

Original Research Article

Tyrosinase inhibition, antioxidant and antibacterial activity of commercial daisy extract (*Bellis perennis*)

ABSTRACT

Extracts obtained from plant material have found wide application in the chemical and pharmaceutical industries because they contain significant concentrations of biologically active substances. Commercial daisy extract (*Bellis perennis*) was used in this paper for in vitro testing of tyrosinase enzyme inhibition, antioxidant and antimicrobial activity. Inhibition of the tyrosinase enzyme was determined by monitoring the formation of dopachrome at a wavelength of 492 nm. Antioxidant activity was tested using FRAP and DPPH methods, while antibacterial activity was tested by diffusion technique on reference strains from the ATCC collection. The results showed that daisy extract inhibits tyrosinase enzyme in dose-dependent manner. The extract effectively neutralized DPPH radicals and also showed good reducing ability. Bacterial strains used for in vitro antimicrobial activity testing did not show sensitivity to the extract concentrations used in this study.

Keywords: daisy extract; tyrosinase; FRAP; DPPH; antimicrobial activity

1. INTRODUCTION

Daisy (*Bellis perennis* L.) is a traditional medicinal plant from the Asteraceae family. It is used to treat eye diseases, eczema, gastritis, enteritis, diarrhea, rheumatism, upper respiratory tract infections and to heal wounds [1-4]. It is native to western, central and northern Europe, but is commonly found as an invasive plant in North America [5]. The species has a very long flowering season, with flowers being produced mainly from about March to November. The dominant components in daisies are essential oils, flavonoids and triterpenoid saponins [3,6-8]. The phenolic constituents of *Bellis perennis*, in addition to flavonoids, include anthocyanins (three glucuronylated and malonylated cyanidin-3-glucosides) tannins, and phenolic acids [9]. The following flavonoids were described in daisy flowers: quercetin, apigenin, kaempferol, isorhamnetin, apigenin-7-O- β -D-glucoside, apigenin-7-O- β -D-glucuronide, apigenin-7-O-(6''-E-caffeoyl)- β -D-glucoside, apigenin-7-O- β -D-methylglucuronide, isorhamnetin-3-O- β -D-galactoside, isorhamnetin-3-O- β -D-(6''-acetyl)-galactoside, and kaempferol-3-O- β -D-glucoside [9-11]. Daisy has a strong inhibitory effect on melanogenesis (the process of creating the pigment melanin). Inhibits tyrosinase activity. In addition to inhibiting properties, it regenerates skin cells, brightens melanin in the epidermis and protects the skin from the formation of new freckles and oxidative stress.



Fig. 1. *Bellis perennis*

2. MATERIAL AND METHODS

The commercial extract is accompanied by relevant physico-chemical data from the manufacturer, which are available in Table 1.

Table 1. Values of selected physicochemical parameters for daisy extract

Parameter	Request	Results	Measure unit
Refraction index	1.337 - 1.341	1.3392	-
Density	1.008 - 1.015	1.009	g/ml
pH	4.8 - 5.8	4.8	-
Dry residue	2 - 3	2.5	%
Color	8 - 12.5	8.6	-
2-phenoxyethanol	0.8 - 1	0.95	%
Potassium sorbate	0.2 - 0.3	0.25	%
Total aerobic bacteria	≤100	< 100	CFU/g
Total yeasts and molds	≤ 10	<10	CFU/g

All chemicals used were of analytical grade and were used as received, without any further purification. The 2,2-diphenyl-1-picrylhydrazyl, sodium acetate, ferric chloride and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich (St. Louis, USA). Ultrapure water, prepared with a Smart2Pure device, was used to prepare the aqueous solutions. Spectrophotometric measurements in the analysis of antioxidant capacity were performed on Perkin Elmer Lambda 25 spectrophotometer.

2.1. Tyrosinase inhibition assay

Tyrosinase enzyme activity was examined in 96 wells microtiter plates, monitoring the formation of dopachrome at a wavelength of 492 nm, using a microtiter plate reader (Tecan Sunrise). Prepared stock solution of daisy extract in phosphate buffer (0,1 M, pH=6,8) was mixed with phosphate buffer, 40 µl of substrate (L-DOPA, 0,1 mM) and 40 µl of enzyme (mushroom tyrosinase 35 U/MI-Sigma-Aldrich in phosphate buffer) to obtain final daisy extract concentrations (5% - 183,5 mM, 2%- 73,4 mM, 1%- 36,7 mM, 0,5%- 18,3 mM and 0,2%- 7,2 mM) in 200 µl of reaction mixture. Assay mixture containing daisy extract, phosphate buffer and substrate was first incubated at 25°C for 10 minutes. After incubation period, 40 µl of enzyme was added to reaction mixture and the amount of dopachrome production in the reaction mixture was determined spectrophotometrically at 492 nm every 60 seconds for 30 minutes in microplate reader.

The extent of inhibition by the tested daisy samples was expressed as the concentration of sample needed to inhibit 50% of the enzymatic activity (IC₅₀). Kojic acid (90 µmol/L) dissolved in phosphate buffer was used as a positive control. Blank test with and without

tyrosinase enzyme were also performed. Each measurement was performed at least in triplicate.

The percentage inhibition of the tyrosinase enzyme is calculated according to the equation:

$$\% \text{ tyrosinase inhibition} = (A-B) - (C-D) / (A-B) \times 100 \quad (1)$$

where A is the absorbance of the blank with enzyme, B is the absorbance of the blank without enzyme, C is the absorbance of samples with enzyme and D is the absorbance of samples without enzyme.

2.2. DPPH radical scavenging activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [13]. Different volumes of the extract were mixed with methanol to 2 ml, after which 0.5 ml of 0.5 mM DPPH radical solution was added. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 ml of 0.5 mM DPPH dilution, diluted with 4 ml of methanol, was used as a control sample.

The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$[(A_c - A_s) / A_c] \times 100 \quad (2)$$

where A_s is the absorbance of the solution containing the sample at 517 nm and A_c is the absorbance of the DPPH solution.

2.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of extract, which reflects the antioxidant activity, was determined following the protocol [14]. 3 ml of prepared FRAP reagent was mixed with 100 μ l of diluted extract. Absorbance at 593 nm was recorded after a 30 min incubation at 37°C. The FRAP value was calculated from the calibration curve of iron(II) sulfate heptahydrate.

2.4. *In vitro* antibacterial activity testing

The *in vitro* antibacterial activities of daisy extract were investigated using two Gram-positive bacteria (*S. aureus* and *E. faecalis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) by applying the diffusion technique. Pure extract and dilutions in the range of 3-5% were used to test the susceptibility of bacterial strains. The sample volumes applied to the agar wells were 100 μ l. Ciprofloxacin were used as a control.

2.5. Heavy metal content

The content of bioelements in such prepared parent sample was determined using ICP OPTIMA 2100 DV (Perkin Elmer) spectrometer. Daisy extract were directly analysed, without prior preparation.

3. RESULTS AND DISCUSSION

3.1. Tyrosinase inhibition

Tyrosinase enzyme plays an important role in melanogenesis. It converts L-tyrosine to DOPA and also oxidizes DOPA to dopachrome which is responsible for melanin pigments

production [15]. In this study L-DOPA was used as the substrate to evaluate diphenolase activity. All analyzed daisy extracts showed inhibitory potential. The tyrosinase inhibitory potential of the extracts increased as the concentrations were increased from 7,2 mM to 183,5 mM. Calculated concentration of sample needed to inhibit 50% of the enzymatic activity (IC₅₀) was 179,19 mM. The obtained results were comparable to that of the standard tyrosinase inhibitor kojic acid, used as a positive control in a tyrosinase inhibitory assay.

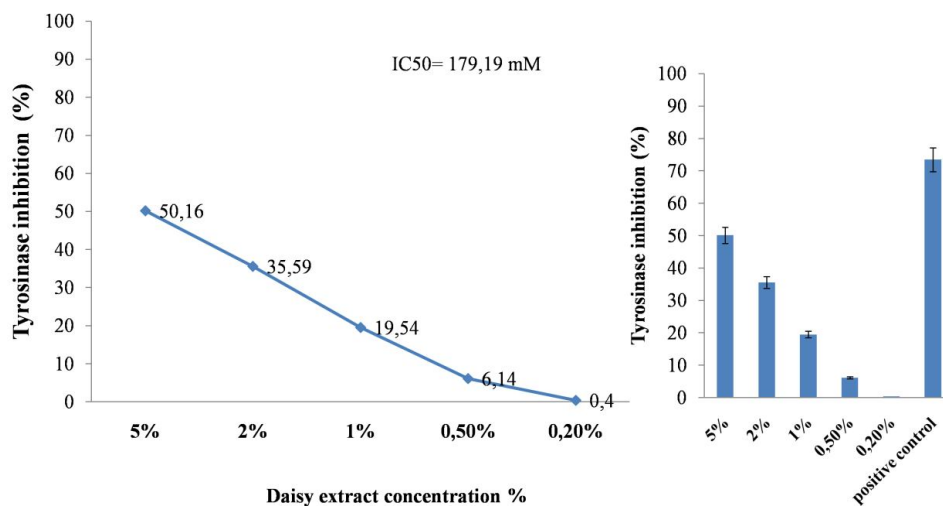


Fig. 2. Results of tyrosinase inhibition analysis with daisy extracts

3.2. *In vitro* antioxidant activity

FRAP and DPPH methods were used in the antioxidant capacity test, which showed that daisy extract has a high antioxidant capacity in vitro. An FRAP value of 742.11 $\mu\text{mol/g}$ indicates a good reducing ability of the sample, but still significantly weaker than the ascorbic acid used as a control, whose FRAP value is 14250 $\mu\text{mol/g}$. The reducing power of bioactive compounds is associated with their electron donating capacity and this is reflected with their antioxidant activity.

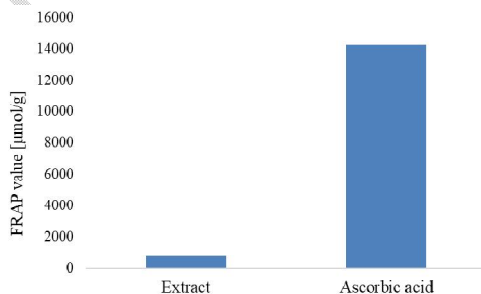


Fig. 3. Graphical comparison of FRAP values of extract and ascorbic acid

Daisy extract has a high power to neutralize DPPH radicals. A calibration series was used to construct a calibration direction from which the IC₅₀ value for the extract, which was 0.097 mg/ml, was determined. The obtained value is significantly higher than the IC₅₀ value of the control, which is 0.03 mg/ml, which indicates a weaker antioxidant capacity of the sample.

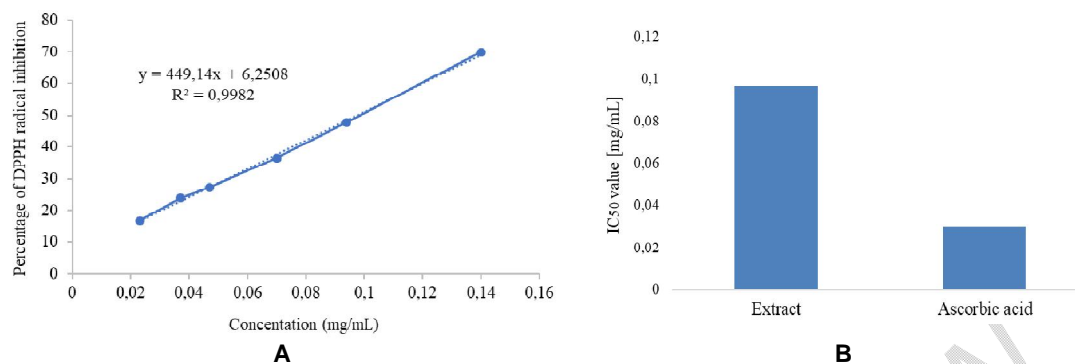


Fig. 4. Results of DPPH radical inhibition by daisy extract (A) and graphical comparison of IC₅₀ values for extract and ascorbic acid (B)

Siatka and Kašparova [10] examined seasonal variations in the total phenol and flavonoid content and the activity of removing DPPH daisy radicals, collected at three sites in the Czech Republic. A slightly better ability to neutralize DPPH radicals was found compared to our study. It was also found that the content of phenols and flavonoids, as well as the radical activity of cleaning daisy flowers vary relatively little during the year and do not depend on the time of collection.

3.3. *In vitro* antibacterial activity

The parent extract as well as its dilutions did not inhibit the growth of the bacteria used in this study. The reason for this may be the geographical origin of the sample from which the extract was obtained, as well as the method of production and storage conditions. The antibacterial activity of extracts of different parts of daisies was investigated by the diffusion technique of Karakas et al [1]. Gram-positive bacteria (*S. aureus*, *S. pyogenes* and *S. epidermidis*) showed greater sensitivity to extracts of *B. perennis* flowers than Gram-negative bacteria. The susceptibility of Gram-positive bacteria may depend on the structure of their cell wall consisting of a single layer, while the cell wall of Gram-negative bacteria is a multilayered formation.

3.4. Heavy metal content

The content of heavy metals in the commercial daisy extract is shown in Table 2. The presence of arsenic, cadmium, cobalt, vanadium, zinc and selenium was not detected in the sample, while other elements were found in very small quantities. The most common element is manganese, with a content of 1.267 mg/l. Typically, manganese can enter the environment from the industries of steel, fireworks, dry cell batteries, fertilizers and paints. Heavy metals are present in spices and herbs in various concentrations. The share of some heavy metal in the plant, ie extracts and essential oils of these plants certainly depends on the locality where the plant is grown, the way the plant is treated with various fertilizers and pesticides, as well as environmental contamination by industrial activities.

Table 2. Content of heavy metals in daisy extract

Heavy metal	Content (mg/l)
As	0,000
Cd	0,000
Cu	0,083

Cr	0,028
Co	0,000
Fe	0,545
Ni	0,273
Sn	0,031
Pb	0,002
Mn	1,267
V	0,000
Sr	0,335
Zn	0,000
Se	0,000

4. CONCLUSION

Daisy extract has shown significant effectiveness in neutralizing DPPH radicals and good reducing ability, which is characterized by an effective natural antioxidant. Antibacterial activity was not recorded on the reference bacterial strains used in this study, but a more detailed analysis should certainly be done on other reference and clinical strains, which will certainly be the subject of further research. Daisy extract in all concentrations showed inhibitory activity on the enzyme tyrosinase. As the concentration of daisy extract increased, so did the percentage of inhibition. Based on the chemical composition, it was determined that daisy extract is safe but also effective in terms of inhibition of the enzyme tyrosinase, and as such could be found in emulsions intended for the treatment of hyperpigmentation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Karakas FP, UcarTurker A, Karakas A, Mshvildadze V, Pichette A, Legault J. *In vitro* cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers of common daisy - A medicinal plant. *Journal of Herbal Medicine*. 2017;8:31-39.
2. Karakas FP, Karakas A, Mshvildadze V, Legault J, Pichette A, Ucar Turker A. Bioassay guided fractionation of extracts from flowers of *Bellis perennis* L. for their anticancer activity. *Planta Medica*. 2011;77:PM216.
Available: <https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0031-1282974?device=desktop&innerWidth=412&offsetWidth=412>
3. Karakas FP, Karakas A, Boran C, Ucar Turker A, Yalcin FN, Bilensoy E. The evaluation of topical administration of *Bellis perennis* fraction on circular excision wound healing in Wistar albino rats. *Pharmaceutical Biology*. 2012;50:1031-1037.
4. Leporatti ML, Ivancheva S. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. *Journal of Ethnopharmacology*. 2003;87:123-142.

5. Cakılcıoğlu U, Sengun MT, Turkoglu I. An ethnobotanical survey of medicinal plants of Yazıkonak and Yurtbaşı districts of Elazığ province, Turkey. *Journal of Medicinal Plants Research*. 2010;4:567-572.
6. Morikawa T, Li X, Nishida E, Ito Y, Matsuda H, Nakamura S, Muraoka O, Yoshikawa M. Perennisosides I-VII, acylated triterpene saponins with antihyperlipidemic activities from the flowers of *Bellis perennis*. *Journal of Natural Products*. 2008;71:828-835.
7. Kavalcioglu N, Açık L, Demirci F, Demirci B, Demir H, Baser KH. Biological activities of *Bellis perennis* volatiles and extracts. *Natural Product Communications*. 2010;5:147-150.
8. Gudej J, Nazaruk J. Flavonol glycosides from the flowers of *Bellis perennis*. *Fitoterapia*. 2001;72:839-840.
9. Siatka T, Kašparova M. Seasonal Variation in Total Phenolic and Flavonoid Contents and DPPH Scavenging Activity of *Bellis perennis* L. Flowers. *Molecules*. 2010;15:9450-9461.
10. Nazaruk J, Gudej J. Qualitative and quantitative chromatographic investigation of flavonoids in *Bellis perennis* L. *Acta Poloniae Pharmaceutica*. 2001;58:401-404.
11. Toki K, Saito N, Honda T. Three cyanidin 3-glucuronylglucosides from red flowers of *Bellis perennis*. *Phytochemistry*. 1991;30:3769-3771.
12. Horozić E, Zukić A, Kolarević L, Bjelošević D, Ademović Z, Šarić-Kundalić B, Husejnagić D, Kudumović A, Hamzić S. Evaluation of antibacterial and antioxidant activity of methanol needle extracts of *Larix Decidua* Mill., *Picea Abies* (L.) H. Karst. and *Pinus Nigra* J. F. Arnold. *Technics Technologies Education Management*. 2019;14:14-19.
13. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*. 1999;299:15-27.
14. Zengin G, Uysal S, Ceylan R, et al. Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A phytochemical study. *Industrial Crops and Products*. 2015;70:1-6.