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FULGA Ala

BIOCHEMICAL ACTIVITY OF EXTRACTS FROM SPECIA TARAXACUM OFFICINALE G.H. WEBER ex WIGGERS

315.01 – MEDICAL BIOCHEMISTRY

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Scientific advisor
Tagadiuc Olga,
MD, PhD, dr. hab. med. sci., prof.
Scientific co-advisor
Todiras Mihail,
MD, PhD, dr. hab. med. sci., assoc. prof.

Guidance committee members:
Gudumac Valentin,
MD, PhD, dr. hab. med. sci., prof.
Andronache Lilia,
MD, PhD, res. sci.
Protopop Svetlana,
MD, PhD, assoc. prof.

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The specialized Scientific Council:
Chairman:
Parii Sergiu,
MD, PhD, dr. hab. med. sci., assoc. prof.

Members:
Tagadiuc Olga,
MD, PhD, dr. hab. med. sci., prof.
Todiras Mihail,
MD, PhD, dr. hab. med. sci., assoc. prof.
Globa Pavel,
PhD, assoc. prof.

Official reviewers:
Gudumac Valentin,
MD, PhD, dr. hab. med. sci., prof.
Lisii Dan,
MD, PhD, assoc. prof.
Bulimestru Ion,
PhD, assoc. prof.

Author
Fulga Ala

SUMMARY

THE RESEARCH CONCEPTUAL FRAMEWORK

Actuality and importance of the researched problem. Oxidative stress is considered one of the key mechanisms of many pathological processes, such as neoplasms, diabetes, neurodegenerative, cardiovascular diseases, etc. Pro-oxidants are frequently associated with damage to proteins, lipids and nucleic acids, and the prevention or treatment of these processes depends on the mobilization of the body's own antioxidant systems or the external use of substances capable of improving the situation in oxidative stress [1].

The use of synthetic antioxidant agents is often faced with the development of adverse reactions. The latter can be considered a norm in the chemotherapy of neoplasms, widely causing genetic mutations, myelosuppression, immunosuppression, cardiac, pulmonary, hepatic, renal toxicity, etc. Thus the development of new drugs, characterized by targeted functions and minor adverse problems remains a challenge for contemporaneity.

Natural compounds can exert prophylactic or therapeutic functions, the effectiveness of which has been proven throughout the evolution of mankind. In the specialized literature, multiple substances are described, generously provided by nature, such as flavonoids, carotenoids, vitamins, phenolic acids (caffeic, ferulic, gallic), which alone or in combination show antioxidant, anti-inflammatory, immunomodulatory, antitumor properties, etc.

Taraxacum officinale G.H. Weber ex Wiggers (TO) is a plant, whose chemical content and therapeutic applications prompt modern medicine for in-depth investigations in oncology, gastroenterology and hepatology, nephrology, geriatrics, etc. Being considered a simple weed, thanks to the presence in its composition of flavonoids, polyphenols, vitamins and minerals, it has proven its effectiveness not only in traditional medicine, but also as a generous source of substances with antitoxic, antioxidant, antitumor functions [2–5]. All the component parts of the plant have proven to be beneficial for health: the root, the leaves, the stem, the flower and the seeds, whose chemical content is extremely variable, depending on the climatic conditions, the harvest season, the quality of the soil, etc.

This plant represents a promising source of drugs thanks to its involvement in multiple signaling mechanisms through its action on interleukins, NF-κB, Akt, MEK, ERK, sVCAM-1, MAPK, MMP, TNF, *etc.* [1-7]. Due to the multitude of components, the content of which is influenced by multiple factors, the results of the TO action presented in the specialized literature often refer to a single component part of the plant, to a single type of extractant and mechanism of action. At the moment, there is a lack of a comprehensive study, which would highlight and compare simultaneously several mechanisms of action of TO extracts, made from various component parts of the plant with various solvents.

The **aim** of the research was to study the chemical composition and *in vitro* action of TO extracts in order to elucidate the biochemical mechanisms of action and the therapeutic potential.

The objectives:

1. Determination of the chemical composition of *Taraxacum officinale* extracts by quantifying the total content of flavonoids, phenols, phytosterols and hydroxychoric acids;
2. Evaluation of the action of *Taraxacum officinale* extracts on antioxidant enzymes and thiol-disulfide homeostasis;
3. Elucidation of the action of *Taraxacum officinale* on markers of oxidative stress, free radical potential, total antioxidant capacity, mass and average activity of antioxidants;
4. Study of the action of *Taraxacum officinale* extracts on cell viability in glial tumor cultures;
5. Establishing correlations between the chemical content and the mechanism of action of *Taraxacum officinale* extracts.

Scientific research methodology. A multidimensional study of the chemical composition of TO leaf and root extracts and their in vitro biological effects was performed. To achieve the proposed goal and objectives, the content of flavonoids, polyphenols, phytosterols and hydroxycinnamic acids was determined in the hydro-ethanolic and DMSO extracts from leaves and roots of TO. Subsequently, the action of the extracts on the enzymatic antioxidant system, thiol-disulfide homeostasis, markers of oxidative stress, antiradical potential, total antioxidant capacity, mass of antioxidant substances, average antioxidant activity and viability of human glioma cells U-251 MG was deciphered.

The doctoral scientific project was positively approved by the Research Ethics Committee of SUMPh „Nicolae Testemitanu" (decision no. 14 of 21.11.2019).

The work was carried out within the Department of biochemistry and clinical biochemistry, the Laboratory of biochemistry and the Scientific Center for Drug Research of SUMPh „Nicolae Testemitanu".

The novelty and scientific originality of the obtained results. A complex, single-moment study was carried out that allowed the elucidation and detailing of the chemical composition of TO and the biochemical mechanisms of action of extracts from TO.

It has been established that extracts from TO possess modulatory action on markers of oxidative stress and the antioxidant system, enzymatic and non-enzymatic, which is dependent on the content of phenolic acids. The quantification of the content of TFC, TPC, phytosterols and hydroxycinnamic acids, allowed the correlation with the impact on the biochemical markers studied and the advancement of some hypotheses regarding the molecular bases of the modulatory effects of the extracts from TO.

The dependence of both the chemical content and the biochemical and biological effects on the plant organ (leaves or roots), the type of extractant and its concentration, the exposure time and the content of biologically active substances in the extract was identified. It has been reported that although TO acts in a time- and concentration-dependent manner, the ratio of chemical compounds is more important for the manifestation of either stimulatory or inhibitory effects.

The theoretical significance of the work. The present research allowed detailing of the mechanisms of action of *Taraxacum officinale* extracts, underlining the importance of knowing the concentration and ratio between the bioactive compounds, which determine the modulatory action of this plant within the studied biochemical mechanisms.

The applicative value of the research. The results obtained in the *in vitro* estimation of the biochemical action characteristics of *Taraxacum officinale* extracts justify the need to describe in studies the growth area of the plant and its anatomical organs, of the extractants of various polarity, of the methods used in the extraction of the bioactive compounds for the relevant analysis of the results and their comparison.

Implementation in practice. The obtained results were implemented in the didactic activity of the Department of biochemistry and clinical biochemistry, as well as in the scientific activity of the Laboratory of biochemistry of SUMPh „Nicolae Testemitanu".

Approval of scientific results. The results of this study were presented at national and international scientific forums, such as: *Annual Scientific Conference Biomedical and Health Research: Quality, Excellence and Performance* SUMPh „N. Testemitanu", Chisinau, 2020, 2021; *The 8th International Medical Congress for Students and Young Doctors* SUMPh „N. Testemitanu", Chisinau, 2020; *The 7th International Conference Ecological & Environmental Chemistry*, Chisinau, 2022; *Medical drugs for humans. Modern issues of pharmacotherapy and*

prescription of medicine. Materials of the V International Scientific and Practical Conference, Kharkiv, Ukraine, 2021; *Middle East International Conference on contemporary scientific studies-V*, Ankara, Turkey, 2021; *Applications of Chemistry in Nanosciences and Biomaterials Engineering-NanoBioMat* 2021, Bucharest, Romania, 2021; *IV International Icontech Symposium on Innovative Surveys in Positive Sciences*, Adana, Turkey, 2021; *Euroasia International Congress on Scientific Research and Recent Trends – VIII*, Philippine Merchant Marine Academy, Zambale, Philippines, 2021; *2. International Scientific Research and Innovation Congress*, Istanbul, Turkey, 2021; *International Harran Health Sciences Congress-III*. Harran University, Sanlıurfa, Turkey, 2021; *3rd International Sukurova Agriculture and Veterinary Congress*, Adana, Turkey, 2021; *4th International African Conference on Current Studies*, Bani Waleed University, Libya, 2021; *International Anatolian Congress on Medicinal and Aromatic Plants*, Arapgir Municipality, Malatya, Turkey; *3.Uluslararasi Palandöken Bilimsel Çalişmalar Kongresi*, Erzurum, Turkey, 2021; *3.International Gobeklitepe Scientific Studies Congress*, Şanliurfa, Turkey, 2021; *International Modern Scientific Research Congress – II.* Istanbul, Turkey, 2021; *ISPEC 8th International Conference on Agriculture, Animal Science and Rural Development.* Bingol, Turkey, 2021; *2nd International Conference on Coffee and Cocoa*, Bogota, Colombia, 2022; *Cukurova 8th International Scientific Researches Conference*, Adana, Turkey, 2022; *Tashkent I st-International Congress on Modern Sciences Tashkent Chemical-Technological Institute*, Tashkent, Uzbekistan, 2022; *Hodja Akhmet Yassawi 6th International Conference on Scientific Research,* Lankaran, Azerbaijan, 2022; *2. International Dicle Scientific Studies and Innovation Congress*. Diyarbakir, Turkey, 2022; *Applications of Chemistry in Nanosciences and Biomaterials Engineering-NanoBioMat 2022*, Bucharest, Romania, 2022; *Ecological and environmental chemistry. XXth Edition,* Chisinau, 2022; *Applications of Chemistry in Nanosciences and Biomaterials Engineering-NanoBioMat 2022*, Bucharest, Romania, 2022; *RSU RESEARCH WEEK/Knowledge for use in practice,* Riga, Latvia, 27-31 March, 2023; *The 2nd International Electronic Conference on Biomedicines/ Medicinally Active Plants and Phytochemicals*, Switzerland, 01-31 March, 2023.

Publications related to doctoral thesis. The results of the research were presented in 74 scientific papers, including 5 articles in SCOPUS journals and other international databases, 2 articles in journals from the National Profile Register, 1 article in the proceedings of the national scientific conference with international participation. At the same time, 3 innovator certificates, 1 invention patent and 3 innovation implementation documents in the scientific-practical process were obtained.

The volume and structure of the thesis. The thesis was presented on 184 pages, which include all the mandatory elements stipulated by the current Guide (2017), iconographically represented by 26 tables, 22 figures, included in the text and 22 annexes, 220 bibliographic sources.

Keywords: *Taraxacum officinale*, biochemical mechanisms, oxidative stress, antioxidant system, enzymatic antioxidants, thiol-disulfide homeostasis, cell viability, glioblastoma.

1. *TARAXACUM OFFICINALE* – CHEMICAL COMPOSITION AND MECHANISMS OF ACTION

In this chapter, a synthesis of the existing data in the specialized literature was carried out, with the purpose of arguing the study. The results of the analysis of the information regarding the general characteristic of the *Taraxacum* species, the chemical composition of the plant, deciphering the composition depending on the part of the plant, the geographical area and the type of extractant were presented. At the same time, the mechanisms of action of total extracts or solitary compounds extracted from *Taraxacum officinale* were also discussed, emphasizing the controversial results or the absence of certain data.

2. MATERIALS AND METHODS OF THE STUDY OF THE CONTENT OF TARAXACUM OFFICINAL EXTRACTS AND THEIR MECHANISMS OF ACTION

Characteristics of the material included in the study. *Taraxacum officinale* F. H. Wigg plants harvested from the natural environment (spontaneous flora, 47 4' 8" North, 28 40' 47" East) served as raw material. Leaves (TOL) and roots (TOR) separately were dried in the dark at room temperature for 2 weeks. The dry raw material was ground using a coffee grinder (Scarlett Coffee grinder SC-4145). The obtained powder was stored in transparent polystyrene tubes with hermetic stoppers in the dark at room temperature.

Preparation of TO extracts. A series of extracts were prepared separately from the raw material, leaves and roots. For the preparation of solvents were used: deionized water (Adrona Crystal), DMSO (Dimethyl sulfoxide, Sigma, DE), ethyl alcohol – 96% (Luxfarmol, MD), from which ethyl alcohol of different concentrations (20%, 50% and 80%).

The biomass (g)/extractant (mL) ratio used was 1 g/10 mL. The extraction was carried out at room temperature in glass flasks with a volume of 100 mL, for 24 hours.

The final concentration of DMSO (<0.1%) in the extracts was chosen according to literature data [6, 7]. This concentration does not affect cell integrity, does not influence cell proliferation and does not have a cytotoxic effect.

Statistical analysis of the results. The results of the study were stored and grouped in the MS Access 2007 (Microsoft Office 2007) database. Statistical analysis was performed using GraphPad 8.0 (GraphPad Prism Software, v.8, San Diego, CA) and WINSTAT (R. Fitch Software, DE) software. The following were calculated: the means and standard deviations (X±SD), the confidence interval (95% CI), the IC50 index (the molar concentration of a substance required to block a response by 50% of the activity range between the minimum and maximum compared to the control). Correlations were performed by Spearman (r_s), statistical difference between groups being evaluated by Mann-Whitney U test. The management of bibliographic references was performed automatically, using the software integrated in Word 2007 and Chrome, Zotero 6.0.18 (www.zotero.org).

3. CHEMICAL COMPOSITION OF *TARAXACUM OFFICINALE* EXTRACTS

The determination of the total flavonoids content was carried out based on the method developed by Ordonez *et al.* (2006), modified after Fulga *et al.* (2021) [8]. Dihydroquercetin (DHQE) was used as a reference substance. The results were read at 420 nm. The total polyphenols content (TPC) was determined by the spectrophotometric method according to Folin-Ciocalteu. The intensity of the coloring is proportional to the total amount of polyphenolic compounds present in the analyzed sample and was measured at a wavelength of 760 nm [9]. The assessment of phytosterol content was carried out according to the method developed in the Laboratory of biochemistry of SUMPh „Nicolae Testemițanu" [10]. The intensity of the staining is directly proportional to the concentration of β-sitosterols, measured at 500 nm. The dosage of hydroxychoric acids was carried out by the HPLC-UV method, described in the European Pharmacopoeia for „Echinaceae purpureae herba" (Ph. Eur. 01/2008:1823) and „Echinaceae purpureae radix" (01/2008:1824) and modified by the Center's collaborators Scientific of Medicine, for the analysis of TO extracts [11].

Chemical composition of Taraxacum officinale leaf extracts. The highest flavonoid content was determined in the 80% ethanol (LEtOH80) extracts (6.52±0.15 mg/mL), although statistically significant differences with the 50% ethanol (LEtOH50) extracts (6.30±0.07 mg/mL) were not determined (r_s =0.14, p=1.2) (table 1). The lowest content of flavonoids was recorded in the case of the DMSO (LDMSO) extract (0.50±0.03 mg/mL). The lowest total polyphenol content (TPC) was recorded when using 80% alcohol (11.52±0.01 mg/mL) and DMSO (13.88±0.13 mg/mL). The leader in TPC content was the 50% ethanol extract (38.00±1.02 mg/mL).

The evaluation of the phytosterol content allowed to establish that the primacy also went to the extracts on 50% alcohol (2.51±0.45 mg/mL), the minimum concentration being recorded in the case of extraction on DMSO (0.38±0.01 mg/mL).

The highest amount of hydroxychoric acids was recorded in the 50% ethanol extracts (48.80±2.22 mg/mL), and the lowest in the 80% ethanol extract (4.96±0.39 mg/mL) and DMSO (11.40 ±0.06 mg/mL). The extraction of caftaric acid was more productive in the case of 50% ethanol (5.64±0.36 mg/mL), and the increase in ethanol concentration sharply decreased this process (0.18±0.001 mg/mL). The same efficiency of the extracts was determined in the case of chicory acid extraction. For chlorogenic acid, the most effective extractant was 50% ethanol (1.16±0.14 mg/mL), and the minimum content was determined when using 80% ethanol (0.29±0.003 mg/mL) and DMSO (0.29±0.002 mg/ mL).

Table 1. **Content of biologically active substances in *Taraxacum officinale***

The extract	X±DS (mg/mL)						
	TFC	TPC	Phytosterols	The sum of hydroxychoric acids	Caftaric acid	Chicory acid	Chlorogenic acid
LDMSO	0.50±0.03	13.88±0.13	0.38±0.01	11.40±0.06	1.48±0.03	8.16±0.03	0.29±0.002
RDMSO	0.60±0.06	4.04±0.22	0.34±0.001	1.07±0.001	0.10±0.001	0.76±0.01	0.11±0.001
LEtOH20	4.61±0.001	31.40±1.08	1.11±0.12	33.80±1.72	5.16±0.11	23.80±1.11	0.88±0.01
REtOH20	1.74±0.1	2.00±0.01	0.59±0.003	1.68±0.03	0.55±0.02	0.65±0.01	0.10±0.001
LEtOH50	6.30±0.07	38.00±1.02	2.51±0.45	48.80±2.22	5.64±0.36	35.00±0.69	1.16±0.14
REtOH50	0.96±0.09	4.86±0.01	0.44±0.01	8.00±0.93	0.74±0.01	5.34±0.41	0.65±0.05
LEtOH80	6.52±0.15	11.52±0.01	2.34±0.40	4.96±0.39	0.18±0.001	2.26±0.11	0.29±0.003
REtOH80	1.28±0.16	8.30±0.42	0.46±0.03	3.14±0.06	0.11±0.002	1.62±0.01	0.54±0.002

Note: TFC – total flavonoid content, TPC – total polyphenol content. Concentration of extracts: LDMSO – 110,000 μg/L, LEtOH20 – 140,000 μg/L, LEtOH50 – 150,000 μg/L, LEtOH80 – 40,000 μg/L, RDMSO – 145,000 μg/L, REtOH20 – 95,000 μg/L, REtOH50 – 60,000 μg/ L and REtOH80 – 50,000 μg/L.

Chemical composition of Taraxacum officinale root extracts. The flavonoids were best extracted when using 20% (REtOH20) (1.74±0.1 mg/mL) and 80% (REtOH80) (1.28±0.16 mg/mL) ethanol, the minimum amount being recorded in the DMSO (RDMSO) extract (0.60±0.06 mg/mL). The total polyphenols content was the highest when using 80% ethanol (8.30±0.42 mg/mL), and the lowest was in the case of 20% ethanol (2.00±0.01 mg/mL) (table 1). The extraction of phytosterols proved to be more efficient in 20% ethanol (0.59±0.003 mg/mL), the minimum concentration being attested when using DMSO (0.34±0.001 mg/mL).

The total amount of hydroxychoric acids was found to be higher when using high-concentration ethanol, 50% (8.00±0.93mg/mL) and 80% (3.14±0.06 mg/mL), and the extraction on DMSO was found to be less effective (1.07±0.001 mg/mL). The extraction of caftaric acid was more efficient when using 50% ethanol (0.74±0.01 mg/mL), the minimum amounts being recorded in the extracts on 80% ethanol (0.11±0.002 mg/mL) and DMSO (0.10±0.001 mg/mL). Chicoric acid was maximally extracted when using 50% ethanol (5.34±0.41 mg/mL), and the

minimum concentration was determined in extracts on 20% ethanol (0.65±0.01 mg/mL) and DMSO (0.76±0.01 mg/ mL). The extraction of chlorogenic acid proved to be more efficient in high concentration ethanol, 50% (0.65±0.05 mg/mL) and 80% (0.54±0.002 mg/mL), and the use of 20% ethanol (0.10±0.001 mg/mL) and DMSO (0.11±0.001 mg/mL) could not be considered as the best choice.

4. THE INFLUENCE OF TARAXACUM OFFICINAL EXTRACTS ON THE ENZYMATIC ANTIOXIDANT SYSTEM AND THIOL-DISULFIDE HOMEOSTASIS

In this study, we evaluated the action of TO extracts on the enzymatic antioxidant system (SOD, CAT, GPx, GST and GR), as well as the content of total and free thiol groups, total glutathione, as well as its fractions, oxidized and reduced. The erythrocytes of healthy people served as the enzyme source. Determination of superoxide dismutase (SOD) activity was performed according to the procedures described by Дубинина Е.Е. (1983) and Разыграев А.В. (2006), in the modification of Tagadiuc *et al.* (2010) [12], of catalase (CAT, μM/gHb) - according to the procedure described by Королюк *et al*. (1988), in our modification [13], of glutathione peroxidase (GPx) – according to the procedure described by Кулинский *et al.* (2007), in the modification of Tagadiuc *et al.* (2012) [14], of glutathione S-transferase (GST) – according to the procedure described by Habig *et al.* (1974), in the version modified by Tagadiuc *et al.* (2012) [15] and glutathione reductase (GR) was performed according to the method described by Кулинский *et al.* (2009), in the modification of Gudumac *et al.* [16]. Total glutathione dosage was performed according to the procedure described by Akerboom *et al.* (1981), in the modification proposed by Andronache *et al.* (2014) [17], of the content of thiol groups of proteins - according to the evaluation procedure described by Ellman *et al.* (1994) in the modification of Gudumac *et al.* (2012), on erythrocyte lysate [18].

Influence of TO extracts on SOD activity (table 2). TOL extracts stimulated SOD activity. The highest activity of SOD (50.71±0.12 u/gHb) was recorded after the action of the extracts made on 20% ethanol (+85.4%, p=0.05) compared to the control. All TOR extracts stimulated SOD activity statistically significantly. The most imposing influence was recorded in the case of the extract on DMSO (50.10±10.73 u/gHb, +83.9%, p=0.02), followed by the extract made on 50% ethanol (27.26±1.81 u/gHb or +54.9%, p = 0.05).

Action of TO extracts on CAT activity. All investigated extracts decreased CAT activity, except for the 80% ethanol extract of the leaves (43.83±1.18 μM/gHb or +15.2%, p=0.05). The correlation study did not record statistically significant associations between CAT activity and ethanol concentration in TOL extracts (r_s=-0.33, p=0.11). The most impressive decrease was determined following the action of the TOR extract on DMSO (26.17±9.84 μM/gHb or -42.3%, p=0.02). CAT activity was inversely proportional to the ethanol concentration used for TOR extracts (r_s=-0.73, p=0.001).

***Influence of TO extracts on GPx activity*.** TOL extracts mainly stimulate the activity of this enzyme, the highest GPx activity being recorded after the influence of the extracts made on DMSO (255.74±19.60 μM/gHb or +46.3%, p=0.05) and 20% ethanol (236.99±24.58 μM/gHb or +45.7%, p=0.05). We determined the most pronounced stimulatory action in the case of TOR on 80% ethanol (221.97±6.37 μM/gHb or +84%, p=0.05). The statistical study revealed a medium, negative association between GPx activity and the concentration of ethanol used in TOL extracts (r_s=-0.52, p=0.02).

Action of TO extracts on GST activity. All the extracts made from TO exerted stimulatory effects. The most promising actions were the extracts of TOL on 80% ethanol (29.71±1.38 μM/gHb or +160%, p=0.05) and that of TOR on DMSO (24.84±2.45 μM/gHb or +72%, p=0.02), followed by the ethanolic ones of 20% (26.39±0.46 μM/gHb; +28.9%, p=0.05) and 50% (23.32±1.89 μM/gHb; +10%, p=0.04).

Table 2. **Activity of enzymes of the antioxidant system and markers of thiol-disulfide homeostasis in relation to the concentration of the tested chemical compounds**

The extract (A)	Enzyme activity (mean)					Chemical compounds (mg/mL)						
	SOD (u/gHb)	CAT (µM/gHb)	GPOx (µM/gHb)	GST (µM/gHb)	GR (µM/gHb)	TFC	TPC	Phytosterols	Hydroxychoric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LDMSO	**27.74**	**6.84**	**255.74**	22.26	**31.41**	0.5	13.9	0.38	11.4	8.16	0.29	1.48
LEtOH20	**50.71**	23.79	236.99	20.09	**10.06**	4.61	31.4	1.11	33.8	23.8	0.88	5.16
LEtOH50	40.79	43.63	**132.71**	26.29	23.95	6.3	38	2.51	48.8	35	1.16	5.64
LEtOH80	38.90	43.83	212.77	**29.71**	22.66	6.52	11.5	2.34	4.96	2.26	0.29	0.18
RDMSO	**50.10**	26.17	184.16	24.84	18.29	0.6	4.04	0.34	1.07	0.76	0.11	0.1
REtOH20	36.08	**48.04**	182.22	26.39	19.20	1.74	2	0.59	1.68	0.65	0.1	0.55
REtOH50	42.23	41.88	141.07	23.32	20.42	0.96	4.86	0.44	8	5.34	0.65	0.74
REtOH80	38.02	41.71	221.97	**16.15**	13.16	1.28	8.3	0.46	3.14	1.62	0.54	0.11
The extract (B)	**Marker (µM/gHb)**					**Chemical compounds (mg/mL)**						
	Free SH	**Total SH**	**Total glutathione**	**GSH**	**GSSG**	**TFC**	**TPC**	**Phytosterols**	**Hydroxychoric acids**	**Chicory acid**	**Chlorogenic acid**	**Caftaric acid**
LDMSO	3.30	**3.79**	22.44	13.98	8.46	0.5	13.88	0.38	11.4	8.16	0.29	1.48
LEtOH20	3.24	3.47	**18.98**	**10.88**	8.11	4.61	31.4	1.11	33.8	23.8	0.88	5.16
LEtOH50	3.05	3.26	23.35	14.62	8.73	6.3	38	2.51	48.8	35	1.16	5.64
LEtOH80	**3.02**	**3.15**	28.40	18.01	10.39	6.52	11.52	2.34	4.96	2.26	0.29	0.18
RDMSO	3.23	3.53	17.34	13.32	**4.02**	0.6	4.04	0.34	1.07	0.76	0.11	0.1
REtOH20	**3.58**	3.72	**33.95**	**28.03**	5.92	1.74	2	0.59	1.68	0.65	0.1	0.55
REtOH50	3.18	3.38	24.79	13.33	**11.46**	0.96	4.86	0.44	8	5.34	0.65	0.74
REtOH80	3.08	3.24	30.93	20.41	10.52	1.28	8.3	0.46	3.14	1.62	0.54	0.11

Note: TFC – total flavonoid content, TPC – total polyphenol content. Minimum and maximum TO extracts activity results have been selected in **bold**. Concentration of extracts: A) LDMSO – 110,000 µg/L, LEtOH20 – 140,000 µg/L, LEtOH50 – 150,000 µg/L, LEtOH80 – 40,000 µg/L, RDMSO – 145,000 µg/L, REtOH20 – 95,000 µg/L, REtOH50 – 60,000 µg/L and REtOH80 – 50,000 µg/L; B) LEtOH20 – 140,000 µg/L, LEtOH50 – 150,000 µg/L, LEtOH80 – 40,000 µg/L, RDMSO – 145,000 µg/L, REtOH20 – 95,000 µg/L, REtOH50 – 60,000 µg/L and REtOH80 – 50,000 µg/L.

The correlation study revealed a very high association between GST activity and ethanol concentration in TOL extracts (r_s=0.94, p=0.0001) and a reasonable, inverse association between GST activity and alcohol concentration used in TOR extracts (r_s= -0.59, p=0.01).

Influence of TO extracts on GR activity. The extracts made from TO leaves achieved both stimulatory and inhibitory action, depending on the type and concentration of the extractant (table 2). The stimulatory effect reached its peak in the case of ethanol extracts of 50% (23.95±2.19 µM/gHb or +34.7%, p=0.05) and 80% (22.66±0.47 µM/gHb or +34.6%, p=0.05). At the same time, inhibitory action was determined after the use of extracts made on low concentration ethanol, of 20% (10.06±1.30 µM/gHb or -50.4%, p=0.05). GR activity proved to be dependent on the concentration of ethanol used in TOL extracts, registering very high statistical associations (r_s=0.81, p=0.0001).

The roots of TO demonstrated a virtually univocal inhibitory action on GR activity (table 2). The strongest influence was detected in the case of the 50% ethanol extract (20.42±0.001 µM/gHb or -31.4%, p=0.04). The only stimulatory effect recorded by TOR was in the case of the 20% ethanol extract (+2.3%), values that did not reach the statistical threshold (p=0.51). Statistical tests revealed reasonable, negative correlations between GR activity and the concentration of the extractant used for TOR (r_s=-0.49, p=0.03).

The influence of TO on the content of SH groups. The extracts from TOL mostly decreased the content of thiols (table 2). The most pronounced, statistically true decrease of free SH groups was attested under the influence of leaf extracts on DMSO (3.30±0.31 µM/gHb or -14.2%, p=0.05), 20% ethanol (3.24±0.03 µM/gHb or -13.1%, p=0.05) followed by ethanol 80% (3.02±0.01 µM/gHb or -6.7%, p=0.05). In the rest of the cases, the values were not statistically different from the control sample.

Similar to the content of free thiols, the amount of total thiols also changed. The most pronounced decrease was determined after the action of the extracts on DMSO (3.79±0.08 µM/gHb or -10%, p=0.05), 20% ethanol (3.47±0.05 µM/gHb or -11.8%, p=0.05) and 80 % (3.15±0.02 µM/gHb or -9.5%, p=0.05) (table 2).

The calculations allowed us to conclude that free -SH represent the majority of total -SH. Thus, as a result of LDMSO action, they represented 87%, of LEtOH20 – 93.3%, LEtOH50 – 93.7% and 95.9% in LEtOH80.

The importance of the ethanol concentration used for the extractions was reported by negative correlation values of medium strength (r_s=-0.55, p=0.02) both in the case of free thiols and in the case of total thiols (r_s=-0.65, p=0.004) .

The influence of root extracts on the content of free thiols was mostly diminutive. The most pronounced decrease was determined under the influence of 50% ethanol extracts (3.18±0.07 µM/gHb or -17.5%, p=0.05) (table 2). In the case of extracts on DMSO and 80% ethanol, the results were not statistically different compared to the control sample.

The total content of –SH groups decreased under the action of root extracts, the most pronounced decrease being attested in the case of 50% ethanol extracts (3.38±0.17 µM/gHb or -19.7%, p=0.05) (table 2). In the case of the extract on DMSO, a decrease in the total content of –SH was also determined, but these values (3.53±0.18 µM/gHb or -6.2%) were not statistically different from the control sample (p=0.15).

Similar to the leaf extracts, free -SH constituted the majority of total thiols calculated in the case of TOR. After RDMSO action they represented 91.4%, REtOH20 – 96.2%, REtOH50 – 93.9% and 94.9% in REtOH80.

The concentration of ethanol used as an extractant proved to be important also in the case of roots, registering negative, statistically true correlations both with respect to the content of free –SH groups (rs=-0.48, p=0.04) and total –SH (r_s=-0.55, p=0.02).

Action of TO extracts on glutathione content. The leaf extracts altered the GSH content in an inhomogeneous manner (Table 2). The active components of the extracts made on 50% (14.62±2.15 µM/gHb or +47.3%, p=0.03) and 80% (18.01±1.02 µM/gHb or +131.5%, p=0.05) ethanol increased

the concentration of GSH compared to the control sample. In the rest of the tested extracts, the GSH content decreased compared to the control, the most obvious being the effects of the extracts on DMSO (13.98±2.82 µM/gHb or -25.5%, p=0.03).

The GSSG content mostly increased following the action of the bioactive components from the leaves, the most active in this respect being the extracts made on DMSO (8.46±3.48 µM/gHb or +123.1%, p=0.05) and 80% ethanol (10.39±3.16 µM/gHb or +144.9%, p=0.05). Although in the case of 50% ethanol extracts a decrease in GSSG content was attested, these values were not statistically different compared to the control sample.

The action of the leaf extracts on the total content of glutathione was a stimulating one, recording maximum values for the action of the extracts made on ethanol of 50% (23.35±2.79 µM/gHb or +22.4%, p=0.05) and 80% (28.40±2.14 µM /gHb or +136.2%, p=0.05) (table 2). It should be noted that, in the rest of the cases, the results were not statistically different compared to the control sample.

The calculations showed that GSH constitutes the majority in all cases: LDMSO – 62.3%, LEtOH20 – 57.3%, LEtOH50 – 62.6% and 63.4% in LEtOH80, without statistical differences between the actions of the extracts (figure 1).

The increase in ethanol concentration proved to be important, causing a synergistic increase in both the oxidized glutathione fraction (r_s=0.47, p=0.04) and the total content (r_s=0.45, p=0.05). In the case of GSSG, the correlation values did not reach the expected statistical threshold (r_s=0.15, p=0.29).

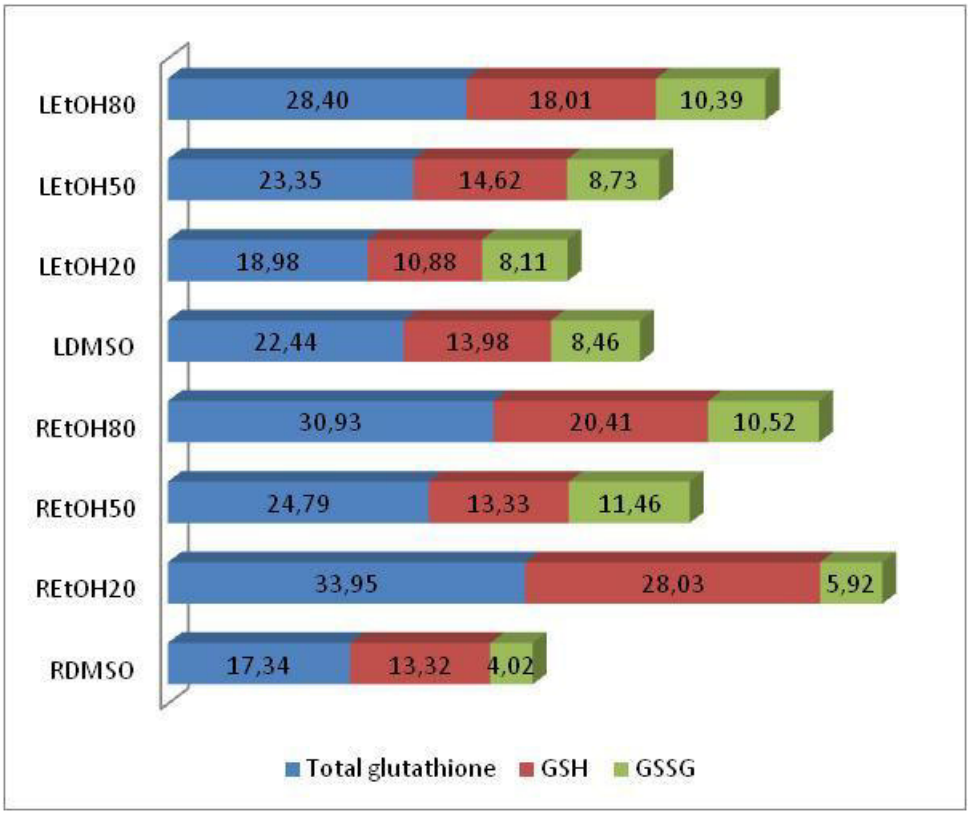

Figure 1. ***Change in the level of glutathione forms as a result of the action of TO extracts.***
Note: results represent means in µM/gHb

The analysis of the results reveals that the root extracts can act both as oxidizing and reducing agents. The amount of GSH increased as a result of the action of the bioactive components from the ethanol extracts of 20% (28.03±1.14 µM/gHb or +98%, p=0.05) and 80% (20.41±0.90 µM/gHb or +105.7%, p =0.05) (table 2). The reduction of GSH was verified under the influence of 50% ethanol extracts (13.33±0.69 µM/gHb or -29%, p=0.05). The values recorded in the case of RDMSO were not statistically different from the control sample. The concentration of GSSG increased, the most impressive being the action of extracts on ethanol of 20% (5.92±0.72 µM/gHb or +91.2%, p=0.05)

and 50% (11.46±3.27 μM/gHb or +202.4%, p =0.05). Total glutathione content increased mostly as a result of the action of roots by TO. The highest concentrations were determined as a result of the action of the extracts on ethanol of 20% (33.95±0.42 μM/gHb or +96.8%, p=0.05) and 80% (30.93±0.40 μM/gHb or +62.1%, p= 0.05) (table 2).

The statistical analysis allowed us to conclude that GSH constituted the majority of the amount of glutathione (figure 1). Thus, after the action of the root extract on DMSO, the percentage share of GSH was 76.8%, after REtOH20 – 82.6%, REtOH50 – 53.8% and 66% in the case of REtOH80.

Unlike the leaf extracts, in the case of the roots no statistically significant correlations were determined between the ethanol concentration and the amount of total glutathione (r_s=-0.15, p=0.29), GSH (r_s=0.01, p=0.48) and GSSG (r_s =-0.19, p=0.25).

5. THE ANTIRADICAL, TOTAL ANTIOXIDANT POTENTIAL AND THE ACTION OF *TARAXACUM OFFICINALE* EXTRACTS ON OXIDATIVE STRESS MARKERS

5.1 Material and methods. The determination of the antiradical activity of the extracts obtained from Taraxacum officinale was carried out by using the radical cation $ABTS^{\cdot+}$ according to Roberta Re *et al.* [2. 3]. As reference substances we used Trolox and Rutin. The reducing activity of study samples was assessed immediately (0 minutes), and after 15 and 30 minutes of incubation. The determination of the antioxidant capacity (TAC) and the total mass of antioxidant substances (MSA) per unit mass in the research samples was carried out based on the method described by Meijuan Zhang *et al.* (2014), in the modification proposed by Tagadiuc *et al.* (2018) [24,25]. The dosage of nitric oxide (NO) metabolites in the samples to be investigated was performed after their deproteinization according to the procedure described by Метельская *et al.* (2005), in the modification of Gudumac *et al.* [18,26,27]. To determine eNOS activity, erythrocyte lysate containing eNOS (endothelial nitric oxide synthase) was initially prepared [28]. With the Griess reagent, the amount of the metabolite of nitric oxide, nitrite, was determined. Its amount served as a measure of enzyme activity. The calculation was performed according to the calibration curve built on the basis of 10 mM sodium nitrite stock dilutions, expressed in μM/L blood serum. Assessment of the intensity of oxidation reactions with free radicals in the samples to be investigated was carried out by dosing the final product of lipid peroxidation (DAM) according to the procedure described by Галактионова *et al.* (1998), in the modification proposed by Gudumac *et al.* (2012) [29].

5.2 Results of the evaluation of the action of Taraxacum officinale on the antiradical potential, total antioxidant and markers of oxidative stress. Upon immediate measurement, the most active samples were: Trolox 15x105 μg/L (92.45%, SD – 0.39, 95% CI: 91.69-93.21), Trolox 75x104 μg/L (91.93%, SD – 3.56, 95% CI: 84.95-98.91), REtOH50 15,000 μg/L (91.81%, SD – 1.01, 95% CI: 89.83-93.79), Trolox 3x106 μg/L (91.57%, SD – 3.43, 95% CI: 84.85-98.29), REtOH50 30,000 μg/L (91.04%, SD – 2.1, 95% CI: 86.92-95.16) and Rutin 3x106 μg/L (90.64%, SD – 2.05, 95% CI: 86.62-94.66) (table 3). The least effective were REtOH20 of 742 μg/L (1.89%, SD – 0.26, 95% CI: 1.38-2.4) and Rutin 46.88 x103 μg/L (1.78%, SD – 0.25, 95% CI: 1.29-2.27).

After 30 minutes of incubation, among the leaders in reducing the radical ABTS with Trolox and Rutina were the root extracts REtOH50 7,500 μg/L (93.81%, SD – 1.18, 95% CI: 91.5-96.12), REtOH50 15,000 μg/L (91.47%, SD – 1.77, 95% CI: 88-94.94), RDMSO 72,500 μg/L (91.8%, SD – 2.19, 95% CI: 87.51-96.09), REtOH50 30,000 μg/L (91.98%, SD – 1.86 , 95% CI: 88.33-95.63) and RDMSO 36,250 μg/L (91.61%, SD – 0.16, 95% CI: 91.30-91.92) (table 3).

In the comparative analysis, overall the most effective extracts from TO, able to compete with reference substances, were REtOH50 at 30 minutes and 7,500 μg/L (93.81%, SD – 1.18, 95% CI: 91.5-96.12), as well as and REtOH50 15 minutes 7,500 μg/L (93.51%, SD – 1.03, 95% CI: 91.49-95.53). Root extracts on DMSO, 20% and 50% ethanol were the least effective, along with Trolox and Rutin, at various incubation time intervals (table 3).

Table 3. **Antiradical activity of TO extracts in relation to the concentration of the compounds**

The extract	TO dilution (µg/L)	ABTS inhibition (%)				Chemical compounds (mg/mL)						
		0 min	15 min	30 min	Mean	TFC	TPC	Phytosterols	Hydroxycho ric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LDMSO	55,000	59.30	58.86	55.25	57.80	0.14	3.82	0.1	1.57	1.12	0.04	0.2
	27,500	70.28	69.20	62.52	67.33	0.07	1.91	0.05	0.78	0.56	0.02	0.1
	13,750	75.63	77.29	78.52	77.14	0.03	0.95	0.03	0.39	0.28	0.01	0.05
	6,875	84.95	83.91	82.11	83.66	0.02	0.48	0.01	0.2	0.14	0.005	0.03
	3,438	86.81	89.74	88.08	88.21	0.01	0.24	0.01	0.1	0.07	0.002	0.01
	1,719	41.41	69.67	81.69	64.26	0.004	0.12	0.003	0.05	0.04	0.001	0.01
	859	16.91	31.68	37.92	28.84	0.002	0.06	0.002	0.02	0.02	0.001	0.003
LEtOH20	70,000	77.91	86.27	84.05	82.74	1.61	10.99	5.92	0.39	4.17	0.15	0.9
	35,000	88.84	85.92	83.57	86.11	0.81	5.5	2.96	2.96	2.08	0.08	0.45
	17,500	90.48	89.01	87.36	88.95	0.4	2.75	1.48	1.48	1.04	0.04	0.23
	8,750	89.78	87.27	85.08	87.38	0.2	1.37	0.74	0.74	0.52	0.02	0.11
	4,375	89.54	89.32	88.00	88.95	0.1	0.69	0.37	0.37	0.26	0.01	0.06
	2,188	66.90	87.41	84.23	79.51	0.05	0.34	0.18	0.18	0.13	0.005	0.03
	1,094	25.10	47.64	58.87	43.87	0.03	0.17	0.09	0.09	0.07	0.002	0.01
LEtOH50	75,000	72.44	34.15	10.71	39.1	2.36	14.25	0.94	9.15	6.56	0.22	1.06
	37,500	79.90	49.04	12.23	47.06	1.18	7.13	0.47	4.58	3.28	0.11	0.53
	18,750	82.90	59.13	44.00	62.01	0.59	3.56	0.24	2.29	1.64	0.05	0.26
	9,375	83.78	64.80	30.64	59.74	0.3	1.78	0.12	1.14	0.82	0.03	0.13
	4,688	87.09	71.96	39.00	66.02	0.15	0.89	0.06	0.57	0.41	0.01	0.07

Table 3. **Continued**

The extract	TO dilution (µg/L)	ABTS inhibition (%)				Chemical compounds (mg/mL)						
		0 min	15 min	30 min	Mean	TFC	TPC	Phytosterols	Hydroxycho ric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LEtOH50	2,344	86.41	68.71	33.06	62.72	0.07	0.45	0.03	0.29	0.21	0.01	0.03
	1,172	63.48	66.56	28.19	52.74	0.04	0.22	0.01	0.14	0.1	0.003	0.02
LEtOH80	20,000	44.15	33.10	40.92	39.39	0.65	1.15	0.23	0.25	0.11	0.01	0.01
	10,000	56.58	50.27	53.08	53.31	0.33	0.58	0.12	0.12	0.06	0.01	0.004
	5,000	73.48	70.61	74.99	73.03	0.16	0.29	0.06	0.06	0.03	0.004	0.002
	2,500	68.08	75.40	79.63	74.37	0.08	0.14	0.03	0.03	0.01	0.002	0.001
	1,250	45.66	83.67	87.61	72.31	0.04	0.07	0.01	0.02	0.01	0.001	0.001
	625	24.27	54.54	62.23	47.01	0.02	0.04	0.01	0.01	0.004	0.0004	0.0003
	312,5	7.59	14.81	16.67	13.02	0.01	0.02	0.004	0.004	0.002	0.0002	0.0001
RDMSO	72,500	64.20	91.94	91.80	82.65	0.22	1.46	0.12	0.19	0.14	0.02	0.02
	36,250	66.87	91.90	91.61	83.46	0.11	0.73	0.06	0.1	0.07	0.01	0.01
	18,125	46.31	82.07	90.45	72.94	0.05	0.37	0.03	0.05	0.03	0.005	0.004
	9,063	28.64	55.99	63.76	49.46	0.03	0.18	0.02	0.02	0.02	0.002	0.002
	4,531	12.47	29.40	35.68	25.85	0.01	0.09	0.01	0.01	0.01	0.001	0.001
	2,266	7.53	9.71	14.54	10.59	0.01	0.05	0.004	0.01	0.004	0.001	0.001
	1,133	4.64	**1.47**	**1.80**	**2.63**	0.003	0.02	0.002	0.003	0.002	0.0003	0.0003
REtOH20	47,500	89.77	85.84	85.93	87.18	0.41	0.48	0.2	0.14	0.08	0.01	0.07
	23,750	75.74	88.48	87.74	83.98	0.21	0.24	0.1	0.1	0.04	0.01	0.03
	11,875	52.96	80.14	86.38	73.16	0.1	0.12	0.05	0.05	0.02	0.003	0.02

Table 3. **Continued**

The extract	TO dilution (µg/L)	ABTS inhibition (%)				Chemical compounds (mg/mL)						
		0 min	15 min	30 min	Mean	TFC	TPC	Phytosterols	Hydroxycho ric acids	Chicory acid	Chlorogenic acid	Caftaric acid
REtOH20	5,938	23.29	32.82	45.78	33.96	0.05	0.06	0.02	0.02	0.01	0.002	0.01
	2,969	4.51	2.13	27.59	11.41	0.03	0.03	0.01	0.01	0.005	0.001	0.004
	1,484	6.04	5.65	8.69	6.79	0.01	0.01	0.01	0.01	0.002	0.0004	0.002
	742	**1.89**	20.20	3.71	8.6	0.01	0.01	0.003	0.003	0.001	0.0002	0.001
REtOH50	30,000	91.04	91.93	91.98	**91.65**	0.14	0.73	0.07	0.6	0.4	0.05	0.06
	15,000	**91.81**	92.01	91.47	**91.76**	0.07	0.36	0.03	0.3	0.2	0.02	0.03
	7,500	72.11	**93.51**	**93.81**	86.47	0.04	0.18	0.02	0.15	0.1	0.01	0.01
	3,750	36.60	69.90	80.43	62.31	0.02	0.09	0.01	0.07	0.05	0.01	0.01
	1,875	17.97	38.30	48.45	34.91	0.01	0.05	0.004	0.04	0.03	0.003	0.003
	938	4.68	5.46	5.53	5.22	0.005	0.02	0.002	0.02	0.01	0.002	0.002
	469	3.13	1.63	3.12	**2.63**	0.002	0.01	0.001	0.01	0.01	0.001	0.001
REtOH80	25,000	48.89	43.33	42.15	44.79	0.16	1.04	0.06	0.2	0.1	0.03	0.01
	12,500	62.83	54.15	51.84	56.28	0.08	0.52	0.03	0.1	0.05	0.02	0.003
	6,250	74.51	77.36	76.19	76.02	0.04	0.26	0.01	0.05	0.03	0.01	0.002
	3,125	47.61	83.29	83.01	71.3	0.02	0.13	0.01	0.02	0.01	0.004	0.001
	1,563	35.63	72.36	80.82	62.94	0.01	0.06	0.004	0.01	0.01	0.002	0.0004
	781	18.74	42.38	48.36	36.5	0.005	0.03	0.002	0.01	0.003	0.001	0.0002
	391	6.35	11.67	12.68	10.23	0.003	0.02	0.001	0.003	0.002	0.001	0.0001

Note: TFC – total flavonoid content, TPC – total polyphenol content. Minimum and maximum TO extracts activity results have been selected in **bold.**

The importance of ethanol concentration in the ABTS inhibition process was evaluated by the statistical correlation method. Thus, in the case of the leaf extracts, with the increase in the concentration of ethanol, the decrease in the ability to reduce the ABTS radical was attested, both at the immediate measurement (r_s=-0.60, p=0.0001) and after 15 (r_s=-0.57, p=0.001) and 30 (r_s=-0.36, p=0.001) minutes of sample incubation. However, in the case of root extracts, no statistically significant associations were detected, both at the immediate measurement (r_s=0.12, p=0.18), and after 15 (r_s=0.09, p=0.25) and 30 minutes (r_s=0.02, p=0.44).

5.2.2 Total antioxidant potential of TO extracts. ***Influence of TO extracts on total antioxidant capacity (TAC).*** The TAC evaluation of the leaf extracts of TO revealed that only the extract on DMSO increased statistically significantly (13.16±0.39 u/c or +5.5%, p=0.05) this indicator compared to Trolox (12.48±0.07 u/c) (table 4).

In the case of TOR, extracts based on ethyl alcohol of 20% (12.16±0.21 or +4.2%) and 80% (12.80±0.24 or +0.5%) stimulated TAC capacity compared to the control sample (table 4). Conversely, the TAC value decreased under the influence of root extracts on DMSO (12.49±0.47 or -0.1%) and 50% ethanol (12.46±0.13 or -0.1%). Of all the analyzed extracts, only the 20% ethanol extract reached the expected statistical threshold (p=0.05). The correlation study between the concentration of ethanol used in TOR and TAC extracts revealed strong, statistically significant positive associations (r_s=0.85, p=0.001).

The action of TO extracts on the mass of antioxidant substances (MSA). The leaf extracts mostly induced the increase of MSA (Table 4). However, the stimulating effect was statistically different from the control sample only in the case of the 50% ethanol extract (1.68±0.00 or +0.2%, p=0.05). The decrease of MSA was verified as a result of the action of the extracts on 80% ethanol (1.67±0.00 or -0.4%), a value that did not reach the expected statistical threshold (p=0.28).

Table 4. **Total antioxidant capacity (TAC), mass of antioxidant substances (MSA) and average antioxidant activity (AMA) according to the concentration of the tested chemical compounds**

The extract	Marker (u/c)			Chemical compounds (mg/mL)						
	TAC	MSA	AMA	TFC	TPC	Phytosterols	Hydroxycho ric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LDMSO	**13.16**	**1.75**	7.51	0.5	13.88	0.38	11.4	8.16	0.29	1.48
LEtOH20	12.70	1.75	**7.27**	4.61	31.4	1.11	33.8	23.8	0.88	5.16
LEtOH50	12.73	1.68	7.56	6.3	38	2.51	48.8	35	1.16	5.64
LEtOH80	13.10	1.67	**7.87**	6.52	11.52	2.34	4.96	2.26	0.29	0.18
RDMSO	12.49	**1.65**	7.57	0.6	4.04	0.34	1.07	0.76	0.11	0.1
REtOH20	**12.16**	1.65	7.35	1.74	2	0.59	1.68	0.65	0.1	0.55
REtOH50	12.46	1.69	7.38	0.96	4.86	0.44	8	5.34	0.65	0.74
REtOH80	12.80	1.67	7.68	1.28	8.3	0.46	3.14	1.62	0.54	0.11

Note: TFC – total flavonoid content, TPC – total polyphenol content. Minimum and maximum TO extracts activity results have been selected in **bold**. Concentration of extracts: LDMSO – 110,000 µg/L, LEtOH20 – 140,000 µg/L, LEtOH50 – 150,000 µg/L, LEtOH80 – 40,000 µg/L, RDMSO – 145,000 µg/L, REtOH20 – 95,000 µg/L, REtOH50 – 60,000 µg/ L and REtOH80 – 50,000 µg/L.

Root extracts have been shown to be effective, the action on MSA being dependent on the type and concentration of the extractant. The increase in MSA was verified as a result of the action of the extract on 20% ethanol (1.65±0.01 or +1.2%), reaching the statistical threshold (p=0.05) (table 4). In the case of the other extracts, the reduction of MSA values was determined compared

to the control sample, values statistically different from the control being in the case of roots extracted on 80% ethanol (1.67±0.0 or -0.7%, p=0.05).

The concentration of ethanol used in TOR extracts had a directly proportional effect on MSA, establishing positive statistical associations (r_s=0.55, p=0.02), while in those of TOL they were not statistically true.

Influence of TO extracts on the average antioxidant activity (AMA). No statistically significant changes were attested compared to the AMA control in the action of TOL extracts (table 4). The concentration of the extractant proved to have an influence on AMA, establishing positive, statistically true associations (r_s=0.51, p=0.02).

Evaluating the action of TOR on AMA we determined that the average antioxidant activity increased in most cases (table 4). The greatest increase, of the average antioxidant activity, statistically veridical, was attested following the action of the 20% ethanolic extract (7.35±0.10 or +3%). AMA activity proved to be dependent on the ethanol concentration used in the study, establishing positive associations (r_s=0.69, p=0.002).

5.2.3 The result of the action of *Taraxacum officinale* extracts on markers of oxidative stress. *Influence of TO extracts on DAM*. The statistical evaluation of the results revealed that the leaf extract on DMSO considerably decreased the level of DAM 0.81±0.00 µM/gHb, which represented -8.1% compared to the control (p=0.05) (table 5). The 50% ethanolic extract (0.83±0.00 µM/gHb or -3.6% vs. control, p=0.05) was the only ethanolic leaf extract that decreased DAM concentration. Both mentioned extracts produced changes that reached the proposed statistical threshold (p<0.05).

Table 5. **The value of oxidative stress markers in relation to the level of the tested chemical compounds**

The extract	Marker						Chemical compounds (mg/mL)						
	DAM (µM/gHb)	NOS (µM/L)	NO_2^- (µM/gHb)	NO_3^- (µM/gHb)	NO_2^-/NO_3^-	$NO_2^-+NO_3^-$ (µM/gHb)	TFC	TPC	Phytosterols	Hydroxychloric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LDMSO	0.81	2.98	1.75	1.33	1.32	3.08	0.5	13.88	0.38	11.4	8.16	0.29	1.48
LEtOH20	**0.70**	**2.15**	1.84	1.02	1.87	2.86	4.61	31.4	1.11	33.8	23.8	0.88	5.16
LEtOH50	0.83	**3.63**	1.87	1.35	1.41	**3.22**	6.3	38	2.51	48.8	35	1.16	5.64
LEtOH80	0.88	2.77	**1.51**	1.28	**1.18**	2.79	6.52	11.52	2.34	4.96	2.26	0.29	0.18
RDMSO	0.75	2.57	1.84	**0.92**	**2.07**	**2.76**	0.6	4.04	0.34	1.07	0.76	0.11	0.1
REtOH20	**0.97**	2.52	**2.05**	1.10	1.87	3.15	1.74	2	0.59	1.68	0.65	0.1	0.55
REtOH50	**0.97**	3.24	1.72	1.36	1.26	3.08	0.96	4.86	0.44	8	5.34	0.65	0.74
REtOH80	0.92	2.81	1.70	**1.51**	**1.18**	3.21	1.28	8.3	0.46	3.14	1.62	0.54	0.11

Note: TFC – total flavonoid content, TPC – total polyphenol content. Minimum and maximum TO extracts activity results have been selected in **bold**. Concentration of extracts: LDMSO – 110,000 µg/L, LEtOH20 – 140,000 µg/L, LEtOH50 – 150,000 µg/L, LEtOH80 – 40,000 µg/L, RDMSO – 145,000 µg/L, REtOH20 – 95,000 µg/L, REtOH50 – 60,000 µg/ L and REtOH80 – 50,000 µg/L.

The increase in DAM content was determined following the action of TOL extracts made on 80% ethanol (+9.6%) at a p=0.05. In the case of all extracts from TOR, increased values of DAM were recorded, all reaching the expected statistical threshold.

Action of TO extracts on nitric oxide (NO) metabolites. The TOL extracts made on 80% ethanol and on DMSO significantly decreased the level of NO_2^- compared to the control, by -19.5% (p=0.05) and -13.7% (p=0.05) respectively (table 5). The increase in ethanol concentration correlated negatively with the nitrite content (r_s=-0.6, p=0.009). All analyzed TOR extracts

decreased the NO_2^- content compared to the control. In the case of extracts on DMSO (1.84±0.10 or -3.2%), 20% ethanol (2.05±0.04 or -12%) and 50% (1.72±0.02 or -15.2%) values were statistically different from the control (p =0.02, p=0.05, p=0.05). Ethanol concentration showed negative associations with nitrite content (r_s=-0.57, p=0.013).

The concentration of nitrates (NO_3^-) increased abundantly and statistically significantly as a result of the action of TO leaf extracts on ethanol of 50% (1.35±0.15 or +67.7%, p=0.05), 80% (1.28±0.02 or +79.4, p=0.05) and DMSO (1.33±0.11 or +26.7%, p=0.05) (table 5). Unlike nitrites, the content of nitrates proved to be directly dependent on the concentration of ethanol (r_s=0.67, p=0.003). TO root extracts spectacularly increased nitrate levels in the case of ethanolic extracts of 20% (1.10±0.10 μM/ gHb or +46.4%, p=0.05), 50% (1.36±0.02 or +29.6%, p=0.05) and 80% (1.51±0.25 or +86.9%, p=0.05) and in the case of roots determining a positive correlation between nitrate content and ethanol concentration (r_s=0.74, p=0.001).

During the action of TOL, the total concentration of NO_2^-+ NO_3^- increased as a result of the action of all extracts, however, statistically significant values were determined only in the case of 20% ethanolic extracts (2.86±0.07 or +6.5% *vs* control, p=0.05), 50% (3.22±0.04 or +22.8%, p=0.05) and 80% (2.79±0.03 or +7.7%, p=0.05). When evaluating the summary content of NO_2^- and NO_3^- as a result of the action of TOR, it was established that the influence of the roots was an inhomogeneous one. The stimulatory effect of the roots on the summary content of NO_2^- and NO_3^- was detected in the case of 80% ethanol extracts (3.21±0.04 or +22.4%, p=0.05). In the rest of the cases, the differences with the control samples did not reach statistically significant values. The summary content of nitrites and nitrates showed positive correlations with ethanol concentration (r_s=0.56, p=0.01).

The ratio between nitrites and nitrates decreased statistically significantly compared to the control after the action of leaf extracts in ethanol of 50% (1.41±0.29 or -37.5%, p=0.05), 80% (1.18±0.06 or -57.7%, p=0.05) and DMSO (1.32±0.12 or -32.4%, p=0.05). This ratio showed statistically significant negative associations with ethanol concentration (r_s=-0.6, p=0.001). TOL decreased the ratio between nitrites and nitrates statistically significantly under the action of all ethanolic extracts (20% – 1.87±0.20 or -40.3%; 50% – 1.26±0.03 or -37.5%; 80% – 1.18±0.33 or – 47.7%; p=0.05 in all cases). In the case of roots, the ratio between nitrites and nitrates showed negative, statistically true associations with ethanol concentration (r_s=-0.87, p=0.0001).

Evaluation of TO action on nitric oxide synthase (eNOS) activity. eNOS activity increases following the influence of TOL extracts, made on 50% ethanol (3.63±0.20 or +28.6%, p=0.05) and DMSO (2.98±0.07 or +19.2%, p=0.05) and decreases after the action of the extract of 20% (2.15±0.06 or -9.8%, p=0.05). The activity was moderately dependent on the ethanol concentration (r_s=0.45, p=0.05). The activity of eNOS at the action of TOR increased following the action of the 50% ethanolic extracts (3.24±0.05 or +29.6%, p=0.05) and on DMSO (2.57±0.12 or +7.7%, p=0.04). In the case of roots, eNOS activity was also dependent on ethanol concentration (r_s=0.74, p=0.0001).

6. THE ACTION OF *TARAXACUM OFFICINALE* EXTRACTS ON CELL VIABILITY

6.1 Material and methods. Investigation of the antitumor activity of the tested compounds on human glioma cells U-251 MG (Cell Lines Service, DE) was carried out by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium) and Resazurin assays. The biological activities of the tested compounds (TO extracts) were compared with the antitumor activity of Doxorubicin ((7S, 9S)-7-[(2R, 4S, 5S, 6S)-4-amino-5-hydroxy-6-methyloxane- 2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione or Doxo) as reference compound. Prior to use, stock solutions of Doxo (105 and 54,000 μg/L) were diluted, depending on the type of the experiment, with cell culture medium or saline to the required concentrations.

6.2 The result of the action of reference compounds and *Taraxacum officinale* extracts on cell viability.

The MTT test. Action of Doxorubicin. In the case of this experiment, we used Doxo of 10^5 µg/L, later successively diluted 7 times (table 6). The most pronounced antitumor activity was determined when using the stock solution of 10^5 µg/L (70.61%, SD – 15.66, 95% CI: 39.92-101.3). The decrease in the concentration of the drug in question led to an increase in tumor cell viability, results supported by the correlation test (r_s=-0.67, p=0.000). The mean inhibitory concentration in the case of Doxo was equivalent to 49.76 µg/L.

TOL action. In the case of the leaf extract on DMSO, the most promising antitumor action was determined at the concentration of 110,000 µg/L (58.85%, SD – 9, 95% CI: 39.35-78.35) (table 6). In the case of the 20% ethanolic extract, the most pronounced inhibition of tumor viability was determined at the concentration of 140,000 µg/L (76.6%, SD – 6.22, 95% CI: 64.41-88.79). When using the leaf extract on 50% ethanol, viability was most strongly decreased at the concentration of 150,000 µg/L (36.6%, SD – 4.92, 95% CI: 26.96-46.24). Similar to the previous extracts, when using TO leaves on 80% ethanol, the most promising antitumor activity was detected at the maximum concentration of 40,000 µg/L (44.98%, SD – 5.21, 95% CI: 34.77-55.19). The importance of extractant concentration was proven by establishing negative, statistically true associations between ethanol concentration and tumor viability (r_s=-0.51, p=0.000).

TOR action. The most promising antitumor action in the case of root extracts on DMSO was established at the concentration of 145,000 µg/L (72.04%, SD – 2.7, 95% CI: 66.75-77.33) (table 6). In the case of the 20% ethanolic extract, the most obvious inhibition of tumor viability up to 76.81% (SD – 6.11, 95% CI: 64.83-88.79) was established when using the 95,000 µg/L extract. When using the 50% ethanolic extract, the most pronounced inhibitory activity was determined at the concentration of 60,000 µg/L (84.86%, SD – 1.61, 95% CI: 81.70-88.02). In the case of ethanol of maximum concentration, 80%, the most promising antitumor activity was determined at the concentration of 50,000 µg/L (48.61%, SD – 3.89, 95% CI: 40.99-56.23). The importance of the extractant concentration was supported by establishing negative associations between the concentration of ethanol used in the experiment and tumor viability (r_s=-0.30, p=0.004).

When arranging the extracts and reference substances according to the antitumor activity (concentration – viability) we determined that Doxo is the 12th, after the extracts from TO: LEtOH50 (150,000 µg/L – 36.60%) – LEtOH80 (40,000 µg/L – 44.98 %) – RetOH80 (50,000 µg/L – 48.61%) – LEtOH80 (320 µg/L – 51.75%) – LEtOH80 (8,000 µg/L – 51.90%) – LEtOH80 (1,600 µg/L – 52.55%) – LEtOH80 (64 µg/L – 53.73%) – LDMSO (110,000 µg/L – 58.85%) – LDMSO (22,000 µg/L – 68.80%) – LEtOH80 (12.8 µg/L – 69.02%) – REtOH80 (10,000 µg/L – 69.26%) – Doxo100 (100,000 µg/L – 70.61%). The least active solution in the case of this type of tumors were the root extracts on DMSO at the concentrations of 9.28 µg/L (96.22%) and 1.86 µg/L (97.40%).

When comparing the IC50 values, we determined that the lowest average inhibitory dose was in the case of the leaf extract on 80% ethanol (4.36 µg/L), and the highest in the case of the leaf extract on 50% ethanol (70.99 µg/L).

Resazurin method. Doxo action. Tumor cell viability decreased most pronounced (59.52%, SD –5.92 , 95% CI: 47.92-71.12) when using the highest concentration of Doxo.

TOL action. A drastic decrease in the viability of tumor cells was attested when using the leaf extract on DMSO, at the concentration of 110,000 µg/L (6.15%, SD – 1.69, 95% CI: 2.84-9.46) (table 6). In the case of the leaf extract on 20% ethanol, the most pronounced inhibition of tumor viability was determined at the concentration of 140,000 µg/L (54.95%, SD – 8.43, 95% CI: 38.43-71.47).

Table 6. **Antitumor activity of TO extracts depending on the concentration of the compounds**

The extract	Dilution (µg/L)	MTT	Resazurin	Chemical compounds (mg*10^6 /mL)						
		Viability (Mean)		TFC	TPC	Phytosterols	Hydroxychoric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LDMSO	110,000	58.85	**6.15**	550	15,268	1,628	12,540	316.8	316.8	8,976
LDMSO	22,000	68.8	57.72	110	3,053.6	325.6	2,508	63.4	63.4	1,795.2
LDMSO	4,400	79.21	83.31	22	610.7	65.1	501.6	12.7	12.7	359
LDMSO	880	79.72	85.52	4.4	122.1	13	100.3	2.5	2.5	71.8
LDMSO	176	83.26	84.26	0.9	24.4	2.6	20.1	0.5	0.5	14.4
LDMSO	35.2	83.3	87.78	0.2	4.9	0.5	4	0.1	0.1	2.9
LDMSO	7.04	84.54	88.55	0.04	0.98	0.1	0.8	0.02	0.02	0.57
LDMSO	1.408	90.31	90.98	0.01	0.2	0.02	0.16	0.004	0.004	0.11
LEtOH20	140,000	76.6	54.95	6,454	43,960	7,224	47,320	1,232	1232	33,320
LEtOH20	28,000	80.47	76.48	1,290.8	8,792	1,444.8	9,464	246.4	246.4	6,664
LEtOH20	5,600	86.06	85	258.2	1,758.4	289	1,892.8	49.3	49.3	1,332.8
LEtOH20	1,120	87.13	85.08	51.6	351.7	57.8	378.6	9.9	9.9	266.6
LEtOH20	224	89.78	82.59	10.3	70.3	11.6	75.7	2	2	53.3
LEtOH20	44.8	91.87	86.94	2.1	14.1	2.3	15.1	0.4	0.4	10.7
LEtOH20	8.96	92.55	94.9	0.4	2.8	0.5	3.03	0.08	0.08	2.1
LEtOH20	1.792	95.6	**99.74**	0.1	0.6	0.09	0.6	0.02	0.02	0.4

Table 6. **Continued**

The extract	Dilution (µg/L)	MTT	Resazurin	Chemical compounds (mg*10^6 /mL)						
		Viability (Mean)		TFC	TPC	Phytosterols	Hydroxychoric acids	Chicory acid	Chlorogenic acid	Caftaric acid
LEtOH50	150,000	**36.6**	79.96	9,450	57,000	8,460	73,200	1,746	1,746	52,500
LEtOH50	30,000	74.09	88.02	1,890	11,400	753	14,640	10,500	349.2	1,692
LEtOH50	6,000	85.77	84.83	378	2,280	150.6	2,928	2,100	69.8	338.4
LEtOH50	1,200	89.84	88.69	75.6	456	30.1	585.6	420	14	67.7
LEtOH50	240	91.53	97.76	15.1	91.2	6	117.1	84	2.8	13.5
LEtOH50	48	89.5	95.11	3	18.2	1.2	23.4	16.8	0.6	2.7
LEtOH50	96	91.52	97.67	6	36.5	2.4	46.8	33.6	1.1	5.4
LEtOH50	1.92	91.56	96.71	0.1	0.7	0.05	0.9	0.7	0.02	0.1
LEtOH80	40,000	44.98	26.39	2,608	4,608	936	1,984	904	114.4	70.4
LEtOH80	8,000	51.9	75.19	521.6	921.6	187.2	396.8	180.8	22.9	14.1
LEtOH80	1,600	52.55	78.71	104.3	184.3	37.4	79.4	36.2	4.6	2.8
LEtOH80	320	51.75	81.15	20.9	36.9	7.5	15.9	7.2	0.9	0.6
LEtOH80	64	53.73	80.29	4.2	7.4	1.5	3.2	1.4	0.2	0.1
LEtOH80	12.8	69.02	84.91	0.8	1.5	0.3	0.6	0.3	0.04	0.02
LEtOH80	2.56	85.81	85.17	0.2	0.3	0.1	0.1	0.1	0.01	0.005
LEtOH80	0.512	94.12	85.97	0.03	0.1	0.01	0.03	0.01	0.001	0.001

Table 6. **Continued**

The extract	Dilution (µg/L)	MTT	Resazurin	Chemical compounds (mg*10^6 /mL)						
		Viability (Mean)		TFC	TPC	Phytosterols	Hydroxychoric acids	Chicory acid	Chlorogenic acid	Caftaric acid
RDMSO	145,000	72.04	16.42	870	5,858	493	1,557.3	1,096.2	159.5	139.2
RDMSO	29,000	92.7	34.64	174	1,171.6	98.6	311.5	219.2	31.9	27.8
RDMSO	5,800	90.76	66.84	34.8	234.3	19.7	62.3	43.8	6.4	5.6
RDMSO	1,160	92.47	81.15	7	46.9	3.9	12.5	8.8	1.3	1.1
RDMSO	232	93.95	83.08	1.4	9.4	0.8	2.5	1.8	0.3	0.2
RDMSO	46.4	95.2	84.72	0.3	1.9	0.2	0.5	0.4	0.1	0.04
RDMSO	9.28	96.22	89.44	0.1	0.4	0.03	0.1	0.1	0.01	0.01
RDMSO	1.856	**97.4**	**94.01**	0.01	0.1	0.01	0.02	0.01	0.002	0.002
REtOH20	95,000	76.81	65.32	1653	1900	560.5	1596	615.6	98.8	524.4
REtOH20	19,000	86.85	58.34	330.6	380	112.1	319.2	123.1	19.8	104.9
REtOH20	3,800	88.76	60.76	66.1	76	22.4	63.8	24.6	4	21
REtOH20	760	89.75	64.97	13.2	15.2	4.5	12.8	4.9	0.8	4.2
REtOH20	512	91.11	68.09	8.9	10.2	3	8.6	3.3	0.5	2.8
REtOH20	30.4	92.17	68.1	0.5	0.6	0.2	0.5	0.2	0.03	0.2
REtOH20	6.08	92.81	73.78	0.1	0.1	0.04	0.1	0.04	0.01	0.03
REtOH20	1.216	95.67	86.66	0.02	0.02	0.01	0.02	0.01	0.001	0.01

Table 6. **Continued**

The extract	Dilution (µg/L)	MTT	Resazurin	Chemical compounds (mg*10^6 /mL)						
		Viability (Mean)		TFC	TPC	Phytosterols	Hydroxychoric acids	Chicory acid	Chlorogenic acid	Caftaric acid
REtOH50	60,000	84.86	10.83	576	2,916	264	4,797.6	3,206.4	388.8	446.4
REtOH50	12,000	87.51	70.55	115.2	583.2	52.8	959.5	641.3	77.8	89.3
REtOH50	2,400	90.49	74.34	23	116.6	10.6	191.9	128.3	15.6	17.9
REtOH50	480	91.01	77.95	4.6	23.3	2.1	38.4	25.7	3.1	3.6
REtOH50	96	92.45	75.38	0.9	4.7	0.4	7.7	5.1	0.6	5.1
REtOH50	19.2	94.76	76.67	0.2	0.9	0.1	1.5	1	0.1	1
REtOH50	3.84	96.04	86.41	0.04	0.2	0.02	0.3	0.2	0.02	0.2
REtOH50	0.768	83.3	97.29	0.01	0.04	0.003	0.1	0.04	0.005	0.04
REtOH80	50,000	48.61	37.6	640	4,150	230	1,570	810	269	810
REtOH80	10,000	69.26	61.59	128	830	46	314	162	53.8	162
REtOH80	2,000	73.53	68.84	25.6	166	9.2	62.8	32.4	10.8	32.4
REtOH80	400	74.62	74.23	5.1	33.2	1.8	12.6	6.5	2.2	6.5
REtOH80	80	91.3	76.89	1	6.6	0.4	2.5	1.3	0.4	1.3
REtOH80	16	89.48	83.75	0.2	1.3	0.1	0.5	0.3	0.1	0.3
REtOH80	3.2	91.71	79.33	0	0.3	0.01	0.1	0.1	0	0.1
REtOH80	0.64	93.77	86.03	0	0.1	0.003	0.02	0.01	0.003	0.01

Note: TFC – total flavonoid content, TPC – total polyphenol content. Minimum and maximum TO extracts activity results have been selected in **bold**.

When using the leaf extract on 50% ethanol, the viability of about 20% of the cells (79.96±0%) was inhibited at the concentration of 150,000 µg/L. For the leaf extract on 80% ethanol, the most pronounced antitumor action was determined at the concentration of 40,000 µg/L, cell viability decreasing to 26.39% (SD – 5.33, 95% CI: 15.94-36.84). Increasing ethanol concentration had a significant, negative effect on cell viability (r_s=-0.28, p=0.03).

TOR action. In the case of root extracts on DMSO, the most pronounced suppression of cell viability was attested at the extract concentration of 145,000 µg/L (16.42%, SD – 6.36, 95% CI: 3.95-28.89). When using root extracts on 20% ethanol, the most marked inhibition of tumor viability was determined not at the maximum concentration of 95,000 µg/L (65.32%, SD – 2.34, 95% CI: 60.73-69.91), but at the dilution of second, equivalent to 19,000 µg/L (58.34%, SD – 0.04, 95% CI: 58.26-58.42). In the case of the extract made in 50% ethanol, the most pronounced inhibition of tumor viability was determined at the concentration of 60,000 µg/L (10.83%, SD – 2.85, 95% CI: 5.24-16.42). When using 80% ethanol as an extractant, we determined that the most pronounced suppression of tumor viability occurs at the concentration of 50,000 µg/L (37.6%, SD – 1.82, 95% CI: 34.03-41.17). The concentration of ethanol used as an extractant proved to be important, determining positive, statistically true associations with tumor viability (r_s=0.24, p=0.05).

GENERAL CONCLUSIONS

1. *Taraxacum officinale* represents a valuable source of biologically active compounds (flavonoids, polyphenols and phytosterols) whose content depends on the component part of the plant, the type and concentration of the extractant. The highest content of the mentioned compounds was determined in the plant leaf extracts on 50% and 80% ethanol solution.
2. Extracts from *Taraxacum officinale* exert oxidative/nitrosative stress mitigation activity conditioned by the antiradical potential comparable to that of Trolox and Rutin and the selective influence (stimulation or inhibition) on eNOS activity and NO production, which modulates the intensity of lipid peroxidation revealed by the level variations of DAM.
3. Extracts from *Taraxacum officinale* show a modulatory action on the antioxidant capacity manifested by the variation in the activity of the major antioxidant enzymes – SOD, CAT, GPx, GST and GR, associated with shifts in agreement with thiol-disulfide homeostasis and subsequent changes in the total antioxidant capacity, the mass of substances and the average antioxidant activity.
4. *Taraxacum officinale* extracts possess *in vitro* antitumor activity against human glioma cells U-251 MG conditioned by the deregulation of cellular metabolic processes conclusively induced by leaf extracts on 50% ethanol (MTT), leaf extracts on DMSO and root extracts on ethanol of high concentration (Resazurin assay).
5. The actions exhibited by *Taraxacum officinale* depend on the plant organ, the type and concentration of the extractant, and when used as a reducing agent and by the time of action of the extract.

PRACTICAL RECOMMENDATIONS

It is recommended to:

1. describe in detail the growing area of *Taraxacum officinale*, the harvesting period, the anatomical organs, the extractants of various polarity, and the methods used in the extraction of bioactive compounds in the scientific publications presenting the results of studies of the chemical composition, biological effects and biochemical mechanisms of action of *Taraxacum officinale* extracts;
2. continue researching the antineoplastic action of *Taraxacum officinale*, in order to establish the types of sensitive tumors, as well as to elucidate the molecular mechanisms of the antitumor action;
3. use the optimized research methods, developed within the study, in the didactic process (laboratory work) of students/masters/residents in the disciplines of biochemistry and clinical biochemistry, pharmacognosy and pharmaceutical botany.

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FULGA ALA

BIOCHEMICAL ACTIVITY OF EXTRACTS FROM TARAXACUM OFFICINALE G.H. WEBER EX WIGGERS

315.01 – MEDICAL BIOCHEMISTRY

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