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Volatile Oil Components and Antibacterial Activity of *Achillea biebersteinii* Afan. from Lake Van Basin, Turkey

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Antibacterial activity of the essential oil from dried flowering aerial parts of *Achillea biebersteinii* (Van region of Turkey) was assessed against six microorganism using disc diffusion method. Five microorganisms sensitive to *Alcillea biebersteinii* Afan. were included *Salmonella typhimurium* ATCC 25241 (8-10 mm), *Bacillus subtilis* ATCC 6051 (8-10 mm), *Pseudomonas aeruginosa* ATCC 10145 (10-10 mm), *Staphylococcus aureus* ATCC 12600 (9-10 mm) and *Escherichia coli* ATCC 11775 (8-9 mm). Meanwhile, GC/MS analysis of oil releaved twenty-one compounds with the main compounds as camphor (20.77 %), 1,8-cineol (18.60 %), artemisia ketone (14.69 %), camphene (7.80 %), α-pinene (5.94 %), artemisia alcohol (5.88 %), β-phellandrene (4.87 %) and chrysanthenone (4.33 %), respectively.

Keywords: *Achillea biebersteinii* Afan., Artemisia ketone, *Enterecoccus faecalis*.

INTRODUCTION

The genus *Achillea* L., (Asteraceae) comprises over 100 species worldwide[1] of which 42 species are found in the flora of Turkey[2]. *Achillea* species are used for healing wound and as herbal tea to cure diarrhea, abdominal pain and flatus[3-5]. In addition, some of this species are used as natural dyes due to flavonoids in the content[6]. *Achillea* species are diuretic, emmenagogue agents, antichloristic antispasmodic, antiseptic and infection preventing properties and used to reduce sweating and to stop amarum, stomachicum, cholagolum and carminativum[3,7,8]. *Achillea biebersteinii,* one of the most predominant *Achillea* species in the Mediterranean region, is a perennial herb with erect stems, 30-60 cm high, leaves up to 10 cm, radiate heads and large dense compound corymbs. *A. biebersteinii* Afan., locally named Kiliç otu and Sari çiçek in Turkish, is used as a folk remedy to stop bleeding, treat inappetence, asthma, stomach ache and cancer[9]. Antimicrobial substances are secondary metabolites which is preventing the growth of microorganisms in very small density and these substances have biological origin[10]. Activity of natural antioxidants are closely related to theirs biological functions. The reduction of chronic diseases, DNA damage, mutations, carcinogenesis and Inhibiting of pathogenic bacterial growth is known to be associated with that free radical development in biological systems is terminate[11].

EXPERIMENTAL

The aerial parts of *Achillea biebersteinii* were collected during the vegetative and flowering stages in August 2013 from from lake Van basin of Turkey (38°38′N 42°49′E) at an altitude of 1,640 m. A voucher specimen was deposited in the Herbarium of Field Crops Department, Ynzuncu Yil University.

GC/MS analysis: GC/MC analyses of the essential oils were performed using QP2010 gas chromotography quadrupole mass spectrometry system fitted with an TRB-WAX column (30 m × 0.25 mm i.d., × 0.25 μm film thickness). Carrier gas was helium at a flow rate of 1.2 mL/min. The oven temperature was first kept at 60 °C for 2 min, then raised to 240 °C at the rate of 10 °C/min and held isothermally for 5 min. The components were identified by matching relative retention times and mass spectra with actual samples from essential oil library data (Wiley and Nist) and by comparing relative retention indices (RRI) with published data.

Identification of essential oil components: The shade-dried plant samples (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The oils were extracted with distilled 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.

Determination of antibacterial activity: *In vitro* antibacterial studies were carried out against 6 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. All microorganisms were obtained from the Department of Clinical Microbiology, Faculty of Medicine at Yuzuncu Yil University, Van, Turkey. The antibacterial activity of the essential oils was tested using the disc diffusion method proposed by Bauer *et al.*[12]. Briefly, filter paper disks, 6 mm 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 these cultures was adjusted according to the McFarland 0.5 tubes blur. Activated microorganisms were spread on the surface of predetermined Mueller-Hinton agar (Merck) plates using a sterile swap and incubated for 0.5 h. 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. Microorganisms were incubated in the oven according to the species at the appropriate temperature and time. After the colonies formed around the zones, the zones were measured with the inhibition zone scale in millimeters. The sensitivity of the bacterial species to the essential oils was determined by comparing the sizes of inhibitory zones[13]. 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[13,14]. All tests were done in duplicate/triplicate and repeated 2/3 times. The results were expressed as average values.

RESULTS AND DISCUSSION

Essential oil compounds identified by GC-MS analysis of *Achillea biebersteinii* Afan. is listed in Table-1 along with the relevent retention indices. Twenty-one compounds representing 98.71 % of the total essential oil were identifed. Camphor (20.77 %), 1,8-cineol (18.60 %) and artemisia ketone (14.69 %) were the main components, comprising 54.06 % of the essential oil (Table-1).

Achillea species essential oil has been so far reported to consist mainly of monoterpenes, 1,8-cineole, camphor, borneol and piperitone[15-21]. Likewise, the oil of *A. pachycephala* was found to contain 1,8-cineole and camphor as the major constituents, On the other hand *A. biebersteinii* was rich in camphor and borneol followed by 1,8-cineole[22], whereas 1,8-cineole (32.82 %), carvacrol (10.85 %) and piperitone (7.34 %) were the major components of Iranian samples[23] like the predominant components of Turkish plants including 1,8-cineol (38.09 %) and camphor (23.56 %)[24]. Therefore, in terms of the two first major components of current study, our findings are identical to those from samples of other locations. Though camphor, 1,8-cineole and piperitone were generally found to be the main constituents, there could be seen a considerable variation among the dominant chemical composition of *Achillea* essential oils. In some cases, for instance, the analyzed essential oils were dominated by *cis*-ascaridole (36.20 %), *p*-cymene (31.60 %), carvenone oxide (6.40 %) (Italy) which are either in trace amount or never seen in current study. Similary, the essential oil *A. biebersteinii* growing in Jordan was found to be rich in ascaridole (36.2 %), *p*-cymene (31.6 %), carvenone oxide (6.4 %) and camphor (4.7 %)[16].

TABLE-1
COMPONENTS OF THE ESSENTIAL OILS FROM *Achillea biebersteinii* Afan.

Peak	Component	Retention indices	Rate (%)
1	α-Pinene	937	5.94
2	Camphene	950	7.80
3	β-Pinene	980	1.08
4	Yomogy alcohol	998	2.90
5	α-Terpinene	1018	0.31
6	*p*-Cymene	1025	0.49
7	β-Phellandrene	1029	4.87
8	1,8-Cineol	1033	**18.60**
9	γ-Terpinene	1060	0.94
10	Artemisia ketone	1064	**14.69**
11	Artemisia alcohol	1083	5.88
12	*trans*-Sabinene hydrate	1096	0.70
13	Chrysanthenone	1125	4.03
14	Camphor	1142	**20.77**
15	Pinocarvone	1162	0.66
16	Borneol	1166	2.56
17	Terpinen-4-ol	1176	1.31
18	α-Terpineol	1188	0.95
19	α-Fenchyl acetate	1231	2.42
20	Piperitone	1253	0.79
21	Germacrene D	1480	1.03
Total			98.71

Accordingly, the third most abundant compound of this study was artemisia ketone. However much it was included in *A. oxyodonta* species as predominant, the compound was not detected in almost any studies[22,23,25,26] or found to be low amounts in rare others[27]. Artemisia ketone isolation as a major component from the flowers of *A. biebersteinii* was reported for the first time in the current study.

The presence of piperitone in small amount is another outstanding point to be mentioned while the compound was present in appreciable amounts in some other findings[21-23,26,28]. Baris *et al.*[21], for instance, reported that piperitone as the major component of the essential oil from *A. biebersteinii*. Whereas, this component was found to be very low amount (0.79 %) in the oil extract in the present study.

Variability observed between either major or other constituents of the current study and those reported earlier seems to be arising from altitudes, sampling circumstances and postharvest processing.

Antibacterial activity of *Achillea biebersteinii* essential oil, ampicillin and ofloxacin against 6 bacteria strains are given in Table-2. All of the extracts were active against strains tested ranging from insensitive to extremely sensitive (8-30 mm). Ofloxacin was found to be active to an extreme degree against *Bacillus subtilis* followed by ampicilin on *Enterecoccus faecalis* and *Escherichia coli*. Compared to the antibiotics applied, the antimicrobial activity of *A. biebersteinii* was rather weak. In other words, *A. biebersteinii* exhibited low to mild antibacterial activity against bacterial species, except for *E. faecalis*, in which it was highly active (14-16 mm) (Table-2). *E. faecalis* was moderatley effected by *n*-hexane extract of *A. biebersteinii*

TABLE-2
ANTIMICROBIAL ACTIVITY RESULTS OF *Achillea biebersteinii* Afan. ESSENTIAL OILS COMPARE TO AMPICILLIN AND OFLOXACIN

	SA	BS	PA	EF	ST	EC
Ampicillin	20-22	25-25	22-23	26-28	22-24	26-28
Ofloxacin	24-26	30-30	26-28	20-22	24-26	26-28
A. biebersteinii	9-10	8-10	10-11	14-16	8-10	8-9

SA: *Staphylococcus aureus* ATCC 12600, BS: *Bacillus subtilis* ATCC 6051, PA: *Pseudomonas aeruginosa* ATCC 10145, EF: *Enterecoccus faecalis* ATCC 29212, ST: *Salmonella typhimurium* ATCC 25241, EC: *Escherichia coli* ATCC 11775; 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.

in Karaalp *et al.*[29] study in which methanol extract was even non-active. Paralelly, *A. biebersteinii* was found to be non-active on *E. faecalis*-ATCC-29122 according to Baris *et al.*[21].

A. biebersteinii inhibited the growth of *S. aureus* with the average inhibition zone of 9-10 mm. Comparably, the results of Kharma and Hassawi[30] and Sokmen *et al.*[19] from disc diffusion method indicated that *S. aureus* was almost equally affected by *A. biebersteinii* oil, with a mean inhibition zone of 14.15 mm and 12 mm, respectively, which is rather high to our finding. Nevertheless, our results are identical to those of Baris *et al.*[21] in terms of *S. aureus* in which the oil extract of *A. biebersteinii revealed* moderate inhibition zones of 10 mm. *Escherichia coli* reaction to *A. biebersteinii* has been found to variable in different studies. In current study, for instance, *E. coli* was almost insensitive to *Achillea biebersteinii* Afan. (8-9 mm) (Table-2). And while this zone was 5.8 mm in Kharma and Hassawi[30] study, the strain exhibited broader resistance (14 mm) toward oil extract[19]. The varying degrees of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacteria and the nature and combinations of phytochemicals present in the extracts as observed[22]. The antibacterial activity of the extracts of *A. biebersteinii* toward *Pseudomonas aeruginosa* was surprising. While inhibitory zone of the oil was 10-11 mm (Table-2), it revealed no activity against *P. aeruginosa* in other works[19,30]. The inhibitory effect of *A. biebersteinii* either on differnt *P. aeruginosa* types (*P. aeruginosa*-ATCC9027, *P. aeruginosa*-ATCC27859 and *P. aeruginosa* F5) orits other species (*P. pseudoalkaligenes* F6 and *P. syringae* pv.tomato A35) was zero[21]. We did not come across any activity of *A. biebersteinii* extract against *P. aeruginosa*. *A. millefolium* was only found to be mildly active against *P. aeruginosa*[20].

Conclusion

Fluctuations of major monoterpenes in the essential oils of *Achillea* species seems not to be merely concerned *Achillea* species but is true for most essential oil bearing crops and could be mainly clarified due to genetic diversity, ontogenetic phase and environmental circumstances. Which, in turn, maybe considered as keystones of the further studies in determination of superior populations in terms of antimicrobal agents. The essential oils of *A. biebersteinii* have not been fully evaluated against bacteria in a broad spectrum. Furthemore, multiple discrepancies could be seen concerning bacterial resistance in reports so far. This contradiction likely reflects the differences in plant subspecies, antimicrobial assay, extraction methods and in microbial strains, as wll. Moreover, strains' reaction toward the same volatile oil may be either due to constituents proportion and/or presence or absence of some particular components. Parallely, In most cases, the fractions had the same or greater activity than that of the crude oil. Also, synergistic and/or antagonistic effects might be taken into account for the antomicrobial activity. Either improvement or selection of high-performance *A. biebersteinii* crops could be used as potential antibacterial against infectious agents in new drugs development.

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