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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF TARAXACUM OFFICINALE FLOWERS

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Abstract: Some invasive plant species are a source of bioactive compounds with beneficial antioxidant, antimicrobial, nutraceutical, pharmacological, cosmetic, or therapeutical-related applications. Dandelion (*Taraxacum officinale*) possess several biological effects, including anti-inflammatory, antibacterial, immune-regulating, and anti-tumor properties due to its chemical profile, including phenolic acids, flavonoids, tannins, saponines, tiriterpenes, among others. However, the chemical profile of dandelion is influenced by its planted area, with variations in nutrient and phytochemical content influenced by soil, climate, and pollution levels. For this reason, this study aimed to study dandelion flowers collected in Portugal, by determining the contents of total phenolics and total flavonoids. The antioxidant activity and antibacterial activity against bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and the pathogenic strain (*Streptococcus pneumonia*) were also evaluated. Results justified the therapeutic potential of *T. officinale* to be applied as dietary supplement for food preservation in addition to pharmacological values.

Keywords: Dandelion flowers; Phenolics, Flavonoids, Antioxidant activity, Antibacterial activity.

Introduction

One of the biggest threats to global biodiversity is the spread of exotic plant invasions, which have serious repercussions for the economy, human health, and natural ecosystems. Thus, the ability of invasive plant species to acquire resources is often superior to that of native species, which helps them thrive in new habitats[1]. Assessing invasive plant species' potential for revaluation may encourage their usage as a replacement source of bioactive compounds and, as a result, assess their potential as alternate health-promoting strategies. Recently, there has been a shift in research from eradicating invasive plants to using them as valuable sources of biomass, as plant biomass can be a valuable resource for the bioeconomy[2,3]. For instance, Iyer et al.[4] described *Ulex europaeus*, *Viciasativa*, *Cytisus scoparius*, *Chamaenerion angustifolium*, *Pteridium aquilinum*, and *Buddleja davidii*, globally invasive plant species as valuable alternative protein sources. More recently, Purmalis et al.[5] showed high polyphenols content with high radical scavenging activity in leaves and flowers of *Lupinus polyphyllus*, *Impatiens glandulifera*, *Heracleum sosnowskyi*, *Solidago canadensis*, *Echinocystis lobata*, and *Elodea canadensis*. Despite everything, invasive plants' potential for valorization depends on the composition of their main and secondary metabolites[6,7]. Likewise, *Acacia dealbata* and *Solidago canadensis* are rich in compounds with pesticidal or biostimulant effects suitable for agricultural purposes[8,9]. So, invasive plants biomass exhibits specific properties (functional, biological, medicinal.) that can be used directly or serve as a basis to develop new products.

Taraxacum officinale F.H. Wigg. (dandelion), a member of the Asteraceae family, is nowadays very important for its pharmacological effect and as a food source, due to it containing several bioactive compounds and nutritionally valuable substances[10]. Dandelion is considered a major weed, particularly on lawns, where it is difficult to eliminate because its long taproot easily splits when pulled out, allowing it to remain in the soil

and re-sprout. Despite this, it is edible and frequently appears on lists of unusual edible plants, known as "PANC" (unusual Edible Plants). It has a global distribution, being very common in Portugal and the Iberian Peninsula, where it grows in fields, lawns, roadsides and places with damp soils. Dandelion is traditionally used raw, in salads, dried to make infusions, or cooked. Virtually all its parts are used: leaves, flowers, and roots. Additionally, the root can be roasted for use as a coffee substitute.

Its therapeutic applications are various, owing mostly to its traditional uses. Because it had favorable consequences, it stimulated the repetition of information passed down from generation to generation. *Taraxacum officinale* is a popular medicinal plant used for liver diseases. Vitamin A stimulates the immune system, while vitamin B improves human metabolism. Additionally, this plant contains a lot of minerals, including iron, copper, and potassium. Thus, several caffeic acid derivatives (chicoric, caftaric, chlorogenic, neochlorogenic, 1,5-and 3,5-dicaffeoylquinic acids) and flavonoids (glycosides of apigenin, luteolin, quercetin, isorhamnetin, kaempferol) were identified[11]. Also, sesquiterpene lactones, triterpenoids, polysaccharides, phytosterols, and volatile oils are included in dandelion plants[10]. Multiple pharmacological studies have demonstrated the antiviral, antibacterial, anti-inflammatory, immune-regulating, antioxidant, anti-tumor, and other effects of the *Taraxacum* genus[10-12].

Concerning the necessity to better understand dandelion composition and the potential use of its biomass, the aim of this study was to characterize the total polyphenolic and flavonoid content, antioxidant activity (DPPH, FRAP) and antimicrobial activity of hydroalcoholic extracts from dandelion flowers from Portugal. Compositional analysis of this species provides an addition to existing knowledge, therefore indicating potential differences in comparison to territories where this plant is native. Moreover, for plant biomass usage for bioenergy, typically the whole plant is used, leading to another aim of the study, which is to describe the composition of polyphenols and flavonoids in different parts of the plants, which can foster the development of more precisely aimed biotechnologies and applications of plant extracts in the bioeconomy.

Material and Methods

Plant material

The plants and their parts (flowers) were gathered in the period from May to Jun 2024 in the flowering season. Samples were gathered in the North of Portugal (41º 05' 48.50"N, 8º 33' 21.34"W). After harvest, the samples were delivered to the laboratory (within 3 h of harvesting), dried at 40 °C for 48 h (Gallenkamp Plus II Oven). After confirming dehydration at <10%, the flowers were completely crushed with the help of a mill (GM GrinDomix 200, Retsh, Haan, Germany) for 15 s at 5000 rpm to obtain a fine powder and stored in the freezer (–18 °C).

Extracts Preparation

To prepare the hydroalcoholic extracts, 2.5 g of sample was pulverized in 50 mL of extracting solvent (ethanol:water solution (50:50 v/v)). All samples were subjected to a solid/liquid extraction process for 1 h at 45

°C with constant stirring[13]. Then, filtration was carried out, collecting 10 mL of each of the extract, which were stored at −20 °C until further analysis.

Total Phenolic Content

The TPC determination followed the spectrophotometric methodology described by Vinha et al.[14]. To 30 µL of extract, 150 µL of Folin–Ciocalteu reagent solution 1:10 v/v and 120 µL of sodium carbonate solution (7.5%) were added, homogenizing the resulting solution, which was incubated at 45 °C directly in the microplate reader for 15 min and protected from light. The absorbance values were read at 765 nm. Gallic acid was used as a standard. To obtain the correlation between the absorbance of the samples and the concentration of the standard, a calibration curve was performed (R^2 = 0.9989). The values obtained in the study were quantified in milligrams of gallic acid equivalents per 100 g of dry extract (mg GAE/g of dry weight).

Total Flavonoids Content

Total flavonoids content (TFC) was evaluated using a colorimetric method according to the analytical procedure described by Vinha et al.[14]. A solution was prepared by combining 1 mL of each extract with 300 µL of 5% sodium nitrite ($NaNO_2$) in 4 mL of distilled water. Following a 5-min interval at room temperature, 300 µL of 10% aluminium chloride ($AlCl_3$) were added, and after 1 min, 2 mL of sodium hydroxide (NaOH 1M) and 2.4 mL of distilled water were incorporated in the mixture. Catechin was utilized to plot a standard curve (R^2 = 0.9992). The absorbance at 510 nm was measured using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). Results were expressed as mg of catechin equivalents (CE)/g of dry weight.

Antioxidant Activity by DPPH and FRAP Methods

The samples' ability to scavenge DPPH was assessed using the DPPH• scavenging radical method[14]. The experiment was initiated by combining 30 µL of Trolox standard (562 mg/L)/blank/diluted extract (1:10) with 270 µL of the DPPH• solution (6.1×10^{-5} M) earlier prepared. The decrease of DPPH• was monitored with a microplate reader Synergy HT (Biotek Instruments, Inc., Winooski, VT, USA) in time intervals of 10 min and absorption at 525 nm, to study the kinetic reaction. The reaction endpoint was achieved in 20 min. The following method was used to find the inhibitory percentage for extract.

The used ferric-reducing antioxidant power (FRAP) assay was previously described[14]. Briefly, 265 µL of the FRAP reagent (containing 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM ferric chloride) was mixed with 35 µL of ferrous sulphate standard (5-800 µM)/blank/diluted sample extract (1:10). The final mixture was stored for 30 min at 37°C in the dark, after which the absorbance was measured using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA), at 595 nm. A calibration curve was plotted with ferrous sulphate (5–600 µM; R^2 = 0.9996) and ferric reducing antioxidant power was expressed as µmol of ferrous sulphate equivalents (FSE)/g of dry weight.

Antibacterial Activity Assay

The bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and the pathogenic strain (*Streptococcus pneumonia*) were procured from the laboratory section of IPSN/CESPU - Portugal, and maintained on nutrient agar slants at 4°C. Each of the strains was streaked to a nutrient agar plate and incubated for 5 days at 37°C. A single pure colony of the respective bacterial strains was further inoculated into 5 mL of Nutrient Broth (NB) and incubated at 37°C for 5 days. Following the incubation, the bacterial cultures were adjusted to a concentration of 106 cells/mL for the antimicrobial assay, according to the protocol described by Vinha et al.[15].

The agar disc diffusion method, often called the Kirby-Bauer method, was implemented to determine the antibacterial activities of the hydroalcoholic *T. officinale* flowers extracts. The grown bacterial cultures were inoculated at a concentration of 10^6cfu/mL in 20 mL of molten agar media with a gentle shaking and poured in a Petri dishes (100 mm x 15 mm) and air-dried under laminar air flow (Esco Technologies, Pennsylvania, USA). The filter paper discs (6 mm in diameter) were infused with 10 µL hydroalcoholic extracts, air dried and laid down on the agar media plate inoculated with the bacterial culture. The plates were incubated under aerobic conditions at 37°C for 48h. The antimicrobial activities were measured as the zone of clearance around each bacterial colony by subtracting the size of the infused disk from the zone of clearance observed.

Results and Discussion

The potential of invasive plants for valorization depends on the composition of their primary and secondary metabolites, as well as the soil and climatic conditions where they are found. Thus, the same species may present different levels of bioactive compounds, depending on the location/country where it propagates. The same applies regarding biological activities, which are closely related to the amount of primary and secondary metabolites that plants produce. Table 1 presents the total phenolics and total flavonoids contents, as well as antioxidant activity of *T. officinale* flowers hydroalcoholic extracts.

Analyzing the results obtained in Table 1, it is possible to infer that the total phenolic content is significantly higher than the total amount of flavonoids (765.5 mg

Flowers extracts

TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (%)	FRAP µmol FSE/g
765.5±2.59	102.4±0.99	85.61±1.01	15.8±0.3

TPC - Total phenolic content, TFC – Total flavonoids content, DPPH-2,2-diphenyl-1-picrylhydrazyl, FRAP - ferric-reducing antioxidant power, GAE-gallic acid equivalent. CE – catequin equivalente; FSE - ferrous sulphate equivalents. Results are mean value ± S.D. (N=3)

Table 1. Evaluation of TPC, TFC, DPPH, and FRAP of *T. officinale* flowers hydroalcoholic extracts.

GAE/g and 102.4 mg CE/g, respectively). Our results were significantly superior to those described by Epure et al.[16] in *T. officinale* flowers dichloromethane extracts from Romania (13.15 ± 0.81 mg GAE/g and 6.87 ± 0.34 mg CE/g, respectively). Also, our TPC contents were higher than those obtained by Ivanov[17] (33.90 mg GAE/g, 50% ethanol extract) and by Khan et al. (2019) with 41.47 - 691.6 mg GAE/g, aqueous and hydroalcoholic extracts, respectively. Based on these findings, it can be concluded that the growth zones of this species influence the content of bioactive substances, and that their characterization could improve their application in an assortment of health sectors.

The analysis of the antioxidant capacity of *T. officinale* flowers extracts constitutes a correlation tool with the content of total phenolics and flavonoids and allows the assessment of the specific radical scavenging capacity attributable to the extract condition under study. The results were expressed by carrying out two methods: FRAP, which detects the presence of antioxidants through the reduction of iron, and DPPH, which consists of the reaction of the radical of the molecule with the antioxidant substances in the studied extracts. The results of the antioxidant activity determination showed significant antioxidant capacity. Our results agree with those obtained in similar conditions by Dedić et al.[18] - dried flower extracts prepared in 70% ethanol for 30 min, and higher than those obtained by Petkova et al.[19]. Frolova et al.[20] described similar antioxidant activity (85.51%) in hydroalcoholic extracts of *T. officinale* species. Polyphenol isolation depends largely on extraction as the first step. Polyphenol solubility varies based on polymerization, solvent polarity, and interactions with other components. Thus, thus, antioxidant activity can vary depending on the optimization of the extract.

Developing effective and affordable antimicrobial agents is crucial for maintaining a safe environment and excellent health. Newly discovered antibacterial components must be examined for their properties and methods of action. One of the drawbacks of currently available medicines in the market is the development of resistance by pathogenic microorganisms against them. Plants are considered a rich source of antimicrobial agents that have been shown to be effective against a variety of pathogens. For this reason, this work evaluated the antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia*. The zone of inhibition is Table 2.

Bacteria	100 mg/ mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL
Staphylococcus aureus	20 mm	19 mm	12 mm	8 mm	8 mm
E. coli	24 mm	15 mm	10 mm	7 mm	6 mm
Pseudomonas aeruginosa	25 mm	18 mm	13 mm	8 mm	7 mm
Streptococcus pneumonia	21 mm	15 mm	12 mm	8 mm	6 mm

Disc size = 6 mm; S = low susceptibility (7.0–10.0 mm); S+ = susceptibility (10.5–15.0 mm); S++ = high susceptibility (15.5–18.0 mm); R = resistant (0 mm).

Table 2. Results of the analysis of the antimicrobial activity of hydroalcoholic extracts of *T. officinale* flowers.

The results show the different zone of inhibition between the different bacteria and clove buds. The obtained antibacterial activity shows different zones of inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia*.

In recent years, there has been a drop in microbial susceptibility to the existing antimicrobial agents responsible for drug resistance in hospitals and communities, generating a global epidemic of antibiotic resistance, leading to an ecological disaster of unknown consequences. The studied extracts showed the highest zone of inhibition for 100 mg/mL, against *Pseudomonas aeruginosa* > *E. coli* > *Streptococcus pneumonia* > *Staphylococcus aureus.* The lowest zone of inhibition was observed for concentration lower than 12.5 mg/mL. The maximum diameter of the growth inhibition zone for dandelion extracts was 25 mm (*Pseudomonas aeruginosa*), while for *Staphylococcus aureus* it was 20 mm. A previous study has shown antibacterial activity of *T. officinale* extracts against *Candida* strains[21]. Furthermore, another study has reported hydroethanolic extracts of *T. officinale* showed about 26% inhibition against *H. pylori*[21,22]. The antibacterial activities of the extracts are expected due to the presence of bioactive compounds including phenolic acids, flavonoids, tannins, among others. Thus, the investigated flowers of *T. officinale* represent promising sources of bioactive substances with antibacterial properties.

Conclusion

To conclude, this study revealed that hydroalcoholic extracts of *T. officinale* flowers possess medicinal properties and antibacterial activity that inhibit bacterial growth. The antibacterial activities of these extracts are expected perhaps due to the presence of bioactive compounds. The results of the present study have justified the therapeutic potential of *T. officinale* to be applied as dietary supplement for food preservation in addition to pharmacological values.

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