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RESEARCH ARTICLE

Evaluation of the Repellent Activity of 13 *Achillea* L. Species from Türkiye Against the Virus Vector *Aedes aegypti* (L.) Mosquitoes

Serdar DEMİR [1 (*)] Canan KARAALP [1] Nurhayat TABANCA [2] Ulrich Reginald BERNIER [3]
Kenneth James LINTHICUM [3]

[1] Ege University, Faculty of Pharmacy, Pharmaceutical Botany Department, TR-35030 Izmir - TÜRKİYE

[2] United States Department of Agriculture, Agricultural Research Service, Subtropical Horticulture Research Station, US-33158 Miami, Florida, UNITED STATES of AMERICA

[3] United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, US-32608 Gainesville, Florida, UNITED STATES of AMERICA

ORCIDs: S.D. 0000-0001-6572-8818; C.K. 0000-0002-4787-3779; N.T. 0000-0003-2802-8796; U.R.B. 0000-0002-4710-6801; K.J.L. 0000-0001-6526-7644

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Abstract: Mosquitoes serve as vectors of numerous dangerous animal diseases and some human diseases including malaria, filariasis, yellow fever, dengue, and other viral zoonotic infections in both tropical and temperate countries. Many synthetic chemicals have insecticidal and/or repellent effects that are used to control vectors and protect populations from vector-borne diseases. Since some synthetic chemicals have undesirable effects when used as repellents, attention has turned to developing biodegradable and non-toxic products, including essential oils, extracts, and secondary metabolites of various plants. In this study, to find new and alternative agents to control mosquitoes from natural sources, *n*-hexane, chloroform, and methanol extracts obtained from 13 *Achillea* L. species, including 4 taxa endemic to Türkiye, were evaluated for their insect-repellent activity against an important vector, *Aedes aegypti* (L.) mosquitoes by a cloth-patch assay. Among the tested samples, *n*-hexane extracts of *A. multifida, A. crithmifolia, A. setacea,* and *A. teretifolia* (MED: 0.344, 0.375, 0.409 and 0.437 mg/cm^2, respectively) showed higher repellency. These results indicate that the lipophilic components make a major contribution to repellency and that *Achillea* species can be used as a natural source for insect-repellents.

Keywords: *Achillea, Aedes aegypti, Insect repellent, Vector-borne disease*

Türkiye'den 13 *Achillea* L. Türünün Virüs Vektörü *Aedes aegypti* (L.) Sivrisineklerine Karşı Kovucu Aktivitesinin Değerlendirilmesi

Öz: Sivrisinekler, hem tropikal hem de ılıman ülkelerde çok sayıda tehlikeli hayvan hastalığının ve sıtma, filaryaz, sarı humma, dang humması ve diğer viral zoonotik enfeksiyonlar dahil olmak üzere bazı insan hastalıklarının vektörleri olarak görev yapmaktadırlar. Esas olarak böcek öldürücü ve/veya kovucu etkileri olan birçok sentetik kimyasal, vektörleri kontrol etmek ve halkı vektör kaynaklı hastalıklardan korumak için kullanılmaktadır. Bazı sentetik kimyasalların kovucu olarak kullanıldıklarında istenmeyen etkilerinin olması nedeniyle, dikkatler biyolojik olarak parçalanabilen ve toksik olmayan, bitkisel kaynaklı çeşitli uçucu yağlar, ekstreler ve sekonder metabolitler içeren ürünlerin geliştirilmesine çevrilmiştir. Bu çalışmada, sivrisinekleri kontrol etmek için doğal kaynaklı, yeni ve alternatif ajanlar bulmak amacıyla, 4 taksonu Türkiye'de endemik olmak üzere, 13 *Achillea* L. türünden elde edilen *n*-hekzan, kloroform ve metanol ekstreler, önemli bir vektör olan, *Aedes aegypti* sivrisineklerine karşı böcek kovucu aktiviteleri açısından bez-yama testi ile incelenmiştir. İncelenen örnekler arasında, *A. multifida, A. crithmifolia, A. setacea* ve *A. teretifolia*'dan elde edilen *n*-hekzan ekstreleri daha yüksek kovucu etki göstermişlerdir (sırasıyla MED: 0.344, 0.375, 0.409 ve 0.437 mg/cm^2). Elde edilen sonuçlar, lipofilik bileşenlerin kovucu etkiye büyük katkı sağladığını ve *Achillea* türlerinin böcek kovucular için doğal bir kaynak olarak kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Achillea, Aedes aegypti, Böcek kovucu, Vektörel hastalık*

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(*) Corresponding Author

Phone: +90 232 3113295 Cellular Phone: +90 554 2396183
E-mail: serdar.demir@ege.edu.tr (Demir S)

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Introduction

Mosquitoes live in a wide variety of environments worldwide, and colonize almost every aquatic habitat. Their blood feeding abilities make them the vectors of large variety of pathogens, and they are the most important group of arthropods from the standpoint of public and veterinary health [1]. Mosquitoes are important vectors that can transmit various pathogens that infect humans and animals, and generally, *Anopheles*, *Aedes,* and *Culex* species play an important role in mosquito-borne diseases [2]. *Aedes* mosquitoes are responsible for yellow fever, dengue haemorrhagic fever, and lymphatic filariasis in humans. They also transfer eastern equine encephalitis and setariasis between horses; rift valley fever and lumpy skin disease between cattle; avian malaria between poultry; dirofilariasis (Heartworm) between canines; and tularemia between birds, sheep, horses and pigs [1,3].

The mosquito vector *Aedes aegypti* L. (Diptera: Culicidae) has adapted to the urban environment and is the main cause of human transmission of many mosquito-borne arboviruses, which makes it one of the greatest global public health challenges in the world, and this mosquito continues transmitting diseases like dengue fever, yellow fever, chikungunya and Zika. *Ae. aegypti* mosquitoes are the primary arthropods that serve as vectors for dengue virus, and it is estimated that around 4 billion people live in areas at risk of dengue transmission [4]. Dengue and yellow fever vaccines are the only available vaccines, and no commercial vaccines are available for chikungunya and Zika. Integrated mosquito control strategies, including repellents, attractants, larvicides, adulticides, *Wolbachia*-infected or genetically modified mosquitoes may contribute to control of mosquito-borne diseases [5]. Therefore, reducing the mosquito population by using insecticides, and limiting the biting activities of mosquitoes by repellent compounds are the main control strategies for mosquito-borne diseases. However, new and safer insecticides or topical repellents are needed to provide protection against mosquito bites, as inappropriate and excessive application of these synthetic chemicals have undesirable effects on non-target organisms, on the environment, and on human health [6-8]. Frequent use of any single insecticide class, such as pyrethroids, can lead to non-target effects and the development of insecticide resistance, as numerous studies have shown that *Ae. aegypti* has developed resistance to conventional insecticides. As a solution on these challenges, research on novel mosquitocides with different modes of action such as larvicides, adulticides, pupicides, ovicides, repellents and growth inhibitors are continuing intensively, and plant derived natural products make a major contribution to this area [9-12].

Ethnobotanical and ethnoveterinary studies have reported the use of various plants for protection against vector-borne diseases. In rural regions, people use screens in windows and doors, proper clothing, bed nets, and natural repellents to protect themselves [13]. Hanging or sprinkling plants around the house, spraying plant juices in the house or livestock shelters, rubbing plants or applying plant juices onto skin or fur, and application of smoke by burning of plant parts are the most common practices used to repel mosquitoes and insects [13-15]. Citronella essential oils *(Cymbopogon nardus* and *C. winterianus)* are the most widely used as natural repellents in many repellent products today [16,17]. The synthetic repellent *N*,*N*-diethyl-3-methylbenzamide (DEET) is considered as the gold standard of insect repellents; however, the presence of insecticide-resistant strains and concerns with safety of DEET have prompted the search efforts in medicinal and aromatic plants to develop natural and eco-friendly products, if used in appropriate doses according to the list recommended in The Centers for Disease Control and Prevention (CDC) and the Environmental Protection Agency (EPA) [8,9,18,19].

The genus *Achillea* L. (Asteraceae) is commonly known as yarrow. Yarrow is an important medicinal and aromatic plant in traditional medicine and is generally used for bleeding, gastro-intestinal complaints, and menstrual spasm as an infusion internally, or as a poultice externally [20-22]. The genus is represented by about 115 taxa in the world, and *A. millefolium* L. is among the most widespread species [20]. The genus is represented by 61 taxa, of which 33 are endemic to Türkiye [23]. In the traditional medicine of Türkiye, the genus is generally known as "civanperçemi", "binbiryaprak" or "ayvadana", and herbal teas prepared from various *Achillea* spp. have been used as diuretics, emmenagogues, for abdominal pain, against diarrhea and flatulence [24,25]. Besides having a variety of activities, insecticidal, insect-repellent, and larvicidal activities of essential oils, different extracts and secondary metabolites from various *Achillea* spp. against different insects have been reported [26-29]. A wide variety of biological and pharmacological activities have been mainly attributed to essential oils, proazulenes, flavonoids, guaianolides, polyacetylenes, sesquiterpene lactones, dicaffeoylquinic acids, and alkamides [20-22,30].

Therefore, since there are scientific studies reporting insect-repellent properties of different *Achillea* species, thus, we investigated *n*-hexane, chloroform, and methanol extracts of various *Achillea* species, that were collected from different regions of Türkiye, against *Ae. aegypti* mosquitoes by using a human-based cloth patch repellent bioassay.

Material and Methods

Ethical Statement

This study was approved by the University of Florida Human Use Institutional Review Board (IRB-01, Protocol no: 2005-636).

Plant Material and Extraction

Thirteen *Achillea* taxa were collected during the flowering period from different regions of Türkiye and voucher specimens are deposited in the IZEF Herbarium of Ege University Faculty of Pharmacy, Dept. of Pharmaceutical Botany. The local names and collection sites are given in *Table 1*.

Table 1. *Scientific, and local names, voucher numbers, collection sites, and yields of extracts of Achillea taxa*

#	Plant Material	Local Names*	Voucher Numbers	Collection Sites	Solvents	Yield of Extracts (% of dry weight)
1	*A. multifida* (DC.) Griseb. (Endemic)	Ebulmuluk	(IZEF5598)	Bursa, Uludag	*n*-Hex	1.37
					$CHCl_3$	4.86
					MeOH	4.81
2	*A. teretifolia* Willd. (Endemic)	Beyaz civanpercemi	(IZEF5497)	Nigde, Altunhisar	*n*-Hex	1.08
					$CHCl_3$	4.50
					MeOH	5.22
3	*A. schischkinii* Sosn. (Endemic)	Deli civanpercemi	(IZEF5503)	Sivas, Karacaoren	*n*-Hex	1.37
					$CHCl_3$	2.46
					MeOH	3.08
4	*A. setacea* Waldst. & Kit.	Ayvabala	(IZEF5476)	Kirklareli, Saray	*n*-Hex	1.15
					$CHCl_3$	2.94
					MeOH	3.82
5	*A. crithmifolia* Waldst. & Kit.	Guzel namusotu	(IZEF5477)	Kirklareli, Kiyikoy	*n*-Hex	2.09
					$CHCl_3$	2.61
					MeOH	4.75
6	*A. falcata* L.	Sırcanotu	(IZEF5509)	Burdur, Elmaliyurt	*n*-Hex	0.59
					$CHCl_3$	1.60
					MeOH	2.73
7	*A. arabica* Kotschy	Hanzabel	(IZEF5501)	Konya, Aksaray	*n*-Hex	0.93
					$CHCl_3$	1.44
					MeOH	5.66
8	*A. coarctata* Poir.	Kirpit	(IZEF5473)	Tekirdag, Ganos Mountain	*n*-Hex	1.37
					$CHCl_3$	2.08
					MeOH	5.16
9	*A. pannonica* Scheele	Kurpotu	(IZEF5481)	Kirklareli, Igneada	*n*-Hex	1.78
					$CHCl_3$	2.62
					MeOH	3.39
10	*A. clypeolata* Sibth. & Sm.	Yilancicegi	(IZEF5479)	Kirklareli, Vize	*n*-Hex	0.79
					$CHCl_3$	1.69
					MeOH	2.88
11	*A. kotschyi* Boiss. subsp. *kotschyi*	Ayvadana	(IZEF5505)	Erzurum, Oltu	*n*-Hex	1.36
					$CHCl_3$	1.80
					MeOH	1.97
12	*A. phyrigia* Boiss. & Bal. (Endemic)	Ozge civanpercemi	(IZEF5498)	Kirsehir, Mucur	*n*-Hex	0.84
					$CHCl_3$	2.55
					MeOH	3.48
13	*A. nobilis* L.subsp. *neilreichii* (A. Kern.) Formánek	Binbir yaprak	(IZEF5510)	Burdur, Elmaliyurt	*n*-Hex	1.19
					$CHCl_3$	4.16
					MeOH	4.98

** Local names were provided from the reference* [23]

Dried flower heads of plants (50 g) were extracted using an orbital shaker (150 rpm) sequentially using *n*-hexane (*n*-hex), chloroform ($CHCl_3$), and methanol (MeOH) for 16 h (500 mL for each; all solvents were analytical grade) at room temperature. The extracts were separately filtered and concentrated under reduced pressure at 40°C with a rotary-evaporator and stored at -20°C for further experiments.

Mosquitoes

Aedes aegypti (Orlando strain, 1952) was used in this study. The mosquito colony was maintained at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE-USDA-ARS) in Gainesville, FL. Pupae were obtained from the colony, and nulliparous female mosquitoes aged 6-10 days were maintained on 10% sugar water and kept in laboratory cages at an ambient temperature of 28±1°C and relative humidity of 35-60%. Nulliparous female mosquitoes were preselected from stock cages using a hand-draw box and trapped in a collection trap. After 500 (±10%) females were collected in the trap, they were transferred to a test cage (dimensions 45x37.5x35 cm, ≈59.000 cm^3) and allowed to acclimatize for 17.5 (±2.5) min before testing was initiated [31].

Repellent Bioassay

Repellency was determined as the minimum effective dosage (MED, mg/cm^2) of *Achillea* extracts using human-based cloth patch assay *(Fig. 1)*. The samples were dissolved in *n*-hex, or $CHCl_3$, or MeOH, and each sample was tested by application of a suitable amount to a cloth to produce successive serial dilutions of 1.500, 0.750, 0.375, and 0.187 mg/cm^2. Each concentration was applied to a cloth to determine the point where the repellent failed for each of the volunteers. The test was conducted by having each volunteer affix the treated cloth onto a plastic sleeve to cover a 32 cm^2 window previously cut into the sleeve. Each of the volunteers wore this sleeve/cloth assembly above a nylon stocking that covered the arm, and protected the hand with a glove. The arm with the sleeve/cloth assembly was inserted into a cage where approximately 500 female *Ae. aegypti* mosquitoes (aged 6-10 days) had been preselected as host-seeking using a draw box. Failure of the repellent treatment is predetermined to be 1% bite through, i.e. the volunteer receives 5 bites through the cloth over the sleeve window in the 1 minute assay, and three repetitions were conducted for all samples [32]. During the experiment, solvents were used as negative controls, and DEET (97%, Sigma) was used as a positive control.

Statistical Analysis

All calculations [Analysis of variance (ANOVA), followed by mean separation with the Tukey-Kramer method (α=0.05) and followed by Dunnett's test (α=0.05)] were determined using a standart statistical software (JMP Pro.16.0 software; SAS Institute Inc. Cary, NC, USA).

Fig 1. Cloth patch assay (Photo by Greg Allen, Natasha M. Agramonte, Ulrich R. Bernier, USDA, ARS, CMAVE). **A-** The plastic sleeve that has a 32 cm^2 window previously cut, **B-** The arm with the sleeve/cloth assembly. The sample to be tested is applied to the cloth, **C-** The arm with the sleeve/cloth assembly is inserted into a cage containing approximately 500 female *Ae. aegypti* mosquitoes, and waited for 1 min, **D-** Failure of repellent treatment is determined by 1% bites (i.e. the volunteer receives 5 bites, for 500 mosquitoes) through the cloth on the sleeve window within 1 min

RESULTS

Results of Extraction

In this study the *n*-hexane, $CHCl_3$, and MeOH extracts from 13 *Achillea* sp., of which 4 are endemic to Türkiye were screened for the first time by a cloth-patch assay against *Ae. aegypti* to find new and alternative agents to control mosquitoes from natural sources. Scientific, and local names, voucher numbers, collection sites, and yields of extracts of investigated *Achillea* taxa are given in *Table 1*.

Results of Repellent Bioassay

Minimum Effective Dosage (MED) values of tested *Achillea* samples against *Ae. aegypti* are presented in *Table 2*. The obtained results showed that the hexane extracts were the most active when compared to the chloroform, and to the methanol extracts. Among the tested samples, *n*-hexane extracts of *A. multifida* (endemic), *A. crithmifolia, A. setacea,* and *A. teretifolia* (endemic) showed higher repellency (MED: 0.344, 0.375, 0.409 and 0.437 mg/cm^2, respectively). Additionally, MED values of *Achillea n*-hexane extract samples were compared with previously reported data in our original article [33], including four essential oils obtained from *Tanacetum annuum* (BTEO), *Anthemis scorbicularis* (ASEO), *Caryopteris* x *clandonensis* (CCEO) and *Prangos platychlaena* (PPEO). One-way ANOVA and Tukey's tests (α=0.05) showed no treatment differences among 78 comparisons. Dunnett's test revealed that MED values of *A. clypeolata* (ACLHex), *A. kotschyi* (AKHex), and *A. schischkini* (ASCHex) hexane extracts were different from the mean of control (DEET) but *A. multifida* (AMHex), and *A. crithmifolia* (ACRHex) were close to the ASEO, CCEO, and PPEO (*Fig. 2*). Among the $CHCl_3$ extracts repellent activity of *A. nobilis* and *A. setacea* (MED: 0.312 and 0.375 mg/cm^2 respectively) were notable and the other endemic plant *A. phyrigia* was effective at 500 mg/cm^2.

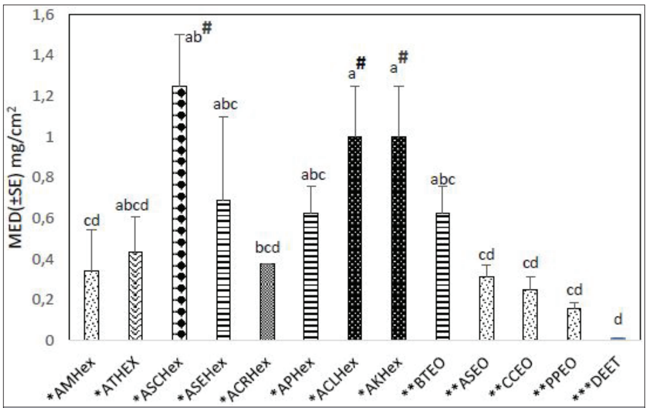

Fig 2. Comparison of minimum effective dosage (MED mg/cm^2 ± SE) values of *n*-hexane extracts of *Achillea* taxa. *A. multifida* (AMHex), *A. teretifolia* (ATHex), *A. schischkini* (ASCHex), *A. setacea* (ASEHex), *A. crithmifolia* (ACRHex), *A. pannonica* (APHex), *A. clypeolata* (ACLHex), and *A. kotschyi* (AKHex) samples were tested on human volunteers against *Ae. aegypti* mosquitoes. Responses were compared with response to known MED values of blue tansy (*Tanacetum annuum*) essential oil (**BTEO), *Anthemis scorbicularis* essential oil (**ASEO), *Caryopteris×clandonensis* essential oil (**CCEO), *Prangos platychlaena* essential oil (**PPEO), and to the standard insect repellent ***DEET (*N,N*-diethyl-3-methylbenzamide). Means followed by the same letter are not significantly different based on the Tukey-Kramer method; α=0.05, with human subjects, *n*=3. Samples with # (ACLHex, AKHex and ASCHex) significantly different from the positive control (DEET) by analysis of Dunnett's test (α=0.05)

Table 2. *Minimum Effective Dosage (MED) values of tested Achillea samples against Ae. aegypti*

#	Plant Material	*MED (± SE) mg/cm^2		
		***n*-Hex**	**$CHCl_3$**	**MeOH**
1	*A. multifida*	0.344±0.204	1.000±0.250	0.750±0
2	*A. teretifolia*	0.437±0.165	1.500±0	0.750±0
3	*A. schischkinii*	1.250±0.250	1.000±0.250	1.250±0.250
4	*A. setacea*	0.687±0.409	0.375±0	1.500±0
5	*A. crithmifolia*	0.375±0	0.750±0	1.500±0
6	*A. falcata*	NT**	0.625±0.125	1.250±0.250
7	*A. arabica*	NT**	0.675±0.125	0.750±0
8	*A. coarctata*	NT**	0.750±0	0.750±0
9	*A. pannonica*	0.625±0.125	0.750±0	1.500±0
10	*A. clypeolata*	1.000±0.250	0.750±0	1.500±0
11	*A. kotschyi*	1.000±0.250	0.750±0	1.500±0
12	*A. phrygia*	NT**	0.500±0.125	1.500±0
13	*A. nobilis*	NT**	0.312±0.062	1.500±0
	DEET (positive control)	0.011±0.001	0.011±0.001	0.011±0.001

**MED:* Minimum Effective Dosage; *SE:* Standard errors; ***NT:* Not Tested; Those samples had not enough quantity to test*

Discussion

Mosquitoes act as vectors of various dangerous zoonotic diseases such as malaria, filariasis, yellow fever, dengue, and other viral infections that threaten public health and the health of animals including livestock, domestic, and wild animals [7,34,35]. Since the synthetic chemicals have undesirable effects on the environment, and human health, interest in medicinal plants has increased to develop natural insecticides and insect-repellents for medicinal and also veterinary purposes [6,27,29].

Plants are a rich source of bioactive chemicals, and in many studies insecticidal, insect-repellent, and larvicidal activities of essential oils, different extracts, and secondary metabolites from various *Achillea* spp. against different insects have been reported. In previous studies, it was reported that the essential oil of *A. millefolium* has toxic and/or repellent effect on several agricultural pest species such as *Tetranychus urticae* [36], *Sitophilus zeamais* [6], *Myzus persicae*, *Plodia interpunctella* [37], *Aegorhinus nodipennis* [38], *Leptinotarsa decemlineata* [39] and *Varroa destructor* [40]. In addition, essential oils of *A. biebersteinii* (currently *A. arabica*), *A. santolina*, and *A. mellifolium* were tested against the *Trogoderma granarium* [26]. Beside essential oil studies, MeOH extract of *A. damascene,* and aqueous extract of *A. biebersteinii* were determined to have insecticidal/repellent activity against *Bemisia tabaci* [41]. Although the ethanol extract of *A. millefolium* provided a good level of repellency against *Acanthoscelides obtectus* [28].

In terms of vector-borne diseases, the repellent activity of the essential oil of *A. wilhelmsii* was evaluated on human subjects against field collected fleas (*Pulex irritans*) and ED_{50}, and ED_{90} values were reported as 0.457 and 2.22 mg/cm^2, respectively [27]. In another study, essential oil of *A. santolina* showed insecticidal and insect repellent activities on both domestic flies and honeybees, while ethanolic extract had no activity at 500 ppm [42].

In various studies, several *Achillea* species were reported to be effective against *Aedes* mosquitoes. Ethyl acetate extract of *A. millefolium* (1%, in acetone) was reported to reduce biting by mosquitoes (including *Ae. communis, Ae. cantans, Ae. cinereus and Ae. diantaeus*) by field studies. The major metabolites in the extract with known repellent and/or insecticide activities were reported as (-)-germacrene D (49%), β-pinene (27%), sabinene (22%), α-pinene (14.1%), 1,8-cineole (10.9%), camphor (6%), β-caryophyllene (5.1%), and *p*-cymene (4.4%) [29]. In another study, field tests revealed that the extracts and oils exhibited promising activity against *Ae. aegypti*, *Ae. communis* and *Ae. cinereus* [43]. Studies performed on ethanolic extracts of *A. millefolium* resulted in a high antifeedant and repellent effect on *Ae. aegypti* [44,45]. Insecticidal activity of *A. millefolium* essential oil was tested against the larvae of the *Ae. albopictus*, and results showed that the essential oil had insecticidal activity at 300 ppm with the mortality rates ranging from 98.3% to 100% [7].

In this study, *n*-hexane extracts of *A. multifida* (endemic), *A. crithmifolia, A. setacea* and *A. teretifolia* (endemic) (MED: 0.344, 0.375, 0.409 and 0.437 mg/cm^2, respectively) showed higher repellency. Among the $CHCl_3$ extracts repellent activity of *A. nobilis* and *A. setacea* (MED: 0.312 and 0.375 mg/cm^2 respectively) were notable and the other endemic plant *A. phyrigia* was effective at 500 mg/cm^2 (*Table 2*). In our previous studies composition of the essential oils from various *Achillea* spp. were investigated, and major components were determined as 1,8-cineole (38.2%), camphor (11.5%), and borneol (9.2%) in *A. setacea* [46]; ascaridol (27.2%), and camphor (18.8%) in *A. crithmifolia*; 1,8-cineole (20.8%) in *A. kotschyi* subsp. *kotschyi* [47]; and piperitone (16.3%), linalool (14.1%), and 1,8-cineole (12.6%) in *A. nobilis* subsp. *neilreichii* [48]. These major components are known to have repellent and/or insecticide activities [29]. Studies comparing the components of essential oils and *n*-hexane extracts from various *Achillea* spp. reported that hexane extracts also include volatile components in varying amounts [49,50]. The amount of the major components were similar in the essential oils and hexane extracts of *A. gypsicola* (camphor: 40 and 25%, and 1,8-cineole: 22 and 16%, in EO and extract, respectively) and *A. biebersteinii*, (camphor: 23 and 18%, and 1,8-cineole: 38 and 15%, in EO and extract, respectively) [49]. In another study, similar results were reported for *A. wilhelmsii* (camphor: 46.6 and 44.7%, and 1,8-cineole: 14.4 and 19.5%, in EO and extract, respectively) [50].

As a result, our findings indicate that the lipophilic components of *Achillea* species make a major contribution to the repellency, which is compatible with literature. Therefore, *n*-hexane extracts are promising to investigate their lipophilic composition. Our results suggest that the *Achillea* species growing in Türkiye would be an important source for biodegradable and non-toxic insect-repellent natural products, or would be a starting point for further studies to find new and alternative agents to control mosquitoes, and vector-borne diseases.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (S. Demir).

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

C.K. and N.T. designed and leaded the study. C.K. and S.D. collected the plant materials and performed extraction. N.T., U.R.B, and K.J.L. performed repellent bioassay and data analysis. All authors participated in the study, and contributed manuscript preparation and reviewed the manuscript.

References

1. Pages N, Cohnstaedt LW: Mosquito-borne diseases in the livestock industry. **In,** Garros C, Bouyer J, Takken W, Smallegange RC (Eds): Ecology and Control of Vector-borne Diseases. Vol. 5: Pests and Vector-Borne Diseases in the Livestock Industry. 195-219, Wageningen Academic Publishers, Netherlands, 2018.

2. Tandina F, Doumbo O, Yaro AS, Traore SF, Parola P, Robert V: Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. *Parasit Vectors,* 11 (1): 467, 2018. DOI: 10.1186/s13071-018-3045-8

3. Inci A, Yazar S, Tuncbilek AS, Canhilal R, Doganay M, Aydin L, Aktas M, Vatansever Z, Ozdarendeli A, Ozbel Y, Yildirim A, Duzlu O: Vectors and vector-borne diseases in Turkey. *Ankara Univ Vet Fak Derg,* 60 (4): 281-296, 2013. DOI: 10.1501/Vetfak_0000002593

4. WHO: Vector-borne diseases. 2020. https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases; *Accessed:* 05 May 2022.

5. Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A, Montarsi F, Rizzoli A: Control methods against invasive *Aedes* mosquitoes in Europe: A review. *Pest Manag Sci,* 71 (11): 1471-1485, 2015. DOI: 10.1002/ps.4044

6. Bertoli A, Conti B, Mazzoni V, Meini L, Pistelli L: Volatile chemical composition and bioactivity of six essential oils against the stored food insect *Sitophilus zeamais* Motsch (Coleoptera Dryophthoridae). *Nat Prod Res,* 26 (22): 2063-2071, 2012. DOI: 10.1080/14786419.2011.607453

7. Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L: Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res,* 107 (6): 1455-1461, 2010. DOI: 10.1007/s00436-010-2018-4

8. Diaz JH: Chemical and plant-based insect repellents: Efficacy, safety, and toxicity. *Wild Environ Med,* 27 (1): 153-163, 2016. DOI: 10.1016/j.wem.2015.11.007

9. Deletre E, Martin T, Dumenil C, Chandre F: Insecticide resistance modifies mosquito response to DEET and natural repellents. *Parasit Vectors,* 12 (1): 89, 2019. DOI: 10.1186/s13071-019-3343-9

10. Polson KA, Brogdon WG, Rawlins SC, Chadee DD: Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. *Acta Trop,* 117 (1): 31-38, 2011. DOI: 10.1016/j.actatropica.2010.09.005

11. Zulfa R, Lo WC, Cheng PC, Martini M, Chuang TW: Updating the insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Asia: A systematic review and meta-analysis. *Trop Med Infect Dis,* 7 (10): 306, 2022. DOI: 10.3390/tropicalmed7100306

12. Sarma R, Adhikari K, Mahanta S, Khanikor B: Combinations of plant essential oil based terpene compounds as larvicidal and adulticidal agent against *Aedes aegypti* (Diptera: Culicidae). *Sci Rep,* 9:9471, 2019. DOI: 10.1038/S41598-019-45908-3

13. Pavela R, Benelli G: Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors - A review. *Exp Parasitol,* 167, 103-108, 2016. DOI: 10.1016/j.exppara.2016.05.010

14. Karunamoorthi K, Husen E: Knowledge and self-reported practice of the local inhabitants on traditional insect repellent plants in Western Hararghe zone, Ethiopia. *J Ethnopharmacol,* 141 (1): 212-219, 2012. DOI: 10.1016/j.jep.2012.02.022

15. Bekele D, Asfaw Z, Petros B, Tekie H: Ethnobotanical study of plants used for protection against insect bite and for the treatment of livestock health problems in rural areas of Akaki District, Eastern Shewa, Ethiopia. *Topclass J Herb Med,* 1 (2): 40-52, 2012.

16. Pohlit AM, Lopes NP, Gama RA, Tadei WP, Neto VFD: Patent literature on mosquito repellent inventions which contain plant essential oils - A review. *Planta Med,* 77 (6): 598-617, 2011. DOI: 10.1055/s-0030-1270723

17. Katz TM, Miller JH, Hebert AA: Insect repellents: Historical perspectives and new developments. *J Am Acad Dermatol,* 58 (5): 865-871, 2008. DOI: 10.1016/j.jaad.2007.10.005

18. Maia MF, Moore SJ: Plant-based insect repellents: A review of their efficacy, development and testing. *Malar J,* 10 (Suppl. 1):S11, 2011. DOI: 10.1186/1475-2875-10-S1-S11

19. Anonymous: Insect repellents. *Med Lett Drugs Ther,* 63 (1628): 109-112, 2021.

20. Nemeth E, Bernath J: Biological activities of yarrow species (*Achillea* spp.). *Curr Phar Des,* 14 (29): 3151-3167, 2008. DOI: 10.2174/138161208786404281

21. Ali SI, Gopalakrishnan B, Venkatesalu V: Pharmacognosy, phytochemistry and pharmacological properties of *Achillea millefolium* L.: A review. *Phytother Res,* 31 (8): 1140-1161, 2017. DOI: 10.1002/ptr.5840

22. Mohammadhosseini M, Sarker SD, Akbarzadeh A: Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. *J Ethnopharmacol,* 199: 257-315, 2017. DOI: 10.1016/j.jep.2017.02.010

23. Arabacı T: *Achillea* L. **In,** Guner A, Aslan S, Ekim T, Vural M, Babac MT (Eds): Türkiye Bitkileri Listesi (Damarlı Bitkiler). 108-112, Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği, İstanbul, 2012.

24. Konyalioglu S, Karamenderes C: The protective effects of *Achillea* L. species native in Turkey against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes. *J Ethnopharmacol,* 102 (2): 221-227, 2005. DOI: 10.1016/j.jep.2005.06.018

25. Karaalp C, Yurtman AN, Yavasoglu NUK: Evaluation of antimicrobial properties of *Achillea* L. flower head extracts. *Pharm Biol,* 47 (1): 86-91, 2009. DOI: 10.1080/13880200802448682

26. Nenaah GE: Chemical composition, insecticidal and repellence activities of essential oils of three *Achillea* species against the Khapra beetle (Coleoptera: Dermestidae). *J Pest Sci,* 87 (2): 273-283, 2014. DOI: 10.1007/s10340-013-0547-1

27. Ghavami MB, Poorrastgoo F, Taghiloo B, Mohammadi J: Repellency effect of essential oils of some native plants and synthetic repellents against human flea, *Pulex irritans* (Siphonaptera: Pulicidae). *J Arthropod Borne Dis,* 11 (1): 105-115, 2017.

28. Jovanovic Z, Kostic M, Popovic Z: Grain-protective properties of herbal extracts against the bean weevil *Acanthoscelides obtectus* Say. *Ind Crops Prod,* 26 (1): 100-104, 2007. DOI: 10.1016/j.indcrop.2007.01.010

29. Jaenson TGT, Palsson K, Borg-Karlson AK: Evaluation of extracts and oils of mosquito (Diptera : Culicidae) repellent plants from Sweden and Guinea-Bissau. *J Med Entomol,* 43 (1): 113-119, 2006. DOI: 10.1093/jmedent/43.1.113

30. Applequist WL, Moerman DE: Yarrow (*Achillea millefolium* L.): A neglected panacea? A review of ethnobotany, bioactivity, and biomedical research. *Econ Bot,* 65 (2): 209-225, 2011. DOI: 10.1007/s12231-011-9154-3

31. Katritzky AR, Wang ZQ, Slavon S, Dobchev DA, Hall CD, Tsikolia M, Bernier UR, Elejalde NM, Clark GG, Linthicum KJ: Novel carboxamides as potential mosquito repellents. *J Med Entomol,* 47 (5): 924-938, 2010. DOI: 10.1603/ME09284

32. Tabanca N, Bernier UR, Agramonte NM, Tsikolia M, Bloomquist JR: Discovery of repellents from natural products. *Curr Org Chem,* 20 (25): 2690-2702, 2016. DOI: 10.2174/1385272820666160421151503

33. Stappen I, Wanner J, Tabanca N, Bernier UR, Kendra PE: Blue tansy essential oil: Chemical composition, repellent activity against *Aedes aegypti* and attractant activity for *Ceratitis capitata*. *Nat Prod Commun,* 16 (2): 1-8, 2021. DOI: 10.1177/1934578X21990194

34. Pirmohammadi M, Shayeghi M, Vatandoost H, Abaei MR, Mohammadi A, Bagheri A, Khoobdel M, Bakhshi H, Pirmohammadi M, Tavassoli M: Chemical composition and repellent activity of *Achillea vermiculata* and *Satureja hortensis* against *Anopheles stephensi*. *J Arthropod Born Dis,* 10 (2): 201-210, 2016.

35. Ari HO, Islek E, Uslu MKB, Ozatkan Y, Karakas F, Yildirim HH, Alp E: The monetary impact of zoonotic diseases on society: The Turkish case. *Ankara Univ Vet Fak Derg,* 69 (1): 9-15, 2022. DOI: 10.33988/auvfd.789598

36. Ahmadi Z, Saber M, Bagheri M, Mahdavinia GR: *Achillea millefolium* essential oil and chitosan nanocapsules with enhanced activity against *Tetranychus urticae*. *J Pest Sci,* 91 (2): 837-848, 2018. DOI: 10.1007/s10340-017-0912-6

37. Magierowicz K, Gorska-Drabik E, Golan K: Effects of plant extracts and essential oils on the behavior of *Acrobasis advenella* (Zinck.) caterpillars and females. *J Plant Dis Prot,* 127 (1): 63-71, 2020. DOI: 10.1007/s41348-019-00275-z

38. Tampe J, Parra L, Huaiquil K, Mutis A, Quiroz A: Repellent effect and metabolite volatile profile of the essential oil of *Achillea millefolium* against *Aegorhinus nodipennis* (hope) (Coleoptera: Curculionidae). *Neotrop Entomol,* 44 (3): 279-285, 2015. DOI: 10.1007/s13744-015-0278-5

39. Alkan M, Gokce A: Toxicological and behavioral effects of some plant extract on Colorado potato beetle, *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae). *Turk Entomol Derg,* 41 (3): 309-317, 2017. DOI: 10.16970/entoted.298995

40. Light M, Faraone N, Shutler D, Cutler GC, Hillier NK: *Varroa destructor* (Mesostigmata: Varroidae) electrophysiological activity towards common yarrow (Asteraceae) essential oil and its components. *Can Entomol,* 153 (2): 211-221, 2021. DOI: 10.4039/tce.2020.65

41. Hammad EAF, Zeaiter A, Saliba N, Talhouk S: Bioactivity of indigenous medicinal plants against the Cotton whitefly, *Bemisia tabaci*. *J Insect Sci,* 14, 1-18, 2014. DOI: 10.1093/jis/14.1.105

42. Twaij HA, Elisha EE, Kery A, Faraj AS: Evaluation of the insecticidal and insect repellent effects of *Achillea santolina*. *Int J Crude Drug Res,* 26 (3): 169-171, 1988. DOI: 10.3109/13880208809053913

43. Thorsell W, Mikiver A, Malander I, Tunon H: Efficacy of plant extracts and oils as mosquito repellents. *Phytomedicine,* 5 (4): 311-323, 1998. DOI: 10.1016/S0944-7113(98)80072-X

44. Thorsell W, Tunon H: Ortho hydroxy-substituted molecules might be of importance for the prevention of bloodsucking by mosquitoes. *Phytomedicine,* 5 (4): 307-310, 1998. DOI: 10.1016/S0944-7113(98)80071-8

45. Tunon H, Thorsell W, Bohlin L: Mosquito repelling activity of compounds occurring in *Achillea millefolium* L. (Asteraceae). *Econ Bot,* 48 (2): 111-120, 1994. DOI: 10.1007/BF02908196

46. Karamenderes C, Karabay NU, Zeybek U: Composition and antimicrobial activity of the essential oils of *Achillea setacea* Waldst.&Kit. collected from different localities of Turkey. *J Fac Pharm Ankara,* 32 (2): 113-120, 2003. DOI: 10.1501/Eczfak_0000000199

47. Karamenderes C, Karabay NU, Zeybek U: Composition and antimicrobial activity of the essential oils of some *Achillea* L. species in Turkey. *Acta Pharm Turcica,* 44, 221-225, 2002.

48. Karamenderes C, Karabay Yavasoglu NU, Zeybek U: Composition and antimicrobial activity of the essential oils of *Achillea nobilis* L. subsp. *sipylea* and subsp. *neilreichii*. *Chem Nat Compd,* 43 (5): 632-634, 2007. DOI: 10.1007/s10600-007-0213-z

49. Kordali S, Cakir A, Akcin TA, Mete E, Akcin A, Aydin T, Kilic H: Antifungal and herbicidal properties of essential oils and n-hexane extracts of *Achillea gypsicola* Hub-Mor. and *Achillea biebersteinii* Afan. (Asteraceae). *Ind Crops Prod,* 29 (2-3): 562-570, 2009. DOI: 10.1016/j.indcrop.2008.11.002

50. Cakir A, Ozer H, Aydin T, Kordali S, Cavusoglu AT, Akcin T, Mete E, Akcin A: Phytotoxic and insecticidal properties of essential oils and extracts of four *Achillea* species. *Rec Nat Prod,* 10 (2): 154-167, 2016.