum province, and reported piperitone (31.06%), camphor (12.46%), eucalyptol (10.98%) and 1,8-cineole (10.93%). Toncer *et al.* (2010) also investigated the essential oil composition of *A. biebersteinii* specimens collected from Bingöl / Genc, Mardin / Midyat, Siirt / Kurtalan and Elazığ / Kömürhan. In the essential oils obtained from the above locations in their study, the components with the highest amounts were as follows: Bingöl / Genc population, 1,8-cineole (15.04%), camphor (14.55%) and d-piperitone (12.53%); from Mardin / Midyat population, 1,8-cineole (31.76%), camphor (27.46%) and d-piperitone (11.97%); from Siirt / Kurtalan population, ascardiol (61.95%) and *p*-cymene (15.61%); from Elazığ / Kömürhan population, 1,8-cineole (42.17%), camphor (15.92%) and α -pinene (4.47%). Rustaiyan *et al.* (1998) examined the essential oil composition of *A. biebersteinii*, which is also spread in Iran. Accordingly, the most commonly found substances were detected as ascidolide (37%), piperitone (17%) and camphor (12%). Ghani *et al.* (2008) studied the composition of the essential oil of *A. biebersteinii* from Golmakan, a district of Razavi Khorasan province (North East of Iran) and they found 1, 8-cineole (32.82%), carvacrol (10.85%) and piperitone (7.34%) components as the most abundant ones. Tabanca *et al.* (2011) investigated the essential oil composition of *A. biebersteinii* populations obtained from various provinces of Turkey. In the essential oil obtained from the Ankara-Kızılcahamam population, 1,8-cineole (36.0%) and camphor (30.0%); from the Ankara-Yenimahalle population, *p*-cymene (27.0%) and camphor (24.5%); from the Konya-Beyşehir population, 1,8-cineole (36.9%) and camphor (15.6%); from the Konya-

Beyşehir-Akşehir population, 1,8-cineole (35.5%) and camphor (35.5%); from the Isparta-Yalvaç population, 1,8-cineole (34.3%) and camphor (21.7%) had the highest values. Typical for camphor are its antispasmodic, diuretic, antirheumatic and sedative effects. The biological function of the chemical components is not limited by their antimicrobial activity; some of them have antioxidant (limonene, pinene), antitumor (linalool, borneol), anti-inflammatory (sabinene, pinene) and analgesic functions (citral) as well (Teneva *et al.*, 2016). Considering the current study reporting essential oils from the *A. biebersteinii* specimens, the major components were detected as; 1, 8-cineole (20.36%), cyclohexanone (8.39%) and spathulenol (4.19%). In conclusion, the findings obtained from various studies were found to be different from the findings of the present study. This may be explained by the fact that the essential oil composition may have different qualities and quantities under different geographical and environmental conditions as well as at different periods of plant growth (Mazandarani *et al.*, 2013).

Antimicrobial activity

The most commonly studied dimension of essential oils is related to antimicrobial activities. These oils have antimicrobial effects against a variety of microorganisms including gram (-) and gram (+) bacteria. As the essential oils are complex mixtures comprising different components, action levels thereof vary depending on diversity and amount of the active substances. Despite having limited information relating to their mechanisms of action, it seems to be associated with lipophilic features and chemical structures of the oils (Bayaz, 2014). For the *in vitro* antimicrobial activity determination of essential oil, disc

diffusion method was used in our study. The results were compared to the standard antimicrobial agents such as gentamycin and tetracycline. Zone diameters of 12 mm -14 mm were detected in disc diffusion methods. *Achillea biebersteinii* essential oil was effective against *Salmonella enterica* serovar typhimurium *Staphylococcus aureus* subsp. *aureus*, *Yersinia pseudotuberculosis*, *Bacillus cereus*, *Enterobacter aerogenes*, *Bacillus subtilis* and *Proteus vulgaris*. The widest resistance zone was against *Bacillus subtilis* with 14 mm diameter while the smallest resistance zone was detected against *Y. pseudotuberculosis*, *E. aerogenes* and *P. vulgaris* with 12 mm diameter (Table 2). The essential oil exhibited lower activity than that of gentamycin, but it exhibited similar activity to that of tetracycline. Barış *et al.* (2006) examined antimicrobial activity of essential oil and methanol extract from *A. biebersteinii*. The methanol extract was not active against any studied microorganisms. On the other hand, essential oil inhibited the growth of 6 bacterial species tested. The essential oils also inhibit antifungal action as reported by Sökmen *et al.* (2004) who assessed antimicrobial activity of essential oil from *A. biebersteinii*. In their study, the essential oil displayed strong activity against *Candida albicans*, *Clostridium perfringens*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Mycobacterium smegmatis* (Sökmen *et al.*, 2004). Likewise, we observed activity against *S. aureus* and *B. cereus* in our study. Kotan *et al.* (2010) examined chemical composition of the essential oil of *A. biebersteinii* by GC-MS and tested antimicrobial effect of essential oil against 25 plant pathogenic bacteria. We also studied antimicrobial efficiency of *A. biebersteinii*, but it was obtained different results when compared to previous studies. These different results may have been obtained due

Table 1. Essential oil composition of *Achillea biebersteinii*

RT (min)	Component	Quantity (%)	RT(min)	Component	Quantity (%)
6.524	β -pinene	0.98	17.301	2-heptyl furan	0.18
6.806	3-octanol	0.23	17.486	5-amino-6-chloro-4-(2-ethenylamino)pyrimidine	1.48
7.606	1,8-cineole	20.36	19.501	bicyclo[4.1.0]heptan-3-ol, 4-hydroperoxy-4,7,7-trimethyl	1.59
7.917	4-hydroxy-6-methyl-2H-pyran-2-one	0.56	20.093	mint furanone 2	0.33
9.902	trans-pinocarveol	0.75	21.568	caryophyllene oxide	2.83
10.035	camphor	0.66	21.367	(+) spathulenol	4.19
10.509	cyclohexanone	8.39	21.989	α -pinene	2.25
10.561	borneol	3.59	22.841	caryophylla-4(12),8(13) diene 5 β -ol	0.18
11.028	menthol	0.25	23.278	t-Murolol	0.36
11.087	3-cyclohexene-1-methanol	0.72	23.686	caryophyllenol-II	0.62
11.590	bicyclo[3.1.1]hept-3-en-2-one	0.16	23.900	1-cyclohexene-1-butanol	0.63
12.057	bicyclo[2.2.1]heptan-2-ol	1.34	28.078	benzothiazole	0.25
15.220	2-cyclohexen-1-one	5.38	30.011	hexadecanoic acid	0.76
16.568	1-methoxy-4-(1-methylethenyl)benzene	0.56	37.121	furane-3-carbohydrazide	0.27
16.886	1,3-dimethoxy-2,5-dimethylbenzene	0.41		Total:	60.26

RT: Retention time

Table 2. Antimicrobial activity (inhibition zones) of the essential oils from *Achillea biebersteinii*

Microorganism	<i>A. biebersteinii</i>	Tetracycline	Gentamycine
	IZ, mm	IZ, mm	IZ, mm
<i>S. enterica</i> serovar <i>typhimurium</i> (-)	13.5±0.70	15±0.00	17.5±0.70
<i>Y. pseudotuberculosis</i> ATCC 911 (-)	12±0.00	NA	20±0.00
<i>E. aerogenes</i> CCM 2531 (-)	12±2.82	11.5±0.70	15.5±0.70
<i>B. subtilis</i> IMG 22 (+)	14±0.00	11±1.41	16±2.82
<i>S. aureus</i> subsp. <i>aureus</i> ATCC 25923 (+)	13.5±0.70	18.5±2.12	19.5±0.70
<i>P. vulgaris</i> FMC 1 (-)	12±2.82	11±1.41	15±0.00
<i>B. cereus</i> 702 ROMA (+)	13±0.00	10±0.00	17±0.00

IZ-Inhibition zones

to using different bacterial species, collecting plant specimens from different locations and using different amounts of essential oil against test microorganisms. Kordali *et al.* (2009) carried out a study about antifungal activity of *Achillea gypsicola* and *Achillea biebersteinii*.

There are many studies related to antimicrobial activity of different *Achillea* species. For example, Bezic *et al.* (2003) investigated volatile constituents and antimicrobial potentials of *Achillea clavennae*, and they found important decline of bacterial growth against *Escherichia coli* and *Proteus mirabilis*. On the other hand, *Bacillus cereus*, *Bacillus subtilis* and *Streptococcus faecalis* were resistant to the essential oil with inhibition zones between 4 mm and 6.5 mm. In a study which was carried out by Candan *et al.* (2003), essential oil of *Achillea millefolium* subsp. *millefolium* manifested antimicrobial activity against *Streptococcus pneumoniae*, *Clostridium perfringens*, *Candida albicans*, *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krusei*. Başer *et al.* (2002) worked out composition and antimicrobial activity of the essential oil of *Achillea multifida*. Plant pathogenic bacteria inhibition potential of *Achillea millefolium* was investigated by Vasinauskiene *et al.* (2006). The strength of the antimicrobial property has been reported to vary according to the used extract type, collecting plant materials from different locations, used extract concentration, and microorganisms tried (Srivastava *et al.*, 2013).

Conclusions

Considering the current study with the essential oils from the of *A. biebersteinii* plants, the major components were detected as, 1,8-cineole (20.36%), cyclohexanone (8.39%), 2-cyclohexen-1-one (5.38%) and spathulenol (4.19%). According to the results obtained in the hereby study, it is possible to conclude that antimicrobial activity of the essential oil is slightly lower in comparison to antibiotics (tetracycline and gentamycine) effect on tested seven different bacteria genus (*S. enterica* serovar *typhimurium*, *Y. pseudotuberculosis*, *E. aerogenes*, *B. subtilis*, *S. aureus* subsp. *aureus*, *P. vulgaris* and *B. cereus*). These findings may be a valuable resource for further biotechnological, biodiversity, pharmaceutical and medical studies. It will also help to understand the importance of the biological diversity and conservation biology efforts.

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