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Antimicrobial activity of rhizomes of *Ferulago trachycarpa* Boiss. and bioguided isolation of active coumarin constituents

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ABSTRACT

Ferulago trachycarpa (Apiaceae) is a plant used traditionally for its sedative, digestive, carminative and aphrodisiac properties with distribution in West, Southwest and South Anatolian part of Turkey. In this study the antimicrobial activities of fractionally n-hexane, dichloromethane, methanol and only methanol extracts from rhizomes of *F. trachycarpa* were screened against *Stapylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus feacalis* ATCC 29212 bacterial strains and fungal strains such as *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750 and *C. parapsilosis* ATCC 22019 by microdilution method. All extracts have been shown to possess antimicrobial activities against bacteria and fungal strains and according to the antimicrobial results, the isolation of the active constituents was made from the most active n-hexane and dichloromethane extracts. So, four pure compounds are known as coumarin derivatives, crenulatin (6-formyl-7-methoxycoumarin), suberosin (7-methoxy-6-prenylcoumarin), marmesin senecioate ((-)-prantschimgin) as dihydrofuranocoumarin derivative and ulopterol [6- (2', 3'-dihydroxy-3'-methylbutyl)-7-methoxy-coumarin] were isolated. Crenulatin (6-formyl-7-methoxycoumarin), suberosin (7-methoxy-6-prenylcoumarin), marmesin senecioate ((-)-prantschimgin) which are pure compounds demonstrated antifungal activity with 625 mg/L MIC against *C. albicans* and antibacterial activity with 1250 mg/L MIC against *S. aureus* (MRSA). These results indicate that extracts and pure compounds obtained from *Ferulago trachycarpa* could be a potential for pharmaceutical products which have antimicrobial activity.

1. Introduction

Plants have a wide variety of phytochemicals which have traditionally used for centuries in ethnomedicines and these chemicals can be used to treat infectious diseases causing by microorganisms (Patra, 2012; Kapoor et al., 2015). The discovery of antibiotic in the early 20th century was seen an important step against bacterial diseases (Kapoor et al., 2015).

Recently, the emergence of multi-drug resistant to bacterial strains with decreased susceptibility to antibiotics has led to the discovery of new antimicrobial agents from natural sources for therapeutic purposes against microbial diseases (Patra, 2012). Therefore, the studies are important to find new antimicrobial agents from plant-derived natural products and provide to exploit new antimicrobial drugs.

The genus *Ferulago* W. Koch. which belongs to the Umbelliferae (Apiaceae) family consists of 49 species in the world. Eighteen *Ferulago* species of which 34 existing in Turkey are endemic (Akalın and Kızılarslan, 2013). Anatolia is acknowledged to the main center of the biodiversity of genus *Ferulago* (Ozhatay and Akalın, 2000) and as taxonomically, it is closely related to *Ferula*, *Peucedanum* and *Prangos* genus (Ozhatay and Akalın, 2000; Akalın and Koçyiğit, 2010–2011).

In folk medicine, *Ferulago* species have been used as sedative, tonic, digestive, carminative, aphrodisiac and in treatment of the intestinal worms and hemorrhoids (Akalın and Koçyiğit, 2010–2011).

The distribution of *Ferulago trachycarpa* have been seen in West, Southwest and South Anatolia of Turkey and while it is known as "Kişniş", "Kuzukulağı", "Kurtkulağı", "Kurtkemirdi", "Kuzubaşı" in Konya, "Kimyon otu" name is called by people in Balıkesir-Edremit region. The fresh leaves of plant are sold in the bazaar and used as a salad in Konya. The mature seeds of the plant are used as a spice in Balıkesir (Akalın and Alpınar, 1994; Akalın and Koçyiğit, 2010–2011).

Previous phytochemical studies have shown that coumarins are the

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main compound on *Ferulago* (De Pascual et al., 1979; Jiménez et al., 2000; Erdurak-Kılıç et al., 2006; Khalighi-Sigaroodi et al., 2006; Basile et al., 2009; Naseri et al., 2013). Coumarins have many pharmacological activities such as antibacterial, antifungal, anticoagulant, anti-inflammatory, anticancer, antihypertensive, antihyperglycemic, antioxidant and anti-inflammatory (Venugopala et al., 2013). There are studies showing that coumarins isolated from *Ferulago* species have antimicrobial activity (Basile et al., 2009; Golfakhrabadi et al., 2016).

The aim of this study is to provide a scientific basis for the antimicrobial activities of isolated coumarins from *F. trachycarpa* rhizomes being the major phytochemical group against various bacteria and fungi.

2. Material and methods

2.1. Chemical materials and instruments

For column chromatography, silica gel (0.2–0.5 mesh, Merck), column (length: 60 cm, size: 3 cm), Sephadex column chromatography LH-20 (Sigma Aldrich), TLC and pTLC plates (Merck 1.05554.001 TLC Kieselgel 60 F_{254} 20x20, aluminium and glass plates) were used.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III, 500 MHz instrument Shimadzu UV-1800 spectrophotometer was used for UV spectra.

2.2. Plant material

F. trachycarpa Boiss. was collected in June 2015 from Konya-Taşkent, Turkey. It was identified by Prof. Dr. Emine Akalın Uruşak and has been deposited at the Herbarium of Faculty of Pharmacy, Istanbul University (ISTE No: 81268).

2.3. Extraction

The rhizomes of *F. trachycarpa* were air dried at room temperature and powdered. The amount of 188 g plant material was extracted with 6 L of n-hexane, dichloromethane and methanol solvents as fractionally and the amount of 10 g plant material was extracted with 300 mL of methanol by percolation method. *n*-Hexane (FTH), dichloromethane (FTD), methanol (FTM) and FTM-s extracts were obtained under vacuum by rotary evaporator and were kept in the freezer at + 4 °C. Also, the amount of them was weighted as 3.49 g, 2.39 g, 15.99 and, 0.101 g, respectively.

2.4. Antimicrobial activity

2.4.1. Microorganism

To determine the antimicrobial activity, the reference bacteria and fungi strains were used: *Staphylococcus aureus* ATCC 6538, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Enterococcus feacalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 10231.

2.4.2. Antimicrobial susceptibility test

Minimum Inhibition Concentration (MIC) values of the extracts and isolated compounds were confirmed against reference strains by microdilution method through Clinical Laboratory Standards Institute (CLSI) criterions (CLSI, 2000, 2006).

In the study, serial two-fold dilutions of the extracts ranging from 5000 µg/ml to 4.9 µg/ml were prepared in medium. The inoculum were prepared using a 4–6 h broth culture of each bacteria and 24 h culture of each yeast strains. Inoculums adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in Mueller-Hinton broth (Difco, Detroit, USA) to give a final concentration of 5×10^5 cfu/ml for bacteria and diluted in RPMI-1640 medium (Sigma-Aldrich) buffered with MOPS buffered to pH 7.0 with 0.165 M MOPS (morpholinepropanesulfonic acid; Sigma-Aldrich, Steinheim, Germany), to give a final concentration 0.5–2.5 $\times 10^3$ cfu/ml for yeast in the test tray. The final volume of the trays was 0,1 ml. Trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton were incubated at 35 °C for 18–20 h and the trays containing RPMI-1640 medium were incubated at 35 °C for 46–50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. Cefuroxime, Ceftazidime, Clotrimazole and Oxacillin were used as reference material and all experiments were performed in triplicate.

2.5. Bioassay-guided fractionation and isolation

According to the results of antimicrobial activity, the active constituent isolation was studied on n-hexane and dichloromethane extracts by column chromatography (40 times amount of extracts SiO_2, 0.2–0.5 mesh) eluting with petroleum ether-dichloromethane-ethyl acetate and ethanol increasing polarity.

For n-hexane extract, total 52 fractions were pooled to volume of 200 mL. Pattern fractions were combined. Fraction 11–13 (with dichloromethane-methanol 3:1; 0.450 g) was subjected to Sephadex LH-20 column chromatography and 33 subfractions were collected. Compound I (toluene-dichloromethane-ethyl acetate-acetonitrile 6:3:2:1; 11.4 mg) was isolated from subfraction 8–12 (99 mg) by pTLC. Fraction 15–18 (with chloroform-methanol 3:1, 1.7632 g) was submitted to Sephadex LH-20 column chromatography and 43 subfractions were collected. Subfraction 8–9 was subjected to Sephadex LH-20 and obtained second subfraction as 43. After pattern fractions were combined, second subfraction 7–14 was purified by preparative TLC to obtain Compound II and III (cyclohexane-ethyl acetate 7:3; 11.6 and 12.5 mg).

For dichloromethane extract, total 52 fractions were pooled to volume of 200 mL. Pattern fractions were combined. Fraction 26–34 (0.0760 g) was applied to pTLC method to produce compound IV (toluene-chloroform-ethyl acetate-acetonitrile 5:3:2:1; 13.5 mg). The procedure from extracts to the isolation of pure compounds is shown in Fig. 1.

The isolated compounds were identified by using spectroscopic techniques UV, ^{1}H and ^{13}C NMR with comparing previously published data for the compounds. The formulas of all pure compounds are shown in Fig. 2.

3. Result and discussion

The extracts (fractionally *n*-hexane-FTH, dichloromethane-FTD, methanol-FTM, and methanol-FTM-s) were obtained from rhizomes of *F. trachycarpa* and the antimicrobial activities of them were investigated against *Stapylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus feacalis* ATCC 29212 bacterial strains and *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750 and *C. parapsilosis* ATCC 22019 fungal strains by microdilution method. All extracts showed antimicrobial activity and according to the antimicrobial activity results, bio-guided isolation was made from most active *n*-hexane and dichloromethane extract. Crenulatin, suberosin, marmesin senecioate ((-)-prantschimgin) and ulopterol were known as coumarin derivatives were isolated.

In literature screenings, marmesin senecioate was detected to be a previously isolated compound from other *Ferulago* species (De Pascual et al., 1979; Doğanca et al., 1992; Köksal, 1999; Jiménez et al., 2000; Öndersev, 2001; Khalighi-Sigaroodi et al., 2006; Razavi et al., 2015; Golfakhrabadi et al., 2016). Suberosin compound was found that isolated in n-hexane extract of *F. carduchorum* (Golfakhrabadi et al., 2013)