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Short communication

Chemical composition and antioxidant properties of *Juniperus communis* L. commercial essential oils from two regions in North Macedonia

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Introduction

Juniperus communis L. (common juniper) is the most exploitable species from the genus *Juniperus* (Cupressaceae). Essential oil (EO) obtained from ripe, non-fermented berry cones is official according to the monograph in the European Pharmacopeia (Ph. Eur.10), where 1% is the minimum required content. Juniper oil is used in the traditional Turkish medicine as a diuretic, for gastrointestinal problems and as a general antiseptic. It is believed to have both anti-inflammatory and analgesic effects. Dissimilarities in the EO chemical composition are observed in the literature and are attributed to the geographical location, age and degree of plant ripeness, harvesting methods and distillation techniques (Harhour et al., 2018). The Juniper EO extracted from berries has been investigated and established for its *in-vitro* antioxidant and anti-radical activities which are mostly dependent on the nature of the oil components and their concentrations.

The aim of the present study was to investigate the chemical composition of the commercially obtained essential oils of juniper berries from Macedonia and to assess their antioxidant activity *in vitro*.

Materials and methods

Essential oil

The essential oil (EO) was a commercial product, originating from two different locations in North Macedonia (Mavrovo and Berovo).

Gas chromatography (GC)

Adams chromatographic conditions given in the book entitled "Identification of essential oil components by gas chromatography/mass spectrometry" were used for GC-MS analysis of the oils. The chromatographic profile was analyzed using the relative percentages of the individual components based on the FID response (Adams, 2007).

Determination of total phenolic and flavonoid content

The total phenolic content (TPC) of the EOs were determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton et al. (1999) and the absorbance was measured at 765 nm where gallic acid was used as positive control.

On the other hand, the total flavonoid content (TFC) of the EOs were estimated using an $AlCl_3$ colorimetric assay by Zhishen et al., 1999, and the absorbance was measured at 430 nm, while quercetin was used as a positive control.

The examined concentration of the EO was 100 µg/mL.

DPPH assay and β-carotene bleaching method for determination of the antioxidant potential

The free radical scavenging abilities of the samples

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were measured after neutralization of the DPPH radical according to the method of Gyamfi et al., 1999, with small adaptations. For this analysis essential oils with a concentration range from 1 - 10 mg/mL were used.

The linoleic acid/*β*-carotene system was used to determine the anti-lipid peroxidation activities of the samples. For this analysis EOs with a concentration range from 0.05 - 1 µg/mL were used.

Ascorbic acid (AA) and butylated hydroxyanisole (BHA) were used as positive controls for both assays.

General experimental procedures

Cary 50 UV-VIS spectrophotometer from Agilent Technologies, was used for absorbance measurements to determine the TPC, TFC, DPPH and *β*-carotene bleaching assay. Anton Paar MCP 200 polarimeter and RM40 Mettler Toledo refractometer were used for optical rotation and refractive index determination, while Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C Mass Quadrupole was used for identification of chemical composition of essential oils.

Results and discussion

The EOs isolated from *J. communis* berries from two different locations in North Macedonia were analyzed using GC/FID/MS. The results showed that the oils' chemical profiles meet the Ph.Eur.10 monograph requirements, and the identified nine components represent 86.82% and 76.85% of the total EOs composition from Berovo and Mavrovo, respectively. Monoterpene hydrocarbons were predominant fraction in the EOs and *α*-pinene (44.19% and 37.70% for EOs from Berovo and Mavrovo, respectively) and *β*-myrcene (20.76% and 18.02% for EOs from Berovo and Mavrovo, respectively) were the main constituents. *β*-Caryophyllene and Germacrene D were identified from the sesquiterepene fraction present with more than 2% in the EOs from both locations. Regardless of the domination of the monoterpene compounds in the oils, there are differences in their quantitative composition preferably due to the different geographical locations (Berovo and Mavrovo) which result with individual biological properties of each oil.

Optical rotation (-5.123 for EO from Berovo and -6.943 for EO from Mavrovo) and refractive index (1.472 for EO from Berovo and 1.476 for EO from Mavrovo) were determined, and the results meet the monograph requirements.

The phenolic content was calculated from the following gallic acid calibration curve y=0.0034x+0.087, R^2=0.97, and expressed as mg of gallic acid equivalents in g of oil (614.60±38.02 mg GAE/g and 930.41±33.09 mg GAE/g for EOs from Berovo and Mavrovo respectively). The flavonoid content was calculated from the following quercetin calibration curve y=0.0102x-0.0637, R^2=0.97 and expressed as mg of quercetin equivalents in g of oil (340.93±6.50 and 340.62±12.55 mg QE/g for EOs from Berovo and Mavrovo respectively). The obtained results suggested that the oil obtained from berries collected from Mavrovo has higher total phenolic and flavonoid content compared to the oil from Berovo. The differences between analyzed samples are probably result of the genetic or environmental factors.

Juniper berry EOs from Berovo and Mavrovo were a DPPH radical reducer with IC_{50} values of 11.9 mg/mL and 20.78 mg/mL, respectively. When compared to BHT and AA, both oil samples were clearly less effective than these synthetic antioxidants. The low antioxidant activity of the examined oils in DPPH assay may be partially due to the dominance of *α*-pinene which in the literature can be found as weak antioxidant (Harhour et al., 2018).

On the other hand, 0.05 µg/mL concentration of the EOs from Berovo and Mavrovo reduced more than 80% of the extent of *β*-carotene bleaching by neutralizing the linoleate-free radical which is formed in the system. However additional analysis should be performed for clarification of the antioxidative potential of the oils.

Conclusion

The purpose of this research was to examine the chemical composition and antioxidant properties of *Juniperus communis* L. essential oils from two regions in North Macedonia (Mavrovo and Berovo). The results from the chemical composition, optical rotation and refractive index meet the Ph.Eur.10 monograph requirements, however both of the oils showed limited potential as antioxidants. Therefore, suitable investigations are essential to evaluate the effectiveness of these oils as antioxidants in food system.

References

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