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**Research Article**

# SCREENING OF *Achillea coarctata* Poir. AND *Achillea setacea* Waldst. & Kit. (Asteraceae) FOR THEIR VOLATILE AND FATTY ACIDS COMPOSITIONS, AND ANTIMICROBIAL ACTIVITIES

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*Achillea coarctata*
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*Additive effect*

**Abstract:** The main aim of this study is antimicrobial screening of the *n*-hexane, ethyl acetate and aqueous extracts obtained from aerial parts of *Achillea setacea* Waldst. & Kit. and *Achillea coarctata* Poir. against the bacterial strains *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 14028 and the yeast *Candida albicans* ATCC 6633 by the microdilution method. In addition, the effect of the aqueous extract of *A. coarctata* combined with fluconazole againts *C. albicans* ATCC 6633, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750 and *C. glabrata* ATCC 90030 was tested with the checkerboard method. The chemical components of the volatiles and fatty acid methyl esters were identified using the Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) techniques. The main components of the volatile oil were found to be 1,8-cineole (16.1%) and *β*-pinene (14.5%) for *A. setacea* and camphor (35.4%) and borneol (12.9%) for *A. coarctata*. The ethyl acetate extracts of *A. setacea* (MIC=156.25 µg/mL) and *A. coarctata* (MIC=312.5 µg/mL) were found more active against the tested *Candida* Berkh. species. The combination of aqueous extract of *A. coarctata* with fluconazole showed additive effect for the tested *Candida* species with FIC values within the range of 0.53-0.625 µg/mL.

**Özet:** Bu çalışmanın ana amacı, *Achillea setacea* Waldst. & Kit. ve *Achillea coarctata* Poir. ‘nın toprak üstü kısımlarından elde edilen *n*-hekzan, etil asetat ve sulu ekstrelerinin *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 14028 ve *Candida albicans* ATCC 6633'e karşı mikrodilüsyon yöntemi ile antimikrobiyal aktivitesinin belirlenmesidir. Buna ek olarak, *A. coarctata*'nın flukonazol içeren sulu ekstresinin *C. albicans* ATCC 6633, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750 ve *C. glabrata* ATCC 90030'a karşı etkisi dama tahtası yöntemiyle araştırılmıştır. Uçucu bileşenlerin ve yağ asidi metil esterlerinin kimyasal bileşimleri Gaz Kromatografisi (GK) ve Gaz Kromatografisi/Kütle Spektrometresi (GK/KS) ile tanımlanmıştır. *Achillae setacea*'nın uçucu yağının ana bileşenleri 1,8-sineol (%16,1) ve *β*-pinen (%14,5), *A. coarctata*’nın ise kafur (%35,4) ve borneol (%12,9) olarak belirlenmiştir. *Achillae setacea* ve *A. coarctata*'nın etil asetat ekstrelerinin (MİK=156,25 µg/mL ve MİK=312,5 µg/mL, sırasıyla) test edilen *Candida* türlerine karşı daha etkili olduğu bulunmuştur. *Achillae coarctata* su ekstresinin flukonazol ile kombinasyonu test edilen *Candida* Berkh. türleri için FIC= 0,53-0,625 µg/mL arasında aditif etki göstermiştir.

## Introduction

The worldwide increase in cases of infection-related diseases and deaths revealed the importance of developing new strategies in fight against infectious diseases. Antimicrobial resistance (AMR) is one of the most serious issues affecting the public health. The overuse of antibiotics and antifungals are largely

attributable for emergence of AMR, and is considered a serious threat in the field of modern medicine applications (Denning *et al*. 2017). *Candida* Berkh. species are known to cause a variety of diseases, ranging from mild mucosal and skin candidiasis to potentially fatal bloodstream infections. All *Candida* species constitute one of the main four factors contributing to infections in the healthcare industry (Arendrup 2010). Studies showed that the prevalence of bloodstream infections caused by *Candida* spp. ranges from 1.2 to 26 cases per 100,000 people, with the United States and other middle- and high-income nations having the greatest rates (Tan *et al.* 2015).

The genus *Achillea* L. (Asteraceae) includes popular and ethnobotanically important species. The most widely distributed representative of the genus is over the world is *A. millefolium* L., also known as Yarrow (Applequist & Moerman 2011). *Achillea* consist of six sections and 58 taxa, and the endemism rate is 53% for Türkiye (Aytaç *et al.* 2016). *Achillea* species were heavily employed in ancient Greek warfare. According to some historians, *Achillea* was named as "Achill," a name that comes from the Greek battle hero Achilles' use of this plant to cure the troops' wounds while they were fighting. Many of the therapeutic properties of *Achillea* spp. are based on data obtained from Greek plant collectors (Nemeth & Bernath 2008). The most common use of *Achillea* species were for respiratory ailments, stomach issues, toothaches, eye issues, and damage to the skin. Topical treatments like washes and poultices were often preferred for dermatological disorders. *Achillae millefolium* is also known for its practical use as a tonic for every ailment, and for liver and renal related issues (Applequist & Moerman 2011).

The use of plants in traditional uses and modern medicine based on the historical public data and modern scientific outputs, are valuable in terms of preventing the inapprotiate uses of antibiotics by public and offers new options in the fight against microorganisms. One of these options may be to consult to the antimicrobial activity properties of herbal extracts from natural sources alone or in combination with an existing antimicrobial agent (Hsieh *et al.* 2001).

When the studies on antimicrobial activities of *Achillea* species are considered, those on *A. setacea* Waldst. Et Kit. and *A. coarctata* Poir. seems to be not much in number, and studies on essential oils are not available. Hence, this study was performed in order to investigate the antimicrobial activity of *n*-hexane, ethyl acetate and aqueous extracts obtained from aerial parts of *Achillea setacea*, and *Achillea coarctata* against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 14028, and *Candida albicans* ATCC 6633 by the microdilution method. The effect of the aqueous extract of *A. coarctata* combined with fluconazole againts *C. albicans* ATCC 6633, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, and *C. glabrata* ATCC 90030 was also tested with the checkerboard method. GC and GC/MS techniques were used to identify the chemical compositions of the volatiles and fatty acid methyl esters.

## Materials and Methods

### *Materials*

Ampicillin, moxifloxacin, clarithromycin, and fluconazole were provided from the Sanovel Pharmaceutical Industry (Istanbul, Türkiye). For antimicrobial activity tests, RPMI-1640 medium containing l-glutamine (Sigma-Aldrich, Germany), 3-[N-morpholino]-propansulfonic acid (MOPS) (Sigma-Aldrich, Germany), and Mueller Hinton Broth (MHB, Sigma-Aldrich, Germany), Mueller Hinton Agar (MHA, Meck), Potato Dextrose Agar (PDA, Merck) were used. All organic solvents and reagents used [*n*-hexane, ethyl acetate (EMSURE® ACS, ISO, Reag. Ph Eur Merck) and dimethyl sulfoxide (DMSO, Emplura®, Merck)] were of analytical or chromatographic grade.

### *Plant material*

*Achillea setacea* was collected from Dereköy, Kırklareli, Türkiye (05.07.2020) by the first author. Identification of the plants was made by Dr. Medine Münevver Uma from Namık Kemal University in Tekirdağ, Türkiye. *Achillea coarctata* Poir. was collected at Hadimköy, Merkez, Edirne, Türkiye (28.06.2020) also by the first author and identified by Dr. Koray Yaylacı from Anadolu University in Eskişehir, Türkiye. The collected material was dried at room temparature and stored in dark before analyses.

### *Preparation of the extracts*

The dried aerial parts of the plant materials (20 g) were ground and macerated at room temperature (24 h) with *n*-hexane (300 mL × 2), and ethyl acetate (300 mL × 2) by mechanical shaking (200 rpm). The liquid extracts were evaporated under vacuo in a rotavapor and stored at +4°C. For infusions, the powdered plant materials (20 g) were brewed using 200 mL hot water, and left rest for cooling, thereafter lyophilized and stored at +4°C until analysis.

### *Extraction of volatiles with microsteam distillation-solid phase microextraction (MSD-SPME)*

The extraction of the volatiles was conducted by MSD-SPME using a previously described assembly (Özek *et al*. 2006). MSD-SPME technique involved concurrent solid-phase microextraction combined with continuous hydrodistillation of the volatiles. This method significantly reduces the time required for the isolation of volatiles. Tandem of MSD-SPME with GC-MS/FID techniques is simple, sensitive, rapid, solvent-less, and non-toxic green technique for analysis of the volatile compounds at microscale level. For extractions, the ground plant material (0.5 g) was placed in 25 mL round bottom flask along with 3 mL water. The flask was fitted with a Claisen distillation head with plug and a condenser set up for refluxing rather than distillation. The threaded plug was used for SPME fiber assembly. A SPME holder equipped with PDMS-DVB “blue type” fiber was used for the extraction of the volatiles. The fiber was conditioned at 250ºC for 10 min before the experiment. After the SPME needle pierced the plug, the fiber was expressed through the needle and exposed to the headspace above

the plant sample. The extraction time for the volatiles was 2 min. After trapping of the volatiles, the loaded SPME fiber was withdrawn into the needle, and then the needle was removed from the plug and subsequently used for GC-MS/FID analyses. Thermal desorption of analytes from the fiber coating was performed by injection of the fiber in the injection port (at 250ºC) for 5 min.

*Gas Chromatography-Mass Spectrometry (GC/MS) Analysis*

The GC-FID and GC/MS studies were performed under the previously described conditions (Özek *et al.* 2006). An Agilent Innowax FSC column (60 m×0.25 mm, 0.25 µm film thickness) was used with He as the carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min, increased to 220°C at a rate of +4°C/min, kept constant at 220°C for 10 min, and then increased to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1, and the injector temperature was 250°C. MS spectra were monitored at 70 eV with a mass range of 35 to 450 *m*/*z*.

*Gas Chromatography (GC-FID) Analysis*

GC analysis was carried out using an Agilent 6890N GC system. To obtain the same elution order as with GC-MS, the line was split for FID and MS detectors, and a single injection was performed using the same column and appropriate operational conditions. Flame ionization detector (FID) temperature was 300°C.

*Identification of volatile constituents*

The essential oil components were identified by comparing their mass spectra by "Baser Library of Essential Oil Constituents", Wiley GC/MS (Wiley & Nist 2011), and Mass Finder Library (Hochmuth 2008), and verified by comparing their retention indices.

*Lipid Extraction and Fatty Acid Derivatization*

Lipid extraction and fatty acid derivatization were carried out using a lipid extraction kit (Möller *et al.* 2019) in conditions reported earlier (Özek *et al.* 2018). The lipids were typically extracted using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent. The lipids were retained in the lower chloroform layer, whereas the aqueous-soluble compounds were retained in the upper methanol-water layer. According to the kit protocol, 0.15 g mill-ground plant material was homogenized with 3 mL extraction solvent consisting of chloroform/methanol (2:1, v/v). After homogenizing and vortexing, 0.5 mL of an aqueous buffer of the kit (composition is not disclosed by the company) was added and the sample was vortexed again. Subsequently, the extraction solution was poured into a syringe system containing a filter (trapping the water). The eluted solvent contained the chloroform phase with total lipids that comprised all extracted lipids from the plant materials. A 200 µL aliquot of the total lipids was dried under a stream of nitrogen for subsequent transesterification. After drying, 1 mL of Boron trifluoride-methanol solution and 0.3 mL of *n*-hexane were added. The mixture was heated at 95°C for 1 hour under reflux. Then, 1 mL of *n*-hexane and 1 mL of distilled water were added to the reaction vessel, vortexed and centrifuged at 500 g for 5 min. The top *n*-hexane layer was transferred into a vial and then injected into GC-MS/FID system without solvent evaporation prior to injection.

*Antimicrobial activity (MIC, µg/mL)*

Microbial strains

*Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 14028*; E. coli* ATCC 8739, *Candida albicans* ATCC 6633; *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, and *C. glabrata* ATCC 90030 were used.

Determination of minimum inhibitory concentrations (MIC)

Antimicrobial activity tests were performed for different extracts of the two *Achillea* species. The extracts were prepared in dimethyl sulfoxide (%10) and water. Determination of MIC values were used with a slight modification of the microdilution method of the Clinical and Laboratory Standards Institute (CLSI 2002, CLSI 2006).

The final concentration range for the extracts was >2500 to 19.53 µg/mL. The standard antibacterial (64-0.125 µg/mL), and antifungal drugs (64–0.125 µg/mL) were prepared in DMSO and water. The activity was evaluated according to the method used by Göger *et al.* (2020).

*The combination of aqueous extract of Achilea coartata with fluconazole*

The antimicrobial activity of aqueous extract of *A. coartata* combined with fluconazole was determined by the checkerboard method. The 10-by-7-well configuration was performed on a 96-well plate. Seven serial dilutions of the extract and ten serial dilutions for fluconazole were prepared using the RPMI medium as in the MIC test. The serial dilutions of fluconazole (0.125-64 µg/mL in sterile medium) were combined with the serial dilutions of the aqueous extract (19.53-2500 µg/mL in sterile medium).

A total of seven rows in the 96-well plate, 200 µL aliquots of extract were serially diluted in medium to the horizontal direction. Similarly, 200 µL aliquots of fluconazole were added in a vertical (column) orientation for anticandidal synergistic activity. Thus, various concentrations of combinations of both extract and fluconazole were formed in the plate. Finally, we made 96-well plates in duplicate and filled each plate with 50 µL including of antibiotic and exract combination and 50 µL of microorganism with a density of $1\text{-}2\times10^3$ CFU/mL. We seeded untreated and dead cell control wells, as well as a column and row of fluconazole or extract alone, as controls. The plates were incubated for twenty-four hour at 37°C. The fractional inhibitory concentration index (FICI), which was calculated using the formula below, was used to effect of analyze extract and fluconazole in the combination (Van Vuuren *et al.* 2009):

$$\text{FIC of extract} = \frac{\text{MIC of extract in combination}}{\text{MIC of extract alone}}$$

$$\text{FIC of antifungal drug} = \frac{\text{MIC of antifungal in combination}}{\text{MIC of antifungal drug alone}}$$

$$\text{FICI} = \text{FIC of extract} + \text{FIC of antifungal drug}$$

The types of effects, based on the calculated FICIs, were classified as follows: ≤0.5: synergistic; 0.5≤1: additive; 1-4 indifferent and ≥4: antagonistic.

## Results

*Yields (%) of the Extracts of Achillea species*

Yields for extracts prepared from *A. coarctata* were calculated as 1.1% for *n*-hexane and ethyl acetate (1.2%) and aqueous (7.5%), and following as *n*-hexane (1.5%), ethyl acetate (2.5%) and aqueous (5.7%) for *A. setacea.*

*Chemical composition of volatiles*

The chemical compositions of the volatiles of *Achillea coarctata* (ACV) and *Achillea setacea* (ASV) were evaluated using simultaneous GC-FID and GC/MS techniques. The summary of the chemical composition of the volatiles is presented in Table 1. Gas-chromatographic analysis of the volatiles resulted with 92 and 90 compounds, accounting for 98.1% and 99.1% of the volatiles from *A. coarctata* and *A. coarctata*, respectively (Table 1).

The categorization of the identified compounds are in Table 2.

Gas-chromatographic profile of ACV is characterized with high abundance of the oxygenated monoterpenes (78.6%) with camphor (35.4%), borneol (12.9%), 1,8-cineole (5.1%), linalool (4.9%), myrtenal (3.1%), and carvacrol (2.6%) as main constituents. The monoterpene hydrocarbons (7.3%) were presented by *β*-pinene (2.8%), camphene (1.9%), and *p*-cymene (1.4%). The oxygenated sesquiterpenes (8.9%) were presented mainly with viridiflorol (5.1%), caryophyllene oxide (0.8%), and caryophylla-2(12), 6(13)-dien-5α-ol (0.7%). The sesquiterpene hydrocarbons were found in low percentages (1.7%).

Gas-chromatographic analysis of ASV was resulted with predomination of the oxygenated monoterpenes (39.3%) with 1,8-cineole (16.1%), camphor (5.9%), *α*-terpineol (4.0%), artemisiaketone (1.7%), borneol (1.1%), *cis*-chrysanthenyl acetate (1.1%), lavandulyl acetate (1.1%), and *cis*-chrysanthenol (0.9%). The monoterpene hydrocarbons (22.3%) were presented mostly with *β*-pinene (14.5%), sabinene (1.9%), *β*-pinene (1.2%), (*E*)-*β*-ocimene (1.2%), and limonene (1.1%). The sesquiterpene hydrocarbons (21.7%) in ASV constituted *β*-caryophyllene (8.2%), *α*-zingiberene (2.5%), *α*-humulene (1.9%), Bicyclosesquiphellandrene (1.4%), ar-curcumene (1.2%), muurola-4,11-diene (1.2%), and *β*-sesquiphellandrene (1.0%). Caryophyllene oxide (2.2%) and caryophylla-2(12),6(13)-dien- 5α-ol (0.6%) together with unknown constituent $C_{15}H_{24}O$ (10.9%) were determined in the group of oxygenated sesquiterpenes (14.9%).

**Table 1.** Chemical composition of the volatiles of *A. coarctata* and *A. setaceae.*

| No | RRI | Compounds | ACV | ASV |
|---|---|---|---|---|
| | | | %[a] | |
| 1 | 1032 | α-Pinene | 0.4 | 1.2 |
| 3 | 1076 | Camphene | 1.9 | 0.2 |
| 5 | 1118 | β-Pinene | 2.8 | 14.5 |
| 6 | 1132 | Sabinene | - | 1.9 |
| 7 | 1145 | Thuja-2,4(10)-diene | 0.1 | 0.3 |
| 8 | 1174 | Myrcene | 0.2 | 0.4 |
| 9 | 1176 | α-Phellandrene | t | - |
| 10 | 1188 | α-Terpinene | - | 0.1 |
| 11 | 1203 | Limonene | 0.4 | 1.1 |
| 12 | 1213 | 1,8-Cineole | 5.1 | 16.1 |
| 13 | 1218 | β-Phellandrene | - | 0.4 |
| 14 | 1225 | (Z)-3-Hexenal | t | 0.1 |
| 15 | 1246 | (Z)-β-Ocimene | t | 0.1 |
| 16 | 1255 | γ-Terpinene | t | 0.1 |
| 17 | 1266 | (E)-β-Ocimene | 0.1 | 1.2 |
| 18 | 1280 | *p*-Cymene | 1.4 | 0.8 |
| 19 | 1285 | Isoamyl isovalerate | - | 0.3 |
| 20 | 1300 | Tridecane | 0.4 | - |
| 21 | 1348 | 6-Methyl-5-hepten-2-one | 0.2 | t |
| 22 | 1358 | Artemisiaketone | - | 1.7 |
| 23 | 1386 | Octenyl acetate | 0.1 | - |
| 24 | 1400 | Nonanal | 0.1 | - |
| 26 | 1439 | γ-Campholene aldehyde | t | - |
| 27 | 1443 | 2,5- Dimethylstyrene | t | - |
| 28 | 1445 | Filifolone | 0.1 | - |
| 29 | 1450 | *trans*-Linalool oxide | t | - |
| 31 | 1474 | Camphenilone | 0.1 | - |
| 32 | 1474 | *trans*-Sabinene hydrate | 0.1 | 0.7 |
| 33 | 1497 | α-Copaene | 0.2 | 0.2 |
| 34 | 1499 | α-Campholene aldehyde | 0.2 | - |
| 35 | 1507 | (*E*,*E*)-2,4-Heptadienal | t | - |
| 37 | 1532 | Camphor | 35.4 | 5.9 |
| 38 | 1535 | β-Bourbonene | 0.1 | 0.3 |
| 39 | 1541 | Benzaldehyde | 0.3 | 0.5 |
| 40 | 1549 | β-Cubebene | - | 0.3 |
| 41 | 1553 | Linalool | 4.9 | 0.3 |
| 42 | 1556 | *cis*-Sabinene hydrate | - | 0.5 |
| 43 | 1562 | Isopinocamphone | 0.3 | - |
| 44 | 1565 | Linalyl acetate | 0.5 | - |
| 45 | 1582 | *cis*-Chrysanthenyl acetate | 0.1 | 1.1 |
| 46 | 1586 | Pinocarvone | 2.1 | 0.6 |
| 47 | 1589 | β-Ylangene | - | 0.2 |
| 48 | 1590 | Bornyl acetate | 0.6 | 0.6 |
| 49 | 1608 | Nopinone (=β-pinone) | 0.8 | - |
| 50 | 1611 | Terpinen-4-ol | 1.1 | 0.7 |
| 51 | 1612 | β-Caryophyllene | 0.5 | 8.2 |
| 52 | 1617 | Lavandulyl acetate | t | 1.1 |
| 53 | 1638 | β-Cyclocitral | 0.1 | - |
| 54 | 1645 | *cis*-Verbenyl acetate | - | 0.1 |
| 55 | 1648 | Myrtenal | 3.1 | 0.8 |
| 56 | 1661 | Alloaromadendrene | 0.9 | - |
| 57 | 1668 | (Z)-β-Farnesene | - | 0.3 |
| 58 | 1669 | Muurola-4,11-diene | - | 1.2 |

**Table 1.** Continued.

| No | RRI | Compound | ACV %[a] | ASV%[a] |
|---|---|---|---|---|
| 59 | 1670 | *trans*-Pinocarveol | 1.4 | - |
| 60 | 1677 | epi-Zonarene | - | 0.8 |
| 62 | 1683 | *trans*-Verbenol | 0.4 | - |
| 64 | 1687 | α-Humulene | 0.1 | 1.9 |
| 65 | 1695 | (*E*)-β-Farnesene | - | 0.6 |
| 66 | 1699 | Amorpha-4,11-diene | - | 0.6 |
| 67 | 1704 | Myrtenyl acetate | 0.5 | - |
| 68 | 1706 | α-Terpineol | 1.3 | 4.0 |
| 69 | 1708 | Ledene | 0.3 | - |
| 70 | 1709 | α-Terpinyl acetate | 0.6 | - |
| 71 | 1719 | Borneol | 12.9 | 1.1 |
| 72 | 1725 | Verbenone | 0.6 | - |
| 73 | 1726 | α-Zingiberene | - | 2.5 |
| 74 | 1733 | Neryl acetate | 0.7 | - |
| 77 | 1747 | *trans*-Carvyl acetate | 0.2 | - |
| 78 | 1751 | Carvone | - | 0.7 |
| 79 | 1764 | *cis*-Chrysanthenol | - | 0.9 |
| 80 | 1768 | Bicyclosesquiphellandrene (=4(15),5,11-muurolatriene) | - | 1.4 |
| 81 | 1771 | γ-Bisabolene | - | 0.3 |
| 82 | 1773 | δ-Cadinene | - | 0.3 |
| 83 | 1782 | Zonarene | - | 0.7 |
| 84 | 1783 | β-Sesquiphellandrene | - | 1 |
| 85 | 1786 | ar-Curcumene | - | 1.2 |
| 86 | 1802 | Cumin aldehyde | - | 0.5 |
| 87 | 1804 | Myrtenol | 1.3 | 0.6 |
| 91 | 1857 | Geraniol | 0.4 | - |
| 92 | 1864 | *p*-Cymen-8-ol | 0.3 | - |
| 93 | 1865 | Isopiperitenone | 0.1 | - |
| 95 | 1948 | *trans*-Jasmone | - | t |
| 96 | 1953 | Palustrol | 0.5 | - |
| 97 | 1969 | *cis*-Jasmone | - | 0.3 |
| 98 | 2008 | Caryophyllene oxide | 0.8 | 2.2 |
| 99 | 2029 | Perilla alcohol | 0.3 | 0.3 |
| 100 | 2037 | Salvial-4(14)-en-1-one | 0.1 | - |
| 102 | 2057 | Ledol | 0.4 | - |
| 103 | 2071 | Humulene epoxide-II | - | 0.3 |
| 104 | 2074 | Caryophylla-2(12),6(13)-dien-5-one | 0.3 | - |
| 105 | 2104 | Viridiflorol | 5.1 | 0.3 |
| 106 | 2108 | C15H24O* | - | 10.9 |
| 108 | 2144 | Spathulenol | 0.1 | 0.1 |
| 109 | 2154 | Muurola-4,10(14)dien-1-ol | - | 0.2 |
| 110 | 2179 | 3,4-Dimethyl-5-pentylidene-2(5H)-furanone | - | 0.1 |
| 111 | 2186 | Eugenol | - | 0.4 |
| 113 | 2198 | Thymol | 0.4 | - |
| 115 | 2232 | α-Bisabolol | 0.1 | - |
| 116 | 2239 | Carvacrol | 2.6 | 0.2 |
| 118 | 2257 | β-Eudesmol | 0.5 | - |
| 119 | 2260 | 15-Hexadecanolide | 0.4 | - |
| 122 | 2324 | Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol II) | 0.7 | 0.6 |
| 124 | 2389 | Caryophylla-2(12),6-dien-5α-ol (=Caryophyllenol I) | - | t |
| 125 | 2392 | Caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II) | - | t |
| | | **Total** | **98.1** | **99.1** |

[a] The data is presented as relative % calculated from flame ionization detector data for each component that was determined in the samples RRI, relative retention index calculated on the basis of retention of n-alkanes. t, trace amounts (were present at <0.1%, ACV, *A. coarctata* volatiles; ASV, *A. setaceae* volatiles.

**Table 2**. Categorization summary of the chemical composition (%) of volatiles from *A. coarctata* and *A. setaceae*.

| | ACV % | ASV % |
|---|---|---|
| Monoterpene hydrocarbons | 7.3 | 22.3 |
| Oxygenated monoterpenes | 78.6 | 39.3 |
| Sesquiterpene hydrocarbons | 1.7 | 21.7 |
| Oxygenated sesquiterpenes | 8.9 | 14.9 |
| Others | 1.6 | 0.9 |

*Fatty acids compositions*

The main components of methyl esters were determined as methyl palmitate (28.8%), methyl nonadecanoate (18.6%), methyl linolenate (15.5%) and methyl linoleate (10.6%). 14 compounds were identified with 95.8% yield for *A. setacea*. As a result of fatty acid analysis, the main components were methyl palmitate (29.8%), methyl linoleate (21.6%) and methyl linolenate (18.8%) for *A. coarctata*. The results of fatty acid analysis are given Table 3 and Table 4.

*Antimicrobial activity (MIC, µg/mL)*

Since the amount of essential oils obtained was not sufficient for antimicrobial activity antimicrobial activities of the plant extracts were investigated (Table 5). The MIC value of *n*-hexane extract of *A. setacea* against *C. albicans* ATCC 6633 was found as 625 µg/mL, and the MIC value of ethyl acetate extract was found at a lower concentration with 156.25 µg/mL. The MIC values of *n*-hexane and ethyl acetate extarcts againts *E. coli* and *S. aureus* were 2500 µg/mL and 1250 µg/mL, respectively. The aqueous extract was found to have MIC value at >2500 µg/mL for the tested strains.

**Table 3.** Fatty acid analysis results of *A. coarctata*

| No | RRI | Compounds | ACFA %[a] |
|---|---|---|---|
| 1 | 1194 | Methyl decanoate (=*Methyl caproate*) | 0.1 |
| 2 | 2018 | Methyl tetradecanoate (=*Methyl myristate*) | 0.9 |
| 3 | 2095 | Methyl pentadecanoate | 0.2 |
| 4 | 2223 | Methyl hexadecanoate (=*Methyl palmitate*) | **29.8** |
| 5 | 2323 | Methyl heptadecanoate | t |
| 6 | 2436 | Methyl octadecanoate (=*Methyl stearate*) | 6.3 |
| 7 | 2468 | (*Z*)-9-Methyl octadecenoate (=*Methyl oleate*) | **15.9** |
| 8 | 2468 | (*Z,Z*)-9,12-Methyl octadecadienoate (=*Methyl linoleate)* | **21.6** |
| 9 | 2572 | Methyl linolenate | **18.8** |
| 10 | 2634 | Methyl eicosanoate (=*Methyl arachidate*) | 3.2 |
| 11 | 2842 | Methyl docosanoate (=*Methyl behenate*) | 2.1 |
| | | **Total** | **98.9** |

**ACFA**, *A. coarctata* fatty acids

**Table 4.** Fatty acid analysis results of *A. setacea*

| No | RRI | Compounds | *ASFA %[a] |
|---|---|---|---|
| 1 | 1810 | Methyl dodecanoate (=*Methyl laurate*) | 3.7 |
| 2 | 2018 | Methyl tetradecanoate (=*Methyl myristate*) | 1.1 |
| 3 | 2020 | Isopropyl myristate | 0.6 |
| 4 | 2095 | Methyl pentadecanoate | 0.5 |
| 5 | 2223 | Methyl hexadecanoate (=*Methyl palmitate*) | **28.8** |
| 6 | 2231 | (*E*)-3-Hexadecanoic acid methyl ester | 1.2 |
| 7 | 2323 | Heptadecanoic acid methyl ester | 0.4 |
| 8 | 2436 | Methyl octadecanoate (=*Methyl stearate*) | 5.2 |
| 9 | 2468 | Methyl oleate | 4.9 |
| 10 | 2509 | (*Z*,*Z*)-9,12-Methyl octadecadienoate (=*Methyl linoleate*) | **10.6** |
| 11 | 2515 | Methyl nonadecanoate | **18.6** |
| 12 | 2572 | Methyl linolenate | **15.5** |
| 13 | 2634 | Methyl eicosanoate (=*Methyl arachidate*) | 2.3 |
| 14 | 2841 | Methyl behenate (=*Methyl docosanoate*) | 2.4 |
| | | **Total** | **95.8** |

[a]**ASFA**, *A. setaceae* fatty acids.

The antimicrobial activities of *n*-hexane, ethyl acetate and aqueous extracts of *A. coarctata* were also tested with the same strains, and additionally tested different *Candida* spp. (Table 6). MIC value of extracts for *A. coarctata* were given in Table 6. The MIC values were found between at 312.5->5000 µg/mL for all extracts. The ethyl acetate extract was found effective with MIC = 312.5 µg/mL for *C. tropicalis*. The MIC value of the aqueous extract was found as >2500 µg / mL for all strains.

**Table 5.** Minimum Inhibitory Concentrations (µg/mL) of *n*-hexane, ethyl acetate and aqueous extracts of *A. setacea.*

| Extracts and standards | *Achillea setacea* | | |
|---|---|---|---|
| | ***E. coli*** ATCC **8739** | ***S. aureus*** ATCC **6538** | ***C. albicans*** ATCC **6633** |
| *n*-hexane | 2500 | 2500 | 625 |
| Ethyl acetate | 1250 | 1250 | 156.25 |
| Aqueous | >2500 | >2500 | >5000 |
| **Ampicillin** | 0.125> | 0.125> | - |
| **Moxifloxacin** | 0.125 | 0.125> | - |
| **Fluconazole** | - | - | 0.25> |
| **Terbinafine** | - | - | - |

*The combination of aqueous extract of A. coarctata with fluconazole*

Since the traditional common use is in aqueous form, we aimed to investigate the combination of this extract with fluconazole. The results of antifungal combination interactions for aqueous extract of *A. coarctata* with fluconazole are given in Table 7. The checkerboard method was used to assess the antifungal effect of the combination against *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. The FICI values were used to evaluate the interaction of the activities.

The combination of aqueous extract of *A. coarctata* with fluconazole showed additive inhibitory effects against *C. albicans* and *C. glabrata* with FICI values calculated as 0.625 and 0.53, respectively. MIC of fluconazole decreased from 64 µg/mL to 8.0 µg/mL in the presence of extract of *A. coarctata* against *C. albicans*, and decreased from 32 µg/mL to 16 µg/mL against *C. glabrata*. When the extract was combined with fluconazole, an indifferent effect (FIC=1.25) was observed against *C. parpsilosis* and *C. tropicalis*.

**Table 6.** Minimum Inhibitory Concentrations (µg/mL) of of *n*-hexane, ethyl acetate and aqueous extracts of *A. coarctata*

| Extracts and standards | ***E. coli*** ATCC 8739 | ***S. aureus*** ATCC 6538 | ***Candida albicans*** ATCC 10231 | ***Candida parapsilosis*** ATCC 22019 | ***Candida tropicalis*** ATCC 750 | ***Candida glabrata*** ATCC 90030 |
|---|---|---|---|---|---|---|
| *n*-hexane | 2500 | 1250 | 625 | 625 | 625 | 625 |
| Ethyl acetate | 1250 | 625 | 625 | 625 | 312.5 | 625 |
| Aqueous | >2500 | >2500 | >5000 | 2500 | 5000 | 5000 |
| **Ampicillin** | 0.125> | 0.125> | - | - | - | - |
| **Cefuroxime** | 16 | 32 | - | - | - | - |
| **Moxifloxacin** | 0.125 | 0.125> | | | | |
| **Fluconazole** | - | - | 8 | 2 | 2 | 32 |
| **Terbinafine** | - | - | 64 | 1 | 128 | 128 |

**Table 7.** Combination of aqueous extract of *A. coarctata* with fluconazole (µg/mL) against *Candida* sp.

| *Candida* sp. | Extract | | | Fluconazole | | | FICI | Results |
|---|---|---|---|---|---|---|---|---|
| | Alone | Combination | 1FIC | Alone | Combination | $^{2}$FIC | | |
| ***Candida albicans* ATCC** 10231 | >5000 | 5000 | 0.5 | 64 | 8 | 0.125 | 0.625 | Additive |
| ***Candida glabrata* ATCC** 90030 | >5000 | 625 | 0.03125 | 32 | 16 | 0.5 | 0.53 | Additive |
| ***Candida parapsilosis* ATCC** 22019 | >5000 | 5000 | 0.25 | 2 | 2 | 1 | 1.25 | Indifferent |
| ***Candida tropicalis* ATCC** 750 | >5000 | 2500 | 0.25 | 2 | 2 | 1 | 1.25 | Indifferent |

## Discussion

The major volatile compounds of *A. setacea* were found to be 1,8-cineole (16.1%), *β*-pinene (14.5%), and camphor (5.9%). In our study, the caryophyllene oxide was identified at a low concentration (2.2%) for *A. setacea*, but an earlier study reported it as the main compound (Maffei *et al.* 1993). The aerial parts of *A. setacea* was reported to comprise a 79.8% rate of essential oils, with 1,8-cineole (18.5%) and sabinene (10.8%) as the main compounds (Ünlü *et al.* 2002). Turkmenoglu *et al.* (2015) reported *α*-bisabolon oxide A (27%) and hexadecanoic acid (16%) as the two main essential oil compounds of *A. setacea*. It is noteworthy that our study did not include data on hexadecanoic acid or *α*-bisabolon oxide A. According to a recent study, the major compounds were borneol (32.97%), 1,8-cineole (14.94%), camphor (10.13%), artemisia ketone (4.70%), α-terpineol (3.23%), and *γ*-eudesmol (3.23%) for *A. setacea* (Marinas *et al.* 2023).

In our study, the major compounds of volatile oil of *A. coarctata* were detected as camphor (35.4%), borneol (12.9%), 1,8-cineole (5.1%), and viridiflorol (5.1%), respectively.

Toker *et al.* (2003) reported the main compounds oil of *A. coarctata* as 1,8-cineole (20.1%), camphor (15.6%), and viridiflorol (11.8%). The essential oils inflorescence and leaves of *A. coarctata* from Greece were analysed and 1,8 cineole (26.9% and 29.1%,), camphor (22.1%, 9.2%) and borneol (5.0%, 6.8%) were determined as the main components of oils of both plant parts (Tzakou *et al.* 2009).

Twenty-eight different compounds have previously been identified in the essential oil of *A. coarctata*, with viridiflorol (25.9%), camphor (9.8%), caryophyllene oxide (9.6%), 15-hexadecanolide (9.4%), hexadecanoic acid (8.2%) and *β*-eudesmol (7.4%) corresponding to 97.3% of the total (Turkmenoglu *et al.* 2015). 15-hexadecanolide (0.4%) and *β*-Eudesmol (0.5%) were detected at low concentration in our study.

There is not enough data on the fatty acid compositions of *Achillea* species, despite the fact that numerous investigations have been done on the chemical and pharmacological aspects of the genus. Fatty acid compositions of *A. setacea* and *A. coarctata* have not been revealed previously. Therefore, we aimed to study fatty acids composition of total lipids in aerial parts of *A. setacea* and *A. coarctata.* The present study shows that studied *Achillea* species are rich sources of valuable nutraceuticals that should be subjected to further research. Vegetable oils are the main source of fatty acids for humans. Two essential fatty acids that have positive impacts on human health are linoleic acid and α-linolenic acid (Lenighan *et al.* 2019). According to several research, fatty acids are significantly present in *Achillea* plants. The most prevalent fatty acids were linoleic acid and oleic acid (Goli *et al.* 2008, Özek *et al.* 2018). As a result, yarrow seeds could serve as a source of edible oil for humans.

Available literature data showed that although there exist some studies on antimicrobial investigation of essential oil and different extracts of *A. setacea* and *A. coarctata*, but most of the antimicrobial activity studies focused on the essential oils of the plants. In a previously reported study, antimicrobial activity of the essential oil of *A. setacea* were evaluated against 14 microorganisms (Ünlü *et al.* 2002). The MIC values of essential oil which showed inhibitory effects on *C. albicans* ranged from 0.28 to 2.25 mg/mL (Ünlü *et al*. 2002). In another study, the antibacterial activity of essential oils of *A. setacea* and *A. coarctata* were evaluated against different microorganisms (Yener *et al*. 2020).

The diffrent polarity of flowers extracts from 13 *Achillea* species, including *A. setacea* and *A. coarctata* were evaluated against antibacterial and antifungal activities. The hexane extracts from *A. coarctata* and *A. setacea* demonstrated antibacterial activity against *E. faecalis* (MIC=31.25 and 62.5 µg/mL, respectively) (Karaalp *et al.* 2009).

The essential oil of *A. coarctata* efficacy to inhibit the growth of Gram-positive and Gram-negative bacteria as well as the yeast *C. albicans* was evaluated. The strongest inhibitory effect of oil was found with MIC 3.25 mg/mL against *C. albicans* (Tzakou *et al.* 2009). The methanol extract of *A. coarctata* was determined MIC=0.78 mg/mL against *K. pneumoniae* (Albayrak *et al.* 2020).

Invasive fungal infections, particularly candidiasis, have dramatically increased over the past few decades. Fluconazole (FLC) is still utilized extensively in clinical settings due to its efficacy and minimal toxicity, despite the development of other antifungal drugs that are more effective (Kriengkauykiat *et al.* 2011). Recent years have seen an increase in efforts to use medication combinations to combat the emergence of resistant fungus. The combinations of antifungal medications have been restricted, however, due to high costs and dangerous side effects (Tragiannidis *et al.* 2013).

Plants have a variety of secondary metabolites that are useful for treating a number of ailments. In comparison to other plant species, *Achillea* plants appear to be especially rich in the types of secondary metabolites. Flavonoids (glucosilated and nonglucosilated), phenolic acids (mainly cinnamic and benzoic acid derivatives), terpenes (including guaianolides, diterpens, sesquiterpenes, and their oxygenated forms), phytosterols, organic acids, fatty acids, and alcohols are the most frequent components (Strzępek-Gomółka *et al*. 2021).

Combinations of herbal substances and extracts have been reported to have synergistic effects (Liu *et al.* 2014, Yang *et al.* 2014). The phenomena of antimicrobial resistance can be effectively managed through the synergistic interaction of natural substances with the currently antimicrobial drugs. Unlike the antimicrobial studies in the literature, in this study we demonstrated the antimicrobial interaction of the extract and fluconazole and found that *A. coarctata* aqueous extract in combination with fluconazole had an additive interaction against *C. albicans* and *C. glabrata* species. MIC values of fluconazole decreased in the presence of the extract. *In vitro* data suggest that new herbal products can be used especially against the resistance mechanisms of fungi to standard antifungal agents.

## Conclusion

In the present study, phytochemical compositions of *A. setaceae* and *A. coarctata* were determined. The antimicrobial activity of aerial parts of different extracts from *A. setaceae* and *A. coarctata*, and the antifungal combination of aqueous extract of *A. coarctata* with fluconazole were evaluated for the first time against *Candida* species. The interactions of antimicrobial drugs with their target inside the pathogen may be improved or made easier by the combination of natural substances, which would stop the development of resistance. Since both drugs can be used in lesser quantities, this method can lessen toxicity. Therefore, the absence of antagonistic effects in combination studies suggests that *A. coarctata* and fluconazole may be effective against *Candida* in possible dermatological skin infections.

**Ethics Committee Approval:** Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

**Data Sharing Statement:** All data are available within the study.

**Author Contributions:** Concept: G.G., Design: G.G., Execution: G.G., E.Ç., A.V., Material supplying: Ö.K.Y., M.M.U., Data acquisition: G.G., E.Ç., A.V. G.Ö., Data analysis/interpretation: G.G., G.Ö., Writing: G.G., G.Ö., Critical review: G.G., G.Ö.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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