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EFFECTS OF *TARAXACUM OFFICINALE* ON OXIDATIVE STRESS MARKERS IN HUMAN ERYTHROCYTES

Ala FULGA

Departamentul de Chimie, Facultatea de Chimie și Tehnologie Chimică, Universitatea de Stat din Moldova, str. Alexei Mateevici 60, MD-2009, Chișinău, Republica Moldova

Abstract

Oxidative stress (OS), caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is implicated in aging and the pathogenesis of cardiovascular, neurodegenerative, and neoplastic diseases. One of the key enzymatic defenses against oxidative damage is catalase (CAT), which decomposes hydrogen peroxide into water and oxygen, thus protecting cells from oxidative injury. Red blood cells (RBCs) / erythrocytes, due to their high oxygen exposure and hemoglobin content, are particularly vulnerable to oxidative stress. Natural antioxidants derived from plants have attracted significant attention for their potential to mitigate oxidative stress and enhance cellular antioxidant defenses. *Taraxacum officinale* (TO), commonly known as *dandelion*, has been widely used in traditional medicine for its anti-inflammatory, hepatoprotective, and antioxidant properties.

The chemical composition of TO varies by plant part: roots are rich in phenolic compounds, terpenes, sesquiterpene lactones, fructans, and inulin, while leaves contain flavonoids, phenolic acids, coumarins, and vitamins, particularly vitamin A. Previous studies indicate that the antioxidant activity of TO depends on multiple factors: including the plant part, extraction solvent, and extraction duration. Despite its well-documented antioxidant properties, the effects of TO extracts on RBC antioxidant enzymes, especially catalase, remain underexplored. Investigating these effects could provide insights into its therapeutic potential for oxidative stress-related disorders. The present study aimed to evaluate and compare the effects of alcoholic extracts from TO roots and leaves on catalase activity in human erythrocytes, contributing to a better understanding of its cellular antioxidant mechanisms.

Keywords: Taraxacum officinale (TO) roots and leaves, oxidative stress (OS), reactive oxygen species (ROS), Catalase (CAT) activity, erythrocytes (RBCs).

EFECTELE *TARAXACUM OFFICINALE* ASUPRA MARKERILOR STRESULUI OXIDATIV ÎN ERITROCITELE UMANE

Rezumat

Stresul oxidativ (SO), rezultat al dezechilibrului dintre speciile reactive de oxigen (SRO) și mecanismele antioxidante ale organismului, se află în centrul multor procese de îmbătrânire și contribuie semnificativ la apariția bolilor cardiovasculare, neurodegenerative și neoplazice. Catalaza (CAT), una dintre principalele enzime protectoare împotriva efectelor oxidative, transformă peroxidul de hidrogen în apă și oxigen, protejând astfel celulele de daunele ireversibile provocate de acești compuși reactivi.

Eritrocitele sunt expuse constant stresului oxidativ datorită conținutului ridicat de hemoglobină și rolului lor în transportul oxigenului, ceea ce le face vulnerabile, în ciuda existenței unor mecanisme antioxidante de protecție. În acest context, antioxidanții naturali, extrași din plante, reprezintă aliați valoroși, capabili să atenueze efectele SRO și să susțină echilibrul antioxidant al celulelor. *Taraxacum officinale* (TO), cunoscută sub numele popular de *păpădie*, se distinge printr-o tradiție îndelungată în medicina populară, fiind apreciată pentru proprietățile sale antiinflamatorii, hepatoprotectoare și antioxidante.

Compoziția chimică a TO este impresionant de diversă și variază în funcție de partea plantei. Rădăcinile abundă în compuși fenolici, terpeni, lactone sesquiterpenice, fructani și inulină, în timp ce frunzele sunt bogate în flavonoide, acizi fenolici, cumarine și vitamine, cu un conținut deosebit de vitamina A. Studiile anterioare arată că potențialul antioxidant al plantei depinde nu doar de partea utilizată, ci și de solventul ales și de durata procesului de extracție. În ciuda multitudinii de dovezi care îi susțin activitatea

antioxidantă, efectele extractelor de TO asupra enzimelor antioxidante din eritrocite, și în mod special asupra catalazei, rămân insuficient explorate. Studiul de față își propune să aducă lumină asupra acestor mecanisme, evaluând și comparând efectele extractelor alcoolice obținute din rădăcinile și frunzele TO asupra activității catalazei în eritrocitele umane. Prin această abordare, ne dorim să evidențiem rolul său protector la nivel celular și potențialul terapeutic în condițiile asociate SO.

Studiul de față a avut drept scop evaluarea și compararea efectelor extractelor alcoolice obținute din rădăcinile și frunzele de TO asupra activității catalazei în eritrocitele umane, contribuind astfel la o mai bună înțelegere a mecanismelor sale antioxidante la nivel celular.

Cuvinte-cheie: Taraxacum officinale (TO) rădăcini și frunze, stres oxidativ (SO), speciile reactive de oxigen (SRO), Catalaza (CAT), eritrocite.

Introduction

Reactive oxygen species (ROS) are naturally produced as byproducts of cellular metabolism and play a dual role in physiology and pathology. Molecules containing unpaired electrons, known as free radicals (FR), exhibit high reactivity and can damage cellular components. Among ROS, the superoxide anion (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are of particular physiological significance [1,2].

Hydrogen peroxide, although generated during normal metabolic processes, can be harmful at elevated levels. It is produced by enzymes such as xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase, as well as during oxygen metabolism in peroxisomes [3,4]. In the presence of transition metals like Fe^{2+} or Cu^{2+}, H_2O_2 can undergo the Fenton and Haber–Weiss reactions, forming highly reactive hydroxyl radicals [5] (fig.1).

1. Oxygen transport by hemoglobin → ROS production
O_2 → partially reduced → superoxide ($O_2^{\bullet-}$) and H_2O_2
2. Iron from hemoglobin
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$ (**Fenton reaction**)
3. Haber–Weiss cycle
$O_2^{\bullet-}$ reduces $Fe^{3+} \rightarrow Fe^{2+}$
Fe^{2+} reacts again with H_2O_2 → more •OH (**the Fenton and Haber–Weiss reactions)**

Figure 1. Fenton and Haber–Weiss reactions

This molecule diffuses easily across membranes, and its redox activity depends on pH: acting as a strong oxidizer in acidic conditions and a reducing agent in basic environments. Excess H_2O_2 contributes to OS, leading to damage of proteins, lipids, and DNA [3].

To protect against oxidative damage, cells have evolved enzymatic and non-enzymatic antioxidant defenses. Glutathione (GSH) reduces H_2O_2 to water and oxygen, while superoxide dismutase converts superoxide anions into H_2O_2 and O_2. Hydrogen peroxide is further detoxified by catalase, a tetrameric heme-containing enzyme predominantly located in peroxisomes [1,6]. Catalase decomposes H_2O_2 into water and oxygen (fig.2) and can oxidize other substrate such as alcohols, phenols, formic acid, and formaldehyde.

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

Figure 2. Hydrogen peroxide decomposition reaction

The enzyme exhibits optimal activity near pH 7, with minimal changes in reaction rate between pH 6.8 and 7.5 [3,7]. The search for natural compounds capable of enhancing cellular antioxidant defenses has gained increasing interest.

Taraxacum officinale (TO), commonly known as dandelion, has been widely used in traditional medicine and shows promising antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial properties. TO contain diverse bioactive compounds, including terpene derivatives (taraxerol, taraxasterol, taraxacoside), sterols (β-sitosterol, stigmasterol), rubber, tannins, fatty acids, flavonoids, carotenoids, saponins, and inulin. It is also rich in minerals such as iron, copper, and potassium, as well as vitamins A, C, B1, PP, and D [8,9]. These compounds collectively contribute to its potent antioxidant activity and therapeutic potential. Despite evidence supporting TO antioxidant properties, the effects of its extracts on RBCs antioxidant enzymes, particularly CAT, remain underexplored. Understanding this interaction is important because RBCs are highly exposed to oxidative stress due to their oxygen transport function. The present study aimed to evaluate and compare the effects of alcoholic extracts of TO roots and leaves on CAT activity in human erythrocytes, providing insights into its cellular antioxidant mechanisms.

Extracts from *Taraxacum officinale* (TO) exhibit significant antioxidant activity, largely attributed to their high content of phenolic compounds. However, in plasma systems, the most pronounced protective effects against H_2O_2/Fe-induced oxidation have been observed in enriched fractions containing flavonoids, sesquiterpene lactone (SL) amino acid adducts, and inositol 4-hydroxyphenylacetate esters [10,11].

The complex chemical composition of TO makes it challenging to fully elucidate its mechanisms of action, which remain largely incompletely, understood. Currently, there is no comprehensive data describing how different types of extractants and their concentrations influence the bioactive content of TO, nor are there detailed comparative studies assessing the effects of root versus leaf extracts on catalase activity. Clarifying these aspects is essential for understanding the therapeutic potential of TO as a natural antioxidant and for optimizing its application in medical and nutraceutical contexts.

Materials and Methods

Leaves and roots of *Taraxacum officinale* F. H. Wigg were collected from a natural habitat in the Republic of Moldova in May 2019. The plant material was air-dried under laboratory conditions at room temperature for two weeks. Once dried, the leaves and roots were manually ground into a fine powder. Ethanolic extracts were prepared at five different concentrations: 20%, 25%, 40%, 50%, and 80%. The biomass-to-solvent ratio was 10:1 (mg/ml). Extraction was performed in 100 ml containers at room temperature for 24 hours. The extracts were subsequently filtered using Whatman No. 1 filter paper and stored at 4°C. Aliquots of 1.5 ml were centrifuged (MPW 370, 5 min, 5000 rpm), and the supernatants were used for further assays. Red blood cell (RBC) catalase activity was determined following the method described by [12]. Blood from healthy volunteers *(all participants gave both, oral and written informed consent according to the World Medical Association Helsinki Declaration of Ethical principles for medical research involving human subjects)* was diluted 1:4 (v/v) with Dulbecco's Modified Eagle Medium (DMEM) and supplemented with gentamicin (100 µg/ml), heparin (2.5 units/ml), and L-glutamine (0.6 mg/ml). For the assay, 0.9 ml of the diluted blood was combined with 0.1 ml of TO ethanol extract in the test wells. Control wells received an equivalent volume of ethanol in isotonic NaCl solution (0.9%). The microplates were incubated for 24 hours at 37°C in a humidified atmosphere containing 3.5% CO_2, followed by centrifugation at 1500 rpm for 5 minutes. The resulting RBC pellet was used to assess catalase activity according to [7]. This method is based on the enzyme's ability to catalyze the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. In the presence of ammonium tetrathiomolybdate, H_2O_2 forms a yellow complex, which loses color as H_2O_2 is cleaved. The degree of color loss, measured spectrophotometrically at 410 nm, corresponds to catalase activity. For control samples, distilled

water replaced H_2O_2. Catalase activity was expressed as µM/g hemoglobin (Hb). All experiments were conducted in triplicate using 24-well microplates.

Statistical Analysis and Ethics

Statistical analyses were performed using GraphPad Prism 8.0. Data are presented as mean ± standard deviation (M±SD). The percentage difference between experimental groups and controls was calculated. Comparisons between groups (control *vs*. experimental and leaves *vs*. roots extracts) were assessed using the Mann–Whitney U test. Spearman's rank correlation coefficient (r_s) was used to evaluate the relationship between ethanol concentration and catalase activity in the tested samples. A significance level of $p \leq 0.05$ was considered statistically significant for all analyses. *Asterisks indicate statistically significant differences between the control and treated groups.

This study was approved by the Research Ethics Committee of the "Nicolae Testemitanu" State University of Medicine and Pharmacy (approval no. 81, dated 19.09.2020).

Results

Catalase activity varied across different ethanolic extracts of *Taraxacum officinale* (TO). For leaf extracts, the enzyme activity measured 23.8 ± 2.3 µM/g Hb at 20% ethanol, representing a 47.2% decrease compared to the control ($p < 0.05$). At 25% ethanol concentration, catalase activity increased slightly to 47.6 ± 0.4 µM/g Hb, which was 5.7% higher than control, though this difference was not statistically significant (p = 0.51). Similar trends were observed for 40% and 50% ethanol extracts, with catalase activities of 43 ± 2 µM/g Hb (2.2% higher than control, p = 0.51) and 43.6 ± 1.1 µM/g Hb (7.3% higher than control, p = 0.51), respectively. For the 80% ethanol leaf extract, catalase activity reached 43.8 ± 1.2* µM/g Hb, which was 15.2% higher than control and achieved statistical significance ($p = 0.05$). The correlation analysis showed a weak, negative, and non-significant relationship between ethanol concentration and catalase activity for leaf extracts (r_s = -0.33, p = 0.11).

For root extracts, catalase activity at 20% ethanol was 48.04 ± 0.73 µM/g Hb, representing a 13.3% decrease relative to control (p = 0.05). At 25% ethanol, enzyme activity significantly increased to 56.12 ± 3.26 µM/g Hb, 47.5% higher than control (p = 0.05). Increasing the ethanol concentration led to reduced catalase activity compared to controls: 40% ethanol—41.72 ± 0.16 µM/g Hb (7.3% lower than control, p = 0.51), 50% ethanol—41.88 ± 11.67 µM/g Hb (15.5% lower than control, p = 0.51), and 80% ethanol—41.71 ± 2.49 µM/g Hb (11.4% lower than control, p = 0.51). Spearman correlation analysis revealed a strong, negative, and statistically significant association between ethanol concentration in root extracts and catalase activity (rs = -0.73, p = 0.001).

By comparing the data between leaves and roots, it was observed that catalase activity in 20% ethanol leaf extracts was 24.3% lower than in root extracts prepared with the same concentration (p = 0.05). Leaf extracts at 25% ethanol showed a catalase activity 8.5% lower than root extracts (p = 0.05). For 40% and 50% ethanol, the differences were minimal, with leaf extracts showing 1.3% (p = 0.51) and 1.8% (p = 0.51) lower activity than root extracts, respectively. At 80% ethanol, catalase activity in leaf extracts was 2.1% lower than in roots (p = 0.28).

Discussion

Taraxacum officinale (TO) has attracted considerable attention as a source of natural phytochemicals due to its promising antioxidant properties, often considered superior to some synthetic antioxidants. Bioactive compounds from TO have been extensively studied for their potential health benefits, including antioxidant, anti-tumor, anti-inflammatory, and anti-diabetic effects[13,14]. Traditionally, TO have been consumed in the form of infusions or decoctions,

functioning as aperients, laxative, stimulant, and mild diuretic. The TO fascinates us with its chemical content of flavonoids and phenolic acids by high-performance liquid chromatography (HPLC). About 43 compounds, without microelements and vitamins, were detected and the most abundant was chicoric acid, that is a dicaffeoyltartaric acid [15].The chemical composition of TO is highly responsive to environmental and physiological factors, including: climate, soil properties, and plant organ specificity. Free 4-dimethylsterol esters peak in winter, correlating negatively with temperature and solar radiation, whereas sitosterol and cycloartenol esters increase under elevated temperature and light exposure [8]. Furthermore, harvest age significantly influences phytochemical complexity and bioactivity. Chloroform extracts from five-month-old TO leaves exhibited a more diverse chemical profile than those from two-month-old leaves and demonstrated dose-dependent inhibition of yellow fever virus replication, with efficacy approximately eightfold higher than that of extracts from younger leaves[16,17].

The distribution of bioactive compounds in TO varies substantially among plant organs. Hydroxycinnamic acids, including chicoric, monocaffeoyltartaric, and chlorogenic acids, are distributed throughout the plant [18], whereas coumarins such as cichoriin and aesculin are predominantly localized in leaves [17]. Comprehensive qualitative and quantitative analyses of aqueous and methanol extracts from roots, stems, and flowers reveal that flavonoid concentrations are highest in flowers, while saponins, phenolics, and alkaloids accumulate primarily in stems and roots. Popescu et al. (2021) reported that flowers and leaves contain significantly higher levels of polyphenols compared to stems and roots, highlighting the importance of organ-specific harvesting strategies for maximizing bioactive compound yield [19]. Future studies exploring the synergistic effects of these compounds, particularly in relation to enzymatic antioxidant defense mechanisms such as catalase activity in erythrocytes, will help elucidate the full therapeutic potential of TO. Most research on TO has focused on phenolic compounds, which play a critical role in plant defense against biotic and abiotic stress, as well as contributing to human nutrition and well-being. However, TO contains a wide spectrum of bioactive molecules—including terpenes, sterols, inulin, vitamins, and amino acids—whose ratios and combined effects on various biochemical processes remain a subject of ongoing investigation [8] .

These observations suggest that the composition and relative abundance of bioactive compounds, as well as their activity under oxidative stress, are likely to differ. This hypothesis was confirmed in our experiments. Specifically, catalase activity was modulated by ethanol concentration in both roots and leaves. In previous studies, we demonstrated that other biochemical pathways associated with oxidative stress are also influenced by TO in an ethanol concentration-dependent manner [8]. Commission monographs recommend the administration of TO three times daily, either as an herbal preparation or as a 1:1 liquid extract in 25% ethanol. This recommendation aligns with our findings, where the highest catalase activity was observed precisely at this ethanol concentration [16]. *Taraxacum officinale* is not a single example which describes how important is the type of solvent, the literature abounds in fresh data which underlines the importance of extractant type and its concentration [20].

Conclusions

Phytotherapeutic herbs and medicinal plants continue to play a crucial role in drug discovery and development. The leaves and roots of *Taraxacum officinale* represent a rich source of bioactive compounds with significant potential for application in nutraceutical and pharmacological fields. The present study demonstrates that *TO* exhibits pronounced antioxidant activity. Notably, the strongest effect on erythrocyte catalase activity was observed for root extracts prepared with 25% ethanol. These effects are likely mediated by a complex mixture of bioactive constituents, the composition and relative abundance of which appear to depend on

ethanol concentration. Further studies are required to comprehensively characterize the biological activities and underlying mechanisms of these extracts.

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Date despre autori:



Ala FULGA, doctor în științe medicale, lector universitar, Departamentul de Chimie, Facultatea de Chimie și Tehnologie Chimică, Universitatea de Stat din Moldova, str. Alexei Mateevici 60, MD-2009, Chișinău, Republica Moldova
E-mail: agfulga@gmail.com
ORCID: 0000-0002-0623-0857