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Short communication

Evaluation of the phenolic content and antioxidant capacity of *Tanaceti herba* from two locations from North Macedonia

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Introduction

Tanacetum vulgare L. (Asteraceae), commonly known as "tansy", is plant native to Europe and Asia, where it grows along roadsides, hedgerows and waste places. The medicinal values of *T. vulgare* preparations include different uses: as food preservatives, insecticides, balsams, as wound healing agents, for treatment of rheumatism, ulcer, digestive, kidney and stomach problems and showed analgesic, antidiabetic, antihelmintic, anti-viral, and antioxidant effect. Medicines are made from the inflorescence and herb of tansy where essential oil (0.1–1.9%), phenolic acids, flavonoids, bitterness and mineral compounds were extracted. Polyphenolic compounds have become the most alluring substances in science throughout the last century due to their potential biological activities, such as antibacterial, antioxidant, and anticancer properties (Aćimović and Puvača, 2020).

Antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals (Nickavar and Esbati, 2012). To provide new insights on extracts from *Tanaceti* herba from two different locations in North Macedonia, we aimed to determinate the total phenols and total flavonoids content as well as to investigate the antioxidant potential by performing DPPH and beta-carotene bleaching assays.

Materials and methods

Plant material

The plant material from *T. vulgare* was harvested from two locations from Kozuf mountain (September 2021) and from Karadzica, near Berovo (October 2021). The plant identity was confirmed by Prof. Dr. Gjose Stefkov, from Faculty of Pharmacy - Skopje.

The herbal plant material was air dried in shadow, packed in paper bags and stored in dark and cold place until further analysis.

Standards and instruments

All chemicals and standards were purchased from Sigma-Aldrich Chemical Co. (France) (2,2-Di-phenyl-1-picrylhydrazyl - DPPH; *β*-Carotene; Butylated hydroxyanisole - BHA, Quercetin; Gallic acid - GA) and/or Merck Company (Germany) (Folin-Ciocalteu's phenol reagent; Ascorbic acid – AA; Linoleic acid and Tween 40). Cary 50 UV-VIS spectrophotometer from Agilent Technologies, was used for absorbance measurements in all assays.

Herbal extracts preparation

0.5 g of dried herbal parts of tansy were extracted twice with 5 mL methanol on ultrasonic bath (50/60 Hz; 720 W) for 10 minutes. The obtained extracts were filtered and filled up to the mark with methanol.

Total phenolic content (TPC)

Singleton procedure from 1999 using the Folin-Ciocalteu reagent with slight modifications was used to determine the TPC of the herbal extracts in concentration

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of 50 mg/mL. The absorbance was measured at 765 nm and gallic acid was used as positive control.

Flavonoid Determination

The total flavonoid contents (TFC) of the extracts were measured spectrophotometric using $AlCl_3$ reagent and calculated as quercetin equivalents by the method of Jiao (2000), slightly modified. The absorbance was measured at 510 nm. Quercetin was used as a positive control and tested samples were in concentration of 50 mg/mL.

Determination of antioxidant potential using the DPPH radical and linoleic acid/β-carotene bleaching assay

The free radical scavenging abilities of the tansy herbal extracts were measured using the DPPH reagent according to the method established by Gyamfi, 1999 with minor modifications. The absorbance values were determined at 517 nm. Linoleic acid/*β*-carotene system was used to determine the initial value of lipid peroxidation. For the assay methanolic tansy herbal extracts with a concentration in a range from 1-10 mg/mL were used. The absorbance was determined at 490 nm. AA and BHA were used as a positive control in the performed assays.

Results and discussions

To characterize the obtained methanolic extracts from *Tanaceti herba*, our first approach was to determine their TPC which was calculated from the following gallic acid calibration curve y=0.0034x+0.087 (R^2=0.97) and expressed as mg of GA equivalents in g of dry plant material - DPM (2.88±0.18 mg GAE/g DPM and 3.01±0.08 mg GAE/g DPM for methanolic herbal extracts from Karadzica and Kozuf respectively).

Additionally, the total flavonoid content was determinate for further specification of the extracts. TFC was calculated from quercetin calibration curve (y=0.0006x+0.0039, R^2=0.99) and expressed as mg of quercetin equivalents in g of dry plant material - DPM (9.98±0.76 mg QE/g DPM and 156.65±12.70 mg QE/g DPM for methanolic herbal extracts from Karadzica and Kozuf, respectively). As it could be observed, in the herbal extract from Kozuf, higher amount of phenolic and flavonoid constituents was determinate. Numerous studies revealed that *T. vulgare* is rich in phenolic acids, flavonoids, and their derivatives, which support the pharmacological effects of the plant (Aćimović and Puvača, 2020).

Antioxidant capacity

The methanolic herbal extracts of *T. vulgare* from Karadzica and Kozuf were investigated for their antioxidant potential and the crude extracts displayed DPPH radical scavenging effects with an IC_{50} value of 6.71 mg/mL and 5.59 mg/mL, respectively. The obtained data suggest that *T. vulgare* showed antioxidant activity in correlation with the TPC and TFC of the extracts, however compared to BHT and AA, were clearly less effective. Additionally, Aćimović and Puvača (2020) in their study investigate the methanolic extracts from aerial parts from tansy with higher DPPH radical scavenging effects (EC_{50} = 37 μg/mL) compared to our results.

On the other hand, 1 mg/mL concentration of the extract from Karadzica reduced more than 50% of the extent of *β*-carotene bleaching by neutralizing the linoleate-free radical which is formed in the system, while 1 mg/mL of the extract obtained from Kozuf, showed higher inhibitory percent (88.25%). However additional analysis should be performed for clarification of the antioxidative potential of these extracts.

Conclusion

In this study, *T. vulgare* from two different locations was evaluated for the antioxidant capacity and phenolic content. The results suggested correlation between phenolic and flavonoid contents and free radical scavenging activities. Therefore, the tested samples might be used as natural sources of antioxidants, however their safety should be thoroughly investigated prior their possible application.

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