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Research Article

Purification of α-pinene, sabinene, and 2,4,5-trimethyl benzaldehyde using NP-Prep-HPLC from *Ferulago setifolia* essential oil and their antibacterial activity

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Abstract

The essential oils from aerial parts of *Ferulago setifolia* K. Koch were obtained by hydro-distillation and subjected to normal-phase-preparative-HPLC (NP-p-HPLC) to yield highly pure α-pinene (**1**), sabinene (**2**), and 2,3,4-trimethyl benzaldehyde (**3**). The structures of isolates were determined by GC/MS analysis. The isolated major components were subjected to antimicrobial assay. The findings demonstrate that whereas the major benzaldehyde molecule (**3**) has even less antibacterial action, the others (**1** and **2**) exhibit strong antimicrobial activity. In this study, the antimicrobial activity of individuals of *Ferulago setifolia* essential oil was first reported.

1. Introduction

The genus *Ferulago* Koch (Apiaceae) is mainly growing in Anatolia and is represented by thirty-four species, among them nineteen species are endemic to Turkey. The Turkish name of the genus is "Şeytanteresi" [1]. *Ferulago* species are known under the names "kişniş" or "çakşır". Many members of the genus are used as decoctions and infusions for alternative medical agents due to their sedative, tonic, and digestive properties as well as used for spice and flavoring agents. The occurrence of coumarins [2, 3], sesquiterpenes [3], and flavonoids [4, 5] was previously reported in *Ferulago* species.

The essential oils are composed mainly of oxygenated and non-oxygenated forms of monoterpenes and sesquiterpenes. Due to the high lipophilicity of essential oils, normal-phase chromatography can be successfully applied to separate individual essential oil components. Preparative HPLC techniques can be used for the efficient separation of essential oils as well as natural extracts with low solvent consumption and timesaving. Purification of pure chemicals present in natural essential oils is critical for determining the molecules responsible for various biological actions. This paper reports the purification of the major components of *Ferulago setifolia* essential oil.

2. Materials and methods

2.1 *Plant Material*

Plant materials were collected at the inflorescence stage from Ergan Mountain, Erzincan/Turkey, in June

2021. The voucher specimens were authenticated by Prof. Dr. Ali Kandemir and deposited at the Herbarium of the Department of Science and Art Faculty, Erzincan Binali Yıldırım University, Erzincan, Turkey (ERZ-HERB No 9062).

2.2 Essential oil extraction

100 g of chopped fresh aerial parts of *F. setifolia* were subjected to Neo-Clevenger apparatus for 2 hours. The oil was obtained with 2.80% yield as pale-yellow liquid and dried by adding anhydrous sodium sulfate and stored in dark vials at +4°C until further analysis.

2.3. Analysis of isolated components

GC/MS analyses were performed using a Thermo Scientific Trace 1310 GC/MS system, equipped with an HP5-MS capillary column (30 m x 0.25 mm and 0.25 µm). Helium (constant flow, 1.2 mL/min) was used as a carrier gas in split mode by 50:1. The injection site and mass transfer line temperature were set at 280 °C. The column oven temperature was programmed as follows: the initial column oven temperature was 60 °C, held for 3 min then ramped to 200 °C at a rate of 3 °C/min and held for 0 min, ramped to 240 °C at a rate of 5 °C/min and held for 5 minutes. The mass spectrometer conditions were as follows: the ion source temperature was 280 °C and the ionization energy was 70 eV in EI mode.

NMR analysis was carried out on a Bruker Avance II, 400 MHz for ^{1}H 100 MHz for ^{13}C. The spectra were recorded in $CDCl_3$.

2.4. Isolation of main components from essential oil

A Shimadzu semi-prep-HPLC instrument equipped with an LC-20AR dual pump, LC-20AT dual channel UV detector, and FRC-10A fraction collector was used for the preparative separation of main components. A silica-packed column (Dr. Maisch, Reprosil) was used for elution (250 mmX20 mm, 5 µm). *n*-hexane (A) and ethyl acetate (B) was used as mobile phase with a 4 ml/min flow rate. The mobile phase program was applied as follows: 90:10 (A: B) for 5 minutes, linear gradient to 50:50 (A: B) for 20 minutes, and finally linear gradient to 0:100 (A: B) for 10 minutes. The elution was monitored at 254 nm.

2.5. Antimicrobial activity

Minimal inhibition concentration (MIC) values were calculated using the microdilution method. The selected microorganisms were adjusted to 0.5 McFarland in Muller Hamilton Broth (MHB). 50 µL of MHB was added to each well of the 96-well plate. 10 µL of isolated individuals were spiked and the mixture was incubated at 37 °C for 6 hours. 50 µL of resazurin solution (0.1%) was added and after 4 hours of additional incubation, the color changes were recorded. The first well where the resazurin blue color turned pink was recorded as the MIC value.

The antimicrobial activities of the isolated components were determined against ten bacterial strains. Five of the ten bacteria strains were gram-positive; *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 51774), *Bacillus cereus* (ATCC 10876), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecium* (ATCC 8459), and other five strains were gram-negative; *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas aeruginos*a (ATCC 15442), *Salmonella enteritica* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076). Tetracycline was used as positive control for bacterial strains.

3. Results and discussion

The crude essential oil was eluted over NP-prep-HPLC to obtain main components. 500 µl essential oil was solved in 1500 µL 90:10 *n*-hexane: ethyl acetate and injected to instrument over the 2 ml sample loop. Four signals which monitored at 254 nm were collected. The separation process was repeated five times. The preparative collection pattern was given in Figure 1 and GC/MS chromatograms of crude essential oil and isolated compounds were given in Figure 2.

After removing solvents under nitrogen atmosphere, the liquid residues for 1 and 4 fractions and white solid crystalline residue for 12th fraction were obtained. The fractions were identified as α-pinene (79 mg) (**1**), sabinene (**2**) (39 mg) and 2,3,4-trimethyl benzaldehyde (**3**) (133 mg), respectively, by comparing MS ionization patterns with those authentic libraries (Nist2005 and Wiley) for **1** and **2** and by NMR analysis for **3**. The ^{1}H-NMR spectra of **3** were recorded in $CDCl_3$. An aldehyde signal at δ_H 10.28 ppm (1H, s, CHO), an AB system δ_H 7.74 (d, *J*=7.8 Hz, 1H, H6) and δ_H 7.13 (d, *J*=7.8 Hz, 1H, H5), and three methyl signals δ_H 3.57 (3H, 4-CH_3), 3.45 (3H, 3-CH_3) and 3.32 (3H, 2-CH_3) ppm related to 2,3,4-

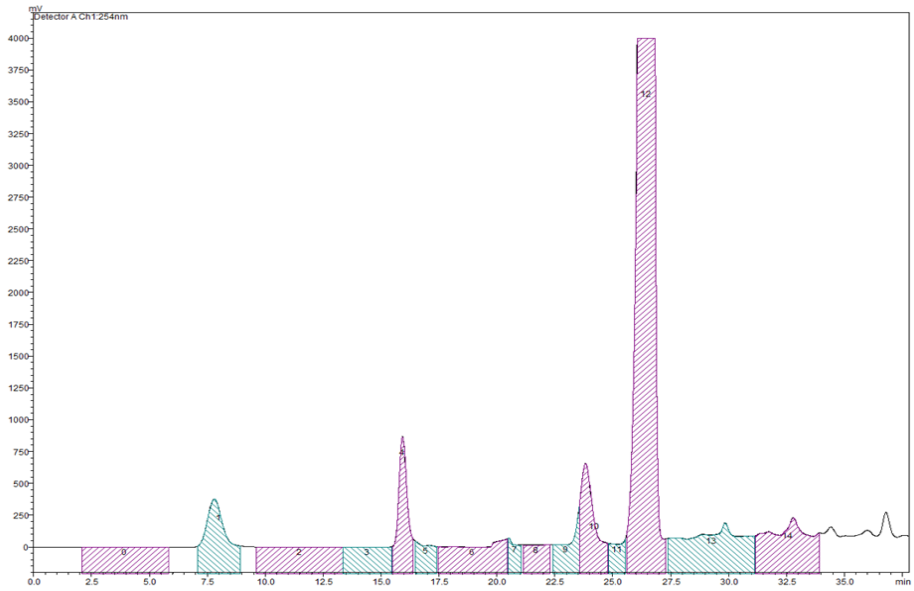

Figure 1. NP-prep-HPLC chromatogram of crude essential oil monitored at 254 nm.

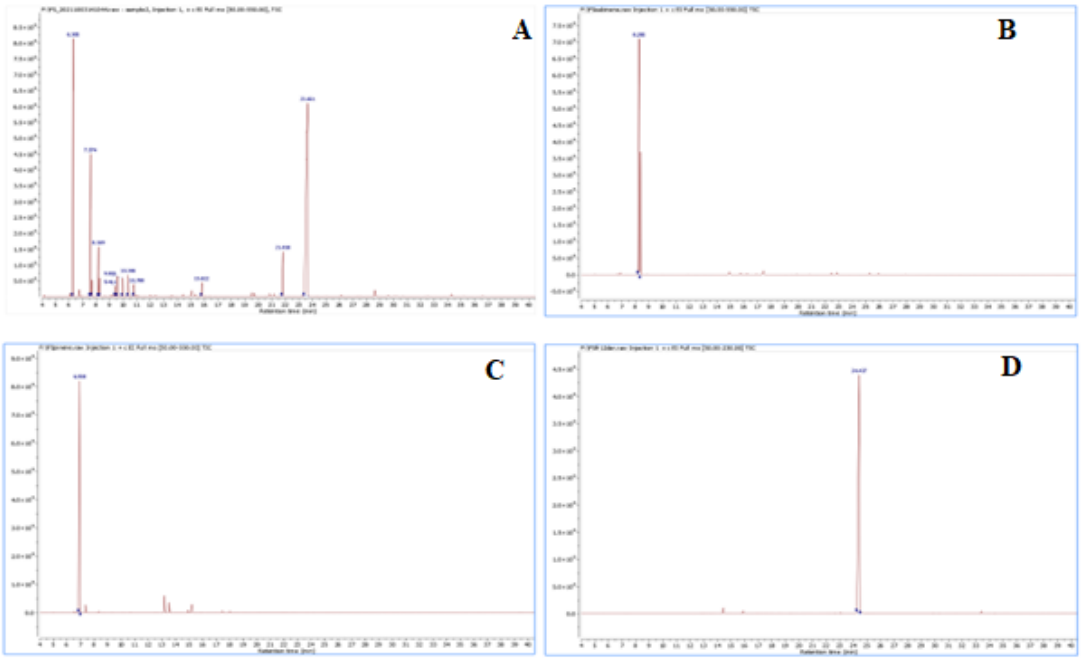

Figure 2. GC/MS chromatograms of crude essential oil and isolated main component A: Essential oil, B: sabinene, C: α-pinene, D: 2,3,4-trimethyl benzaldehyde.

trimethyl benzaldehyde (Figure 3). ^{13}C-NMR of **3** contain 10 carbons including four quaternaries [128.4 (C1), 138.64 (C2), 136.67 (C3) 141.60 (C4)], three methyl [15.78 (2-CH_3), 17.23 (3-CH_3), 20.81 (4-CH_3), three methine one of aldehydic [192.57 (CHO), 127.21 (C5), 127. 16 (C6)] corresponding to $C_{10}H_{12}O$. (Figure 4). The NMR assignments were fully coincident with literature [6]. Several studies reported that the essential oils of some *Ferulago* species contain benzaldehyde derivatives including 2,3,4-trimethyl benzaldehyde, 2,4,5-trimethyl benzaldehyde, and 2,4,6-trimethyl benzaldehyde as a major component. These aldehydes were found in different plant parts of eight *Ferulago* species ranging from %5.25 % to 92.7 [7-12].

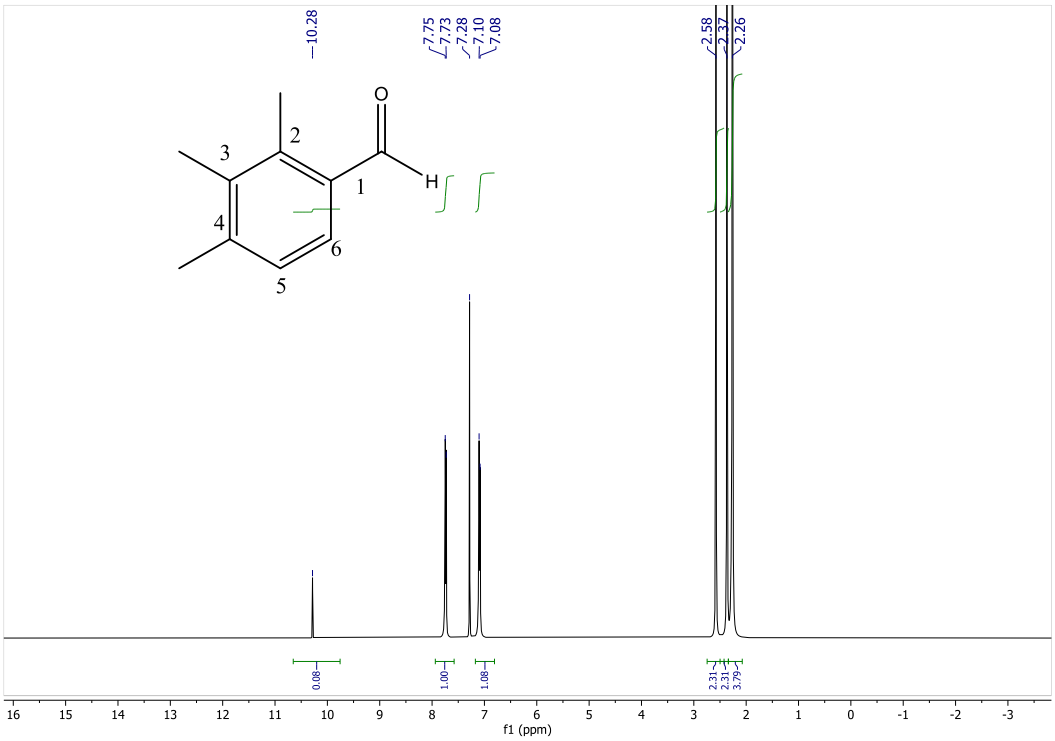

Figure 3. ^{1}H-NMR spectra 3 (400 MHz, in $CDCl_3$)

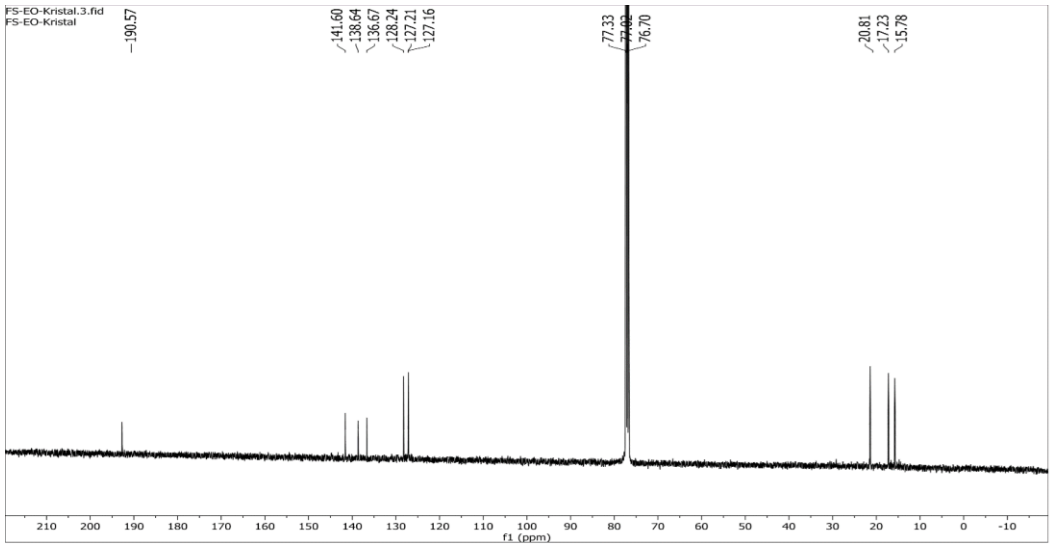

Figure 4. ^{13}C-NMR spectra **3** (100 MHz, in $CDCl_3$)

A literature search showed that the essential oils of *Ferulago setifolia* have not previously been subjected to any biological activity evaluation. In this study, the antibacterial activity of purified main components of essential oil was evaluated by the resazurin based microdilution method. The results are given in Table **1.**

According to our results, pure α-pinene (**1**) was found to be most active compound against *Pseudomonas fluorescence, Listeria monocytogenes, Salmonella enteritica,* and *Bacillus cereus* with the 7.81 µg/ml MIC value. Sabinene (**2**) has a moderate activity against all tested microorganisms. 2,3,4-trimethyl benzaldehyde was

Table 1. Antimicrobial activity of main components of *Ferulago setifolia* essential oil.

Microorganisms	Minimal inhibition concentration (MIC) in µg/ml			
	α-pinene	**Sabinene**	**2,3,4-trimethtyl benzaldehyde**	**Tetracycline**
Pseudomonas fluorescence	7.81	31.25	250	<1.95
Staphylococcus aureus	15.63	31.25	250	<1.95
Listeria monocytogenes	7.81	31.25	250	3.91
Pseudomonas aeruginosa	31.25	31.25	125	3.91
Salmonella enteritica	7.81	15.63	125	1.95
Escherichia coli	31.25	62.5	250	7.81
Bacillus cereus	7.81	31.25	125	3.91
Clostridium perfringens	125	125	250	1.95
Enterococcus faecium	15.63	31.25	250	<1.95
Salmonella enteritis	15.63	62.5	62.5	<1.95

found to have a less activity when compared the **1** and **2**. It seems from the antimicrobial activity of isolated main components was evaluated against the selected microorganisms; the activities of pure compounds (**1**, **2** and **3**) can be sequenced as follows α-pinene (1) > sabinene (2)> 2,3,4-trimethyl benzaldehyde (**3**).

Although there are some studies on the antibacterial activity of pure or α-pinene [13, 14] and sabinene-dominated essential oils [15, 16], there are no data on the activity of pure 2,3,4-trimethyl benzaldehyde. This is the first report on the antimicrobial activity of **3**.

4. Conclusions

The essential oil of *F. setifolia* could be serving as a potential source of α-pinene, sabinene, and 2,3,4-trimethyl benzaldehyde for use in the food, cosmetic and pharmaceutical industries. The results presented in this study showed the antimicrobial activity of 2,3,4-trimethyl benzaldehyde obtained from essential oil of *Ferulago setifolia* growing in Turkey for the first time. The occurrence of 2,3,4-trimethyl benzaldehyde in the essential oil contributed to a better knowledge of this species.

Also, here we report the one-step purification of the major components of essential oils using NP-prep-HPLC. This technique can be recommended to other researchers interested in the purification of essential oil components.

Authors' contributions

All authors contributed equally.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. Karakaya, S.; Şimşek, D.; Özbek, H.; Güvenalp, Z.; Altanlar, N.; Kazaz, C., et al. Antimicrobial activities of extracts and isolated coumarins from the roots of four *Ferulago* species growing in Turkey. Iran J. Pharm. Sci. **2019**, 18, 1516-29
2. Jiménez, B.; Grande, M. C.; Anaya, J.; Torres, P.; Grande, M. Coumarins from *Ferulago capillaris* and *F. brachyloba*. Phytochemistry **2000,** 53, 1025-31
3. Naseri, M.; Monsef-Esfehani, H.; Saeidnia, S.; Dastan, D.; Gohari, A. Antioxidative coumarins from the roots of *Ferulago subvelutina*. Asian J. Chem. **2013**, 25, 1875-8
4. Khanahmadi, M.; Shahrezaei, F.; Alizadeh, A. Isolation and structural elucidation of two flavonoids from *Ferulago angulata* (Schlecht) Boiss. Asian J. Res. Chem. **2011**, 4, 1667-70
5. Kızıltaş, H.; Bingol, Z.; Gören, A. C.; Kose, L. P.; Durmaz, L.; Topal, F., et al. LC-HRMS Profiling and

Antidiabetic, Anticholinergic, and Antioxidant Activities of Aerial Parts of Kınkor (*Ferulago stellata*). Molecules. **2021**, 26, 2469-78

6. Ruberto, G.; Biondi, D.; Renda, A. The composition of the volatile oil of *Ferulago nodosa* obtained by steam distillation and supercritical carbon dioxide extraction. Phytochem. Anal. **1999,** 10, 241-6
7. Demirci, F.; İşcan, G.; Güven, K.; Başer, K. H. C. Antimicrobial Activities of *Ferulago* Essential Oils. Z. Naturforsch C. J. Biosci. **2000**, 55, 886-9
8. Khalighi, S. F.; Haji, A. A.; Shahverdi, A. R.; Mozafarian, V.; Shafiei, A. Chemical composition and antimicrobial activity of the essential oil of *Ferulago bernardii* Tomk. and *M. pimen*. DARU J. Pharm. Sci. **2005,** 13, 100-4
9. Kılıç, C. S.; Özkan, A. M. G.; Demirci, B.; Coşkun, M.; Başer, K. H. C. Essential oil composition of four endemic *Ferulago* species growing in Turkey. Nat. Prod. Commun. **2010,** 5, 1951-4
10. Sanli, A.; Karadogan, T.; Tosun, B.; Erbas, S. Variation of Chemical Composition of Essential Oils in Wild Populations of *Ferulago cassia* Boiss. From Turkey. J. Essent. Oil-Bear. Plants. **2020,** 23, 1386-94
11. Sajjadi, S.; Shokoohinia, Y.; Jamali, M. Chemical composition of essential oil of *Ferulago macrocarpa* (Fenzl) Boiss. fruits. Res. Pharm. Sci. **2012,** 7, 197-204
12. Stamenković, J. G.; Petrović, G. M.; Jovanović, O. P.; Ickovski, J. D.; Palić, I. R.; Stojanović, G. S. Chemical composition of the essential oils and headspace volatiles of *Ferulago sylvatica* (Besser) Reichenb. from Serbia. Nat. Prod. Res. **2020,** 34, 1947-50
13. Rivas da Silva, A. C.; Lopes, P. M.; Barros de Azevedo, M. M.; Costa, D.; Alviano, C. S.; Alviano, D. S. Biological activities of α-pinene and β-pinene enantiomers. Molecules. **2012,** 17, 6305-16
14. Sieniawska, E.; Los, R.; Baj, T.; Malm, A.; Glowniak, K. Antimicrobial efficacy of Mutellina purpurea essential oil and α-pinene against Staphylococcus epidermidis grown in planktonic and biofilm cultures. Ind. Crops Prod. **2013,** 51, 152-7
15. Arunkumar, R.; Nair, S. A.; Rameshkumar, K. B.; Subramoniam, A. The essential oil constituents of *Zornia diphylla* (L.) Pers, and anti-inflammatory and antimicrobial activities of the oil. Rec. Nat. Prod. **2014,** 8, 385
16. Zhou, S.; Wei, C.; Zhang, C.; Han, C.; Kuchkarova, N.; Shao, H. Chemical composition, phytotoxic, antimicrobial and insecticidal activity of the essential oils of *Dracocephalum integrifolium*. Toxins. **2019,** 11, 598.