ISSN 2449-8955

European Journal of Biological Research

Research Article

Achillea millefolium L. subsp. *millefolium* essential oil's antifungal effect

Sinem Aydin[1], Emre Sevindik[2]*

[1] Giresun University, Department of Biology, Faculty of Science and Arts, Giresun, Turkey

[2] Faculty of Agriculture, Department of Agricultural Biotechnology, Adnan Menderes University, South Campus, Cakmar, Aydin, Turkey

*Corresponding author: Emre Sevindik; E-mail: ph.d-emre@hotmail.com

Received: 22 June 2018; **Revised submission:** 20 July 2018; **Accepted:** 16 August 2018

DOI: http://dx.doi.org/10.5281/zenodo.1346338

ABSTRACT

This study was carried out with the aim of determining the antifungal effect of the essential oil isolated from *Achillea millefolium* subsp. *millefolium* plant against pathogenic fungi. In order to test the antifungal effect of the oil, an analysis was conducted on a total of 4 pathogen fungi which included *Candida albicans, Candida tropicalis, Candida parapsilosis* and *Saccharomyces cerevisiae*, and the effect of the essential oil on the growth of these fungi was investigated. The essential oil of *A. millefolium* ssp. *millefolium* had varying degrees of effect on the tested fungi. The highest antifungal effect was found against *S. cerevisiae*; whereas the lowest antifungal effect was found against *C. parapsilosis*. Nystatin showed a higher activity than the essential oil of *A. millefolium* subsp. *millefolium* against the tested fungi. MIC values of the essential oil against the tested fungi ranged from 1.25 µl/ml to 10 µl/ml. The results obtained indicate that essential oil of *A. millefolium* subsp. *millefolium* can be used as an alternative to antifungal agents such as amphotericin, ketoconazole, and fluconazole.

Keywords: *Achillea millefolium* subsp. *millefolium*; Essential oil; Antifungal; Turkey.

1. INTRODUCTION

Since ancient times, raw herbal essences of aromatic plants have been used for different purposes, such as food, perfumery and medicines [1]. Primary and secondary metabolites produced by plants have a wide spectrum of functions. Secondary metabolites have been later utilized by humans due to their beneficial roles [2]. Essential oils are secondary metabolites obtained from plants and have been extensively used since the Middle Ages for bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, medical and cosmetic purposes [3, 4].

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 [5-7]. *Achillea millefolium*, known to the public as "milfoil", "common yarrow", "gordaldo", nosebleed plant" is considered to be one of the oldest medical plants [8]. There are many subspecies of *A. millefolium* species. *A. millefolium* species is represented by two subtypes in Turkey flora. These are *Achillea millefolium* subsp. *millefolium* and *Achillea millefolium* subsp. *pannonica* [9]. *Achillea* species, known as medicinal plants, are used against fever, colds, digestive complaints, slow-healing wounds and dermatitis. However, *A. millefolium* plant has been used be-

cause of its anti-inflammatory, spasmolytic, haemostatic, and cholagogue effects [10]. The herbal tea of *A. millefolium* has been found to be used against diseases of the gastrointestinal tract, especially in the folk medicine. The aim of the present study was to determine the essential oil *A. millefolium* subsp. *millefolium* growing in Ardahan ecological conditions and to investigate their antifungal effect on some strong pathogen fungi.

2. MATERIALS AND METHODS

2.1. Plant material and isolation of essential oils

A. millefolium subsp. *millefolium* samples of the plants were collected as study materials in June 2013 from Ardahan/Turkey surroundings (approximately 2080 m altitude). Extractions were carried out with Clevenger apparatus (Basaran cam, Turkey and Misung Scientific Co., Korea) using water distillation.

2.2. Microorganisms

Candida albicans and *Candida tropicalis* were obtained from Fırat University Department of Biology; *Candida parapsilosis* were obtained from Giresun University Faculty of Education, *Saccharomyces cerevisiae* was obtained from Giresun Province Control Laboratory.

2.3. Antifungal activity

The antifungal activity of the essential oil was determined by disc diffusion method. The essential oil of *A. millefolium* subsp. *millefolium* was sterilized by filtration through a 0.45 µm membrane filter [13]. The turbidity of fungal suspensions were adjusted with 0.5 Mc Farland standard (10^7 CFU/ml fungi concentration), then, the fungal suspension spread on Petri dishes [14]. The discs (6 mm diameter) were put on the inoculated agar and separately impregnated with 20 µl of essential oils. Nystatine disc was used as positive control. Plates were kept at 30°C for 48 h. Antifungal activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters [15].

2.4. Determination of Minimum Inhibition Concentration (MIC) of the essential oils

The MIC was defined as the lowest concentration that completely inhibits the growth of microorganisms. For the determination of values of MIC, a micro-dilution broth assay was utilized. Two-fold serial dilutions (in dimethyl sulphoxide (DMSO)) were prepared from 0.0098 µl/ml to 20 µl/ml of the essential oils of *A. millefolium* subsp. *millefolium* in a 96-well microplate. Plates were incubated at 30°C for 48 h [16, 17].

3. RESULTS AND DISCUSSION

Medical and aromatic plants are rich and important natural sources of biologically active compounds and have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [18]. Table 1 reveals inhibition zones which were created by essential oil of *A. millefolium* subsp. *millefolium* against the test fungi. The highest and the lowest activities were found against *S. cerevisiae* and *C. parapsilosis*, respectively. Nystatin was more active against the test fungi than the essential oil of *A. millefolium* subsp. *millefolium* except for *S. cerevisiae*. In addition to this, DMSO showed no activity.

Table 1. Inhibition zones of essential oil of *A. millefolium* subsp. *millefolium* (mm).

Fungi	*A. millefolium* subsp. *millefolium*	Nystatin	DMSO
C. albicans	17	30	-
C. tropicalis	20	30	-
C. parapsilosis	15	25	-
S. cerevisiae	30	17	-

Table 2. MIC values of essential oil of *A. millefolium* subsp. *millefolium* (µl/ml).

Fungi	*A. millefolium subsp. millefolium*
C. albicans	10
C. tropicalis	5
C. parapsilosis	1.25
S. cerevisiae	2.5

Table 2 shows values of MIC. The values range from 1.25 to 10 µl/ml for *A. millefolium* subsp. *millefolium*. Essential oils exhibited the lowest MIC value against *C. parapsilosis*. El-Kalamouni et al. [19] examined antifungal activity of essential oil of *A. millefolium* collected from France and it was demonstrated that the essential oil were inhibited the growth of *Rhizopus stolonifer*, *Verticillium dahliae*, *Colletotrichum gloesporoides*, *Botrytis cinerae* and *Aspergillus niger*. MIC values were found as 1.6 mg/ml, 3.1 mg/ml, 3.4 mg/ml, 3.6 mg/ml and 4.7 mg/ml, respectively. Karamenderes et al. [20] revealed that essential oil of *A. millefolium* subsp. *millefolium* was active against *C. albicans*. Likewise, we found that essential oil of *A. millefolium* possessed affect on the growth of *C. albicans*. Falconieri et al. [21] studied antifungal activity of the essential oils of flowering aerial parts of wild *A. millefolium* growing on the Mediterranean coast (Sardina Island, Italy) and on the Atlantic Coast. Both of the essential oils inhibited *C. albicans* (MIC: 2.5 µl/ml), *C. tropicalis* (MIC: 2.5 µl/ml) and *C. parapsilosis* (MIC: 2.5 µl/ml). In our study, MIC values were found as 10 µl/ml, 5 µl/ml and 1.25 µl/ml against *C. albicans*, *C. tropicalis* and *C. parapsilosis*, respectively. The difference might be arising from several factors like local, climatic, seasonal, and experimental conditions [22]. Candan et al. [23] reported that essential oil of *A. millefolium* subsp. *millefolium* had activity against *C. albicans*. Similarly, we found activity against *C. albicans*.

4. CONCLUSION

As a result, the antifungal effect of the essential oil obtained from *A. millefolium* subsp. *millefolium* plant was investigated; and it was revealed that it yielded positive results against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *S. cerevisiae.*

AUTHOR'S CONTRIBUTION

Both authors have equally contribution, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare that they have no conflict of interest.

REFERENCES

1. Ekren S, Yerlikaya O, Tokul HE, Akpınar A, Accedil M. Chemical composition, antimicrobial activity and antioxidant capacity of some medicinal and aromatic plant extracts. Afr J Microbiol Res. 2013; 7(5): 383-388.
2. Chishti S, Kaloo ZA, Sultan P. Medicinal importance of genus *Origanum*: a review. J Pharmacognosy Phytother. 2013; 5(10): 170-177.
3. Bayaz M. Esansiyel yağlar: antimikrobiyal, antioksidan ve antimutajenik aktiviteleri. Akademik Gıda. 2014; 12(3): 45-53.
4. Tommasi L, Negro C, Miceli A, Mazzotta F. Antimicrobial activity of essential oils from aromatic plants grown in the Mediterranean area. J Essent Oil Res. 2009; 21(2): 185-189.
5. Arabaci A. *Achillea* L. In: Guner A, Aslan S, Ekim T, Vural M, Babac MT, eds. Türkiye bitkileri listesi (damarli bitkiler) Nezahat Gökyiğit botanik bahçesi ve flora araştırmaları derneği yayını. Istanbul, 2012.
6. Aytac Z, Duman H, Ekici M. Two new *Achillea* L. (Asteraceae) species from Turkey. Turk J Bot. 2016; 40: 373-379.
7. Tabanca N, Demirci B, Aytaç Z, Baser KHC. Chemical composition of *Achillea schischkinii* Sosn., an endemic species from Turkey. Nat Volat Essent Oils. 2016; 3: 24-28.
8. Bayram E, Sönmez Ç, Ekren S, Tatar Ö, Gürel A, Hayta Ş, et al. *Achillea millefolium* L. grubuna ait türlerde verim, uçucu yağ ve chamazulene içeriğinin belirlenmesi. Ege Üniv Ziraat Fak Derg. 2013; 50(1): 87-96.
9. Davis PH. Flora of Turkey and the East Agean Islands. Vol. 5, Edinburg University Press, 1975.
10. Si, XT, Zhang ML, Shi QW, Kiyota H. Chemical constituents of the plants in the genus *Achillea*. Chem Biodiv. 2006; 3(11): 1163-1180.

11. Skocibusic M, Bezic N, Dunkic V, Radonic A. Antibacterial activity of *Achillea calvennea* essential oil against respiratory tract pathogens. Fitoterapia. 2004; 75(7-8): 733- 736.
12. Koçak A, Çakıcı AV, Koçlar G. Variations of essential oil compositions of *Achillea millefolium* L. subsp. *millefolium* taxa growing in Bingol (Turkey). Turkjans. 2016; 3(2): 114-117,
13. Ünal MÜ, Uçan F, Şener A, Dinçer S. Research on antifungal and inhibitory eff ects of DL-limonene on some yeasts. Turk J Agricult For. 2012; 36: 576-582.
14. Ertürk Ö. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia. 2006; 61(3): 275-278.
15. Al Maqtari MAA, Alghalibi SM, Alhamzy EH. Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen. Turk J Biochem. 2011; 36(4): 342-349.
16. Cabarkapa I, Sprinjar M, Milavanovic I, Plavsic D, Palic D, Kokik B, Arsic I. Antimicrobial activity of *Origanum heracleoticum* L. essential oil from Serbia. Agro Food Industry Hi-tech. 2012; 23(5): 55-58.
17. Yiğit D, Yiğit N, Aktaş E, Özgen U. Ceviz (*Junglans regia* L.)' in antimikrobiyal aktivitesi. Türk Mikrobiyoloji Cemiyeti Dergisi. 2009; 39(1-2): 711.
18. Sevindik E, Abacı ZT, Yamaner C, Ayvaz M. Determination of the chemical composition and antimicrobial activity of the essential oils of *Teucrium polium* and *Achillea millefolium* grown under North Anatolian ecological conditions. Biotechnol Biotechnol Equip. 2016; 30(2): 375-380.
19. El-Kalamouni C, Venskutonis PR, Zebib B, Meroh O, Raynaud C, Talou T. Antioxidant and antimicrobial activities of the essential oil of *Achillea millefolium* L. grown in France. Medicines (Basel). 2017; 4(2): 30-39.
20. Karamenderes C, Karabay NÜ, Zeybek U. Composition and antimicrobial activity of the essential oils of some *Achillea* L. species in Turkey. Acta Pharmac Turc. 2002; 44: 221-225.
21. Falconieri D, Piras A, Porcedda S, Marangiu B, Gonçalves MJ, Cabral C, et al. Chemical composition and biological activity of the volatile extracts of *Achillea millefolium*. Nat Prod Communic. 2011; 6(10): 1527-1530.
22. Çetin B, Çakmakçı S, Çakmakçı R. The investigation of antimicrobial activity of thyme and oregano essential oils. Turk J Agricult For. 2011; 35: 145-154.
23. Candan F, Unlu M, Tepe B, Deferara D, Polissiou M, Sökmen A, Akpulat HA. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J Ethnopharmacol. 2013; 87(2-3): 215-220.