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Composition, antimicrobial, antiradical and spasmolytic activity of *Ferula heuffelii* Griseb. ex Heuffel (*Apiaceae*) essential oil

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ABSTRACT

The essential oil from underground parts of *Ferula heuffelii* from N.E. Serbia, was analysed using GC and GC–MS. The main compounds of the essential oil were elemicin (35.4%) and myristicin (20.6%). The essential oil exhibited the best antimicrobial activity against two strains of *Candida albicans* (MIC = 7.0 and 13.7 μg/ml), as well as against *Micrococcus luteus* (MIC = 13.7 μg/ml), *Staphylococcus epidermidis* (MIC = 17.6 μg/ml), *Bacillus subtilis* (MIC = 21.1 μg/ml) and *Micrococcus flavus* (MIC = 28.2 μg/ml). In the DPPH radical scavenging assay, essential oil showed substantial activity with SC_{50} = 22.43 μl/ml. The essential oil was also tested for antispasmodic activity. It inhibited spontaneous contraction of isolated rat ileum dose-dependently, and at the concentration of 86.64 μg/ml exhibited 50% of the maximum effect of atropine. After incubation with 75.00 μg/ml of essential oil, acetylcholine did not induce contractions of ileum, and at 250.00 μg/ml, the essential oil almost completely abolished the spasmodic effect of potassium chloride (80 mM).

1. Introduction

Essential oils have been widely used throughout history for their pharmacological activities such as antibacterial, antifungal, antiviral, antiparasitic, insecticidal and antispasmodic. Today they are being used in pharmaceutical, sanitary, cosmetic, agricultural and food industries (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Teuscher, 2006).

Genus *Ferula* L. (*Apiaceae*) comprises ca. 170 species (Downie, Watson, Spalik, & Katz-Downie, 2000), mostly growing in arid regions of temperate Eurasia, in the Canary Islands and in N Africa. The centre of diversity of the genus is situated in W. and C. Asia (Drude, 1898). Since the classical period, many species of this genus have been used for culinary or medical purposes. For example, fresh stalks of *F. orientalis* L. are used in Turkey as a flavouring agent in pickles (Kartal et al., 2007). Herbal products based on *F. hermonis* Boiss. can be found in the USA as a dietary supplement which is reputed to have aphrodisiac activity (Hadidi, Aburjai, & Battah, 2003). Asafoetida, an oleogum resin from the root of *F. assa-foetida* L. and some other *Ferula* species, is used as a spice in Asia, especially in India, Nepal, Iran and Afghanistan (Eigner & Scholz, 1999; Teuscher, 2006). In Iranian traditional medicine asafoetida extract was used as an antihelminthic, as well as for treatment of abdominal pain, constipation and diarrhoea (Fatehi, Farifteh, & Fatehi-Hassanabad, 2004). Similarly, oleogum resin obtained from *F. gummosa* Boiss. has been used for treating diarrhoea (Sadraei, Asghari, Hajhashemi, Kolagar, & Ebrahimi, 2001).

It was shown that numerous *Ferula* species exhibit antimicrobial and antispasmodic activity, amongst other properties. Asafoetida inhibits growth of *Staphylococcus aureus* and *Shigella sonnei* (Eigner & Scholz, 1999) and the essential oil from underground parts of *F. glauca* L. exhibited good activity against *Bacillus subtilis* (Maggi et al., 2009). Essential oil of *F. gummosa* oleogum resin was active against some Gram (+) and Gram (−) bacteria (Abedi, Jalali, Asghari, & Sadeghi, 2008). *In vitro* experiments revealed that essential oil and water extract of asafoetida can reduce spontaneous contraction of the isolated guinea-pig and rat ileum, as well as contractions induced with acetylcholine (ACh), hystamine, potassium chloride (KCl) and 5-hydroxytryptamine (Fatehi et al., 2004; Sadraei, Ghannadi, & Malekshahi, 2003). It was shown that essential oil and various extracts of underground and aerial parts of *F. gummosa* can significantly inhibit ileum contractions induced by ACh and KCl (Sadraei et al., 2001).

Ferula heuffelii Griseb. ex Heuffel is an endemic and rare W. Moesian perennial species, which predominantly grows in gorges

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and stony places in E. Serbia, and locally in S.W. Romania and W. Bulgaria (Nikolić, 1973).

Considering that the genus *Ferula* is a significant source of plants that are used as spices and that exhibit different pharmacological activities, in this paper we have investigated composition, antimicrobial, antiradical and spasmolytic activity of *F. heuffelii* essential oil for the first time.

2. Material and methods

2.1. Chemicals

Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), alverine (Alverin citrate salt), atropine (Atropinum sulphate salt monohydrate) and potassium chloride were obtained from Sigma Chemical Co. (St. Louis, USA); L-ascorbic acid from Lachema (Neratovice, Czech Republic); Müller–Hinton broth (for bacterial strains) and Sabouraud broth (for two strains of *Candida albicans*), ampicillin, amikacin, ciprofoxacine, nystatin and amphotericin from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia); streptomycin (Streptomicin-sulfat, ampoules 1 g) from Galenika, a.d. (Belgrade, Serbia); acetylcholine (Acetylcholine iodide) from Serva Feinbiochemica (Heidelberg, Germany).

2.2. Plant material and isolation of essential oils

The underground parts of *F. heuffelii* (representative sample) were collected in Serbia from the Djerdap Gorge (Iron Gate) in April 2008. A voucher specimen (ko20080403/1 BEO), was deposited in the Herbarium of the Natural History Museum in Belgrade. Air-dried underground parts were powdered and the essential oil was isolated by hydrodistillation in a Clevenger-type apparatus according to the procedure of the European Pharmacopoeia 6.0, using 1 ml of *n*-hexane as a collecting solvent.

2.3. GC and GC–MS analyses

The essential oil was analysed on the SRI 8610C GC-FID system, equipped with DB-5 capillary column (5% phenyl-methylpolysiloxane, 30 m × 0.32 mm; film thickness 0.25 μm) and a split/splitless injector (split ratio 1:20; injection volume 1 μl of 3% solution of pure oil in *n*-hexane). The column temperature was programmed from 60 °C to 280 °C at a rate of 3 °C/min. The injector and detector temperature was 280 °C. Helium was used as the carrier gas at a flow rate of 1.2 ml/min.

GC–MS analysis was carried out using a Hewlett Packard 6890-5973 GC–MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (split ratio 1:10; 200 °C). The transfer line temperature was 250 °C. Helium was the carrier gas (1 ml/min) and the capillary column used was a HP-5MS (5% phenylmethylpolysiloxane, 30 m × 0.25 mm; film thickness 0.25 μm). The temperature program was the same as that used for the GC analysis. The injected volume was 1 μl of 3% solution of pure oil in *n*-hexane. Identification of the compounds was based on the comparison of their retention indices (KI), retention times (RT) and MS with those obtained from authentic samples and/or the NIST/NBS, Wiley libraries and the literature (Adams, 2001). The linear retention indices (KI) were determined in relation to a homologous series of *n*-alkanes (C_9–C_{24}) under the same operating conditions (Van den Dool & Kratz, 1963).

2.4. Antimicrobial activity

Antimicrobial activity of the isolated oil was assayed using the broth microdilution method (Đorđević et al., 2007). Antimicrobial activity was investigated using a panel that included laboratory control strains from the *American Type Culture Collection* (ATCC): Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 3341), *Micrococcus flavus* (ATCC 10240), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), Gram (–) bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), and two strains of a yeast *Candida albicans* (ATCC 10259 and ATCC 10231). Microorganisms were provided by the Institute for Immunology and Virology, Torlak, Belgrade.

As recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2001), a broth microdilution assay was used for the determination of minimum inhibitory concentration (MIC). Test strains were suspended in the medium to give a final density of 5×10^5 cfu/ml. Pure essential oil was dissolved in DMSO (15 mg/ml), and serial doubling dilutions from 7.0 to 226.5 μg/ml were prepared in a Müller–Hinton broth for bacteria and a Sabouraud broth for yeast *C. albicans*. As a positive control of growth, the wells containing only microorganisms in the broth were used. After incubation for 18 h at 37 °C for bacteria and 48 h at 26 °C for yeast, the growth of the microorganisms was indicated by the presence of a pellet on the bottom of the well. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not demonstrate visible growth. The test was performed two times in duplicate against each microorganism. The MIC of ampicillin, amikacin, streptomycin, ciprofloxacine, nystatin and amphotericin were determined in parallel experiments.

2.5. Radical scavenging activity assay

Three aliquots of the essential oils were mixed with 0.4 ml of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in absolute ethanol, and the final volume was adjusted to 2 ml. Mixtures were vigorously shaken, left for 30 min in the dark and absorbance was measured at 517 nm using absolute ethanol as the blank. One millilitre of 0.5 mM DPPH diluted in 4 ml of absolute ethanol was used as the control. Scavenging of DPPH radical was calculated using the equation: SC (%) = $100 \times (A_0 - As)/A_0$, where A_0 is the absorbance of the control, and As is the absorbance of tested sample. The SC_{50} value represented the concentration of the essential oil that caused 50% of DPPH radical scavenging (Đorđević et al., 2007). Results were compared with the activity of L-ascorbic acid.

2.6. Spasmolytic activity

2.6.1. Isolation of rat-ileum and recording of the contractions

Adult Wistar rats of either sex were used in this study. All experimental procedures with animals where conducted in compliance with The European Council Directive of November 24, 1986 (86/609/EEC). Rats were sacrificed by cervical dislocation and exsanguinations. The ileum portions were isolated out and cleaned off mesenteries. Two centimetre long preparations were mounted in 10 ml tissue baths containing Tyrode's solution (NaCl 136.9; KCl 2.68; $CaCl_2$ 1.8; $MgCl_2$ 1.05; $NaHCO_3$ 11.9; NaH_2PO_4 0.42 and glucose 5.55 mmol/l), maintained at 37 °C and aerated with a mixture of 5% carbon dioxide in oxygen. One end of the segment was attached to the bath bottom and the other to an isotonic force transducer (TSZ-04-E, Experimetria Ltd., Budapest, Hungary). The data were recorded and analysed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd., Budapest, Hungary). The segments were suspended under 1 g tensions and allowed to equilibrate for 30 min. Under these experimental conditions, the segments exhibited spontaneous rhythmic contractions. The essential oil and the control drugs were added directly to the organ bath in volumes not exceeding 5% of the bath volume.