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Preliminary Data Regarding Polyphenols, Carotenoids and Flavonoids Content Correlated with Antioxidant Activity of Some *Taraxacum* Sp. Fluid Extracts

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Abstract. *In the biomedical sciences field of recent years, phytotherapeutic products usage has intensified because they are a safe and sustainable alternative, with less environmentally aggressive molecules that are able to meet therapeutic needs. Mature vegetal product Taraxacum sp., also known as dandelion, has been used for hundreds of years as a traditional remedy for liver, kidney, lung, gastric diseases and even some cancers, due to its anti-inflammatory and antioxidant effects. The aim of this paper was to assess the contents of some bioactive principles and overall antioxidant potential of Taraxacum sp. hydroalcoholic extracts obtained from both plant organs, herba and radix. The vegetal product was collected in the period September-October 2021, from spontaneous flora of South Dobrudja area, Romania. Fresh plant was dried at room temperature on metal sieves, grind to a fine powder and extracted 10% concentration in ethanol 50% and 70% concentrations, using cold maceration and Soxhlet extraction, standard methods, followed by filtration at normal pressure. Obtained hydroalcoholic extracts were analysed by UV-Vis spectrophotometry for determining total carotenoids, flavonoids and total polyphenols content. The total antioxidant capacity was quantified through photochemiluminescence method by comparison with the standard substance used for calibration, Trolox® as tocopherol analogue by ACL (Antioxidant Capacity of Lipid Soluble Substances) procedure using Photochem apparatus, Analytik Jena AG, Germany. Total carotenoids and flavonoids concentration, respectively polyphenols contents, were highest in 70% ethanol extracts, for the two applied extraction methods. Total antioxidant capacity (TEAC) was variable, with increased values in 70% ethanol extracts of both vegetal products, herba and radix. The preliminary valuable obtained results, offer us the*

support for continuation of the studies regarding the therapeutic activity of Taraxacum sp. from Dobrudja spontaneous flora.

Keywords: Taraxacum sp., polyphenols, carotenoids, flavonoids, antioxidant activity.

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Introduction

Plants under the genus *Taraxacum* belong to the family Asteraceae, also named Compositae, subfamily Cichorioideae, tribe Lactuceae, with many varieties and microspecies: 3515 according to *International Plant Name Index* (April, 2022). It is commonly known as dandelion and it has a global spread, being more common in temperate and subtropical regions, from the sea level to alpine elevations, tolerating almost every soil type.

Although, in some parts of the world it is regarded as a weed, dandelion or parts of it are widely used worldwide in a variety of foods. The leaves are eaten as a salad or vegetable, ground roots are used as a substitute for coffee, the heads of young and unopened flowers can be used as capers. Extracts of dandelion flowers are also used in flavouring soft drinks, wine, dairy products, candy and cheese (Khan and Abourashed, 2011; Schütz et al., 2006).

Taraxacum sp. is also a highly valuable plant for medicinal applications, as an herbal remedy for the prevention, management and treatment of various human diseases. Mainly it is considered a choleretic, diuretic and a blood purifier herb, with hypoglycemic, antirheumatic, anti-carcinogenic, hypolipidemic antioxidant and anti-inflammatory properties. Hypolipidemic and hypocholesterolemic effects also, have in association with *Allium sativum* and *Oenothera biennis* extracts (Coprean et al., 2000). So, in the increasing demand for natural products as curative agents and foods, we can include *Taraxacum* sp.

Taraxacum sp. is a perennial laticiferous plant, up to 30 (-50) cm tall, with a long root. The thick, branched taproot, brown, dark-grey externally and white internally, can be up to 2–3 cm in diameter and grow up to 1–2 m in length (von Hofsten, 1954; Solbrig, 1971). The lateral roots are arranged in two rows that wind in a loose spiral around the root and are distributed along its length. The stem is only one to two, maybe three centimetres long, with very short internodes at or below the soil surface. The leaves form a basal, radial rosette (Holm et al. 1997). Leaves can be both horizontal or almost vertical, with a length of 5 to 15-20 cm (Figs. 1. A, C). The leaves are highly variable in shape - oblanceolate, obovate, but more often is pinnatisect, ranging from lobeless forms to highly incised. When leaves are lobed, the lobes point to the leaf base. Margins of the leaves may vary in the depthness of the lobes, from lobeless to triangular dentate edges (giving it the name dandelion from French "dent de lion", meaning lion's tooth). The end lobe, instead, is usually larger than the others.

Before blooming, cylindrical scapes (peduncles), 5–50 cm tall, arise from the rosette. Each of this scape bears a terminal capitulum (inflorescence) of 2–5 cm diameter (Gleason 1963; Holm et al. 1997). Each capitulum is a group of approximately 250

yellow-orangish florets and it is sustained by an oval-cylindrical involucre. Each floret has a corolla of five united petals with one side prolonged, strap-shaped and also has five stamens fused into a tube with a sagittate base, filiform basal lobes. In each floret, the inferior ovary contains one basal, inverted ovule with a single integument and each ovule gives rise to a pale grey-brown to olive-brown, narrowly obovoid-oblong, rough-surfaced cypsela (seed).

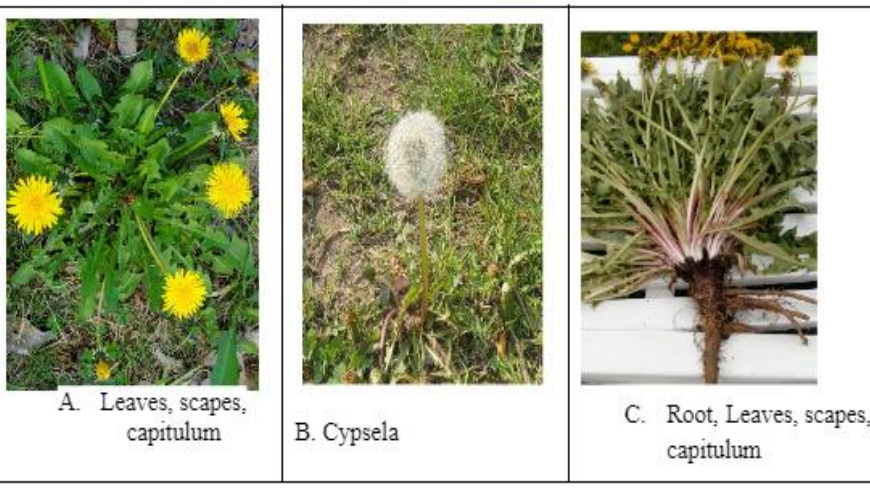

Fig. 1. *Taraxacum* sp. (original photo Tănasă (Acretei) M.V.)

A white pappus atop of each cypsela (Fig. 1. B) allows the wind to disperse it because white pappus has numerous hairs, 3–4 mm in length, mostly white and persistent (Holm et al., 1997). Connecting region of a dandelion seed includes barbs distributed on the inserting region of achene and groove of the inflorescence head. The barbs have conical shape with a length of 42–50 μm, and the groove has plenty of wrinkles to enhance its wrapping ability (Wang et al., 2021).

Phytochemical compositions of dandelion

Some studies have isolated *Taraxacum* sp. phytochemicals, showing that dandelion is rich in bioactive compounds such as phenolic acids, flavonoids, carotenoids (Table 1), coumarin, polysaccharides, sesquiterpene lactones, triterpene, phytosterols, and inulin, but also has high concentrations of fibre, minerals, vitamins and essential fatty acids (Escudero et al. 2003). Phenolic acids and flavonoids content show an increased antioxidant and immune-stimulatory activities. Dandelion sesquiterpene lactones have also, antimicrobial and anti-inflammatory activities. (González-Castejón et al., 2012; Schütz et al., 2006). Due to their various pharmacological properties, the identification, isolation, and characterization of dandelion phytochemicals are topics of major interest.

Table 1. Phytochemicals from dandelion HPLC/UPLC/LCMS analysis (adapted after Singh et al., 2020)

Stationary phase	Mobile phase		Flow (mL min^{-1})	Phyto-chemicals identified	Dandelion cultivar	Refe-rence
	A	B				
C18 Phenomenex Hydro-Synergi (4 µm, 150×3 mm)	2% acetic acid in water	0.5% acetic acid in water: ACN (50:50)	0.4	phenolic acids, flavonoids	Taraxacum officinale Web. Ex Wigg.	(Schütz et al., 2005)
C18 Phenomenex Symmetry (5 µm, 250×4.6 mm)	0.1% acetic acid in water	methanol	0.9	flavonoids, phenylpropa noid, benzoic acid derivatives	Taraxacum mongolicum	(Shi et al., 2008)
Acquity UPLC HSS T3 (1.8 µm, 100×2.1 mm)	0.5% formic acid in water	0.5% formic acid in ACN	-	phenolic acids	Taraxacum officinale	(Kenny et al., 2015)
Luna C18 (5 µm, 250×4.6 mm)	0.1% formic acid in water	aceto-nitrile	1.0	phenolic acids, flavonoids	Taraxacum spp.	(Xue et al., 2017)
YMC C30 (3 µm, 150×4.6 mm)	methanol	TBME	0.5	carotenoids, chlorophylls	Taraxacum officinale	(Gomez et al., 2018)

Antioxidant activity

Some recent studies demonstrated that polyphenols contained in *Taraxacum* determined antioxidant effects in vitro (Aabideen et al. 2020; Jedrejek et al. 2019; Lis et al. 2020) and other studies showed that *Taraxacum* has antioxidant activity *in vivo* (Choi et al. 2010) and *in vitro* (Ivanov et al. 2018; Milek et al. 2019). Two studies emphasize that polysaccharides from this plant have antioxidant activity *in vitro* (Guo et al. 2019; Park et al. 2014). The results of Jedrejek et al. 2017 suggest that for tested *Taraxacum* fractions, especially phenolic fractions from petals, which are recognized as better than other parts of the plant as source of flavonoids, may be a complementary source of natural compounds with a valuable antioxidant activity, representative for diseases-associated with oxidative stress.

The aim of this paper was to assess the concentrations of some key classes of phytochemicals, the contents of valuable bioactive principles and overall antioxidant potential of *Taraxacum* sp. hydroalcoholic extracts, in different molar ratio.

Material and Methods

Mature vegetal product (whole plant) of *Taraxacum* sp. was collected in the period September-October 2021, from spontaneous flora of the South Dobrudja area, Romania. The biological material was represented by roots (coded radix) (Fig. 2. A), scapes, leaves and capitulum (coded mix) (Fig. 2. B) and herba (Fig. 2. C), collected from plants at full maturity and all parts of the plant were separated, washed thoroughly with tap water. Fresh plant was dried at room temperature on metal sieves and grind to a fine powder (Fig. 2. D, E). Vegetal fluid extracts were obtained using two different standard methods, cold maceration and Soxhlet extraction method.

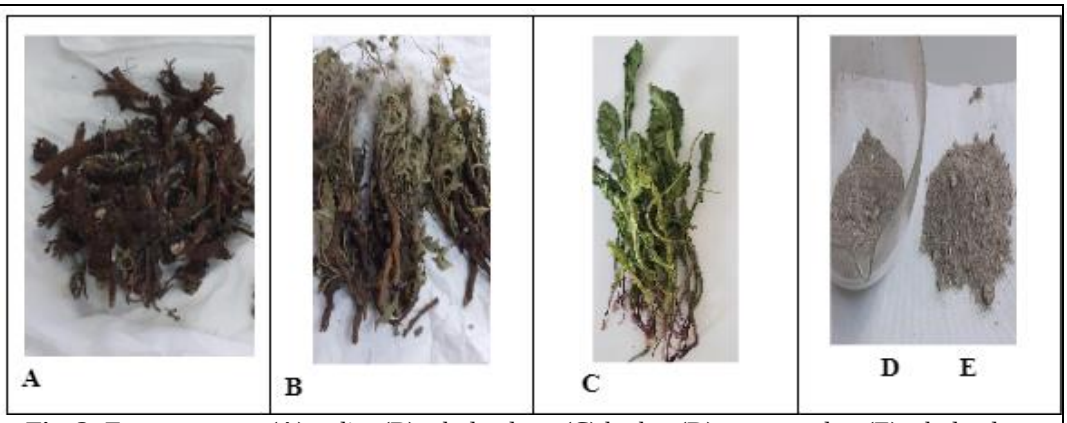

Fig. 2. *Taraxacum* sp: (A) radix; (B) whole plant; (C) herba; (D) root powder; (E) whole plant powder (original photos, Tănasă (Acreței) M.V.)

For *cold maceration*, *Taraxacum* sp. roots powder (radix I) and whole plant powder (herba II) was mixed with 50% and 70% ethanol in a conical flask – 10 g/100 mL. The mixture was stirred thoroughly with a glass rod. The conical flask was kept with intermittent shaking for 12 days, in darkness. The mixture was filtered at normal pressure through quantitative Whatman filter paper, extractive solutions present various colours, from light-brown to green-brown.

Soxhlet extraction technique has been used for a quantity of 10 g *Taraxacum* sp. root powder (radix III), respectivelly 10 g herba powder (herba IV) in 100 mL of 50% and 70% ethanol. A porous thimble loaded with a solid sample is placed inside the main chamber of the Soxhlet extractor (Fig. 3. A). By refluxing the solvent through the thimble using a condenser and a siphon side arm, the extraction cycle was repeated for 4 hours. The extracts were filtered at normal pressure through Whatman blue band filter paper and the filtrate obtained has ocher-brown colour.

(A)

(B)

Fig. 3. (A) Soxhlet extraction equipment; (B) Photochemiluminometer PHOTOCHEM Analytik Jena AG, Germany (original photos, Tănasă (Acreței) M.V.)

Obtained hydroalcoholic extracts were analysed by UV-Vis spectrophotometry method for determining total carotenoids, flavonoids and total polyphenols content.

- For determining the concentration of total carotenoids, 1 mL g vegetal extract was diluted in 9 mL 80% acetone (triplicate samples for each species). The resulting extract was filtered at normal pressure through Whatman blue band filter paper and the spectrophotometric absorbance was read (using a S106 WPA UV-Vis spectrophotometer) against an 80% acetone blank, at 470 nm, 647 nm and 663 nm of wavelengths (Lichtenthaler and Buschmann, 2001). Absorbance values were used to calculate carotenoids concentration, according to the specific trichromatic equations (Lichtenthaler and Buschmann, 2001; Popoviciu et al, 2017).
- For total phenolic compounds determination a UV-Vis spectrophotometric version of the Folin-Ciocâlteu method was used: 1 mL vegetal extract was reacted with 5 mL Folin-Ciocâlteu reagent (10%) and 4 mL sodium bicarbonate solution (7.5%) for 30 min. Spectrophotometric absorbance was read against a blank at 765 nm wavelength. A calibration curve was prepared, by using different gallic acid concentrations (Stankovic and Kragujevac, 2011; Stankovic et al, 2011; Artem et al, 2021; Popoviciu et al, 2020.a; Popoviciu et al, 2020.b). Concentrations were expressed as g/kg dry weight gallic acid equivalent (GAE) for total phenolic compounds.
- For determining the flavonoids content, 1 mL extract were diluted in 4 mL methanol and filtered (triplicate samples). 0.5 mL of extract was diluted in 4 mL water and 8 mL methanol mixture, and the spectrophotometric absorbance was read against a methanol:water blank at 340 nm wavelength (Szabo et al, 2012; Popoviciu et al, 2020.a; Popoviciu et al, 2020.b).
- For determining total antioxidant capacity (*TEAC*), a quantity of 10 g of fine powder of dried vegetal product, was cold-extracted in 50% ethanol (100 mL total volume), at room temperature and darkness, for 12 days, with regular shaking. After decantation,

normal pressure filtration and homogenization (Vortex Velp Scientifica, Italy agitator), 10 µL of supernatant were taken for analysis. Each determination lasted 120 sec. Analyses employed the photochemiluminescence method by ACL (Antioxidative Capacity in Lipid Soluble Substances) procedure Analytik Jena AG and Photochem apparatus Analytik Jena AG, Germany (Fig. 3. B). Triplicate samples of hydroalcoholic extract were quantified by comparison with the standard substance Trolox®, Hoffman-LaRoche's trade name (6-hydroxy-2,5,7,8-tetramethyl - chroman-2-carboxylic acid) vitamin E derivative. For calibration, the standard kit of reagents, Analytik Jena Germany was used: R1 (dilution reagent - methanol), R2 (buffer reagent), R3 (photosensitive reagent), R4 (reagent sized). For the calibration curve (Fig. 5), standard solutions containing 0.5, 1.0, 2.0, 3.0 nmol Trolox were measured (suitable for 5 - 30 µL of R4). The results were expressed as nmol Trolox equivalents/sample volume (Pallag et al, 2018; Negreanu-Pirjol et al, 2014; Negreanu-Pirjol BS. et al, 2014). Samples were prepared, according to Table 2.

Table 2. Working scheme volumes, µL,
[Negreanu-Pirjol, T et al, 2014; Negreanu-Pirjol, BS et al, 2014]

Kit reagent	*Reactive volume R_1 (µL)*	*Reactive volume R_2 (µL)*	*Reactive volume R_3 (µL)*	*Reactive volume R_4 (for the calibration curve) (µL)*	*Sample (µL)*
Blank	2300			0	0
Calibration curve - Trolox standard	2295			5	0
	2290			10	0
	2280			20	0
	2270			30	0
				0	25
Measurement sample	2295			0	10

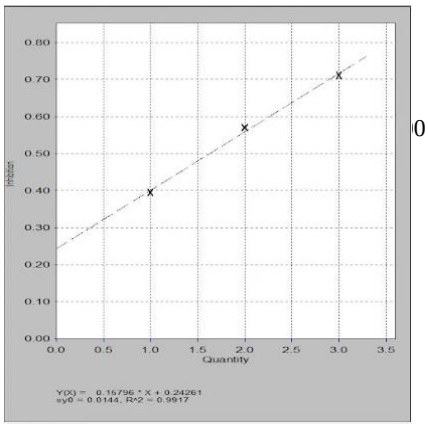

Fig. 4. Calibration curve for Trolox® standard substance (photochemiluminescence method, ACL procedure, Analytik Jena AG, Germany)

Results and Discussions

The results regarding the total antioxidant capacity of the hydroalcoholic extracts of *Taraxacum* sp. (radix, herba and mix) are presented in Fig 5. and Table 3. The voltage (V) recorded is proportional to the luminescence generated as a function of time (sec.); inhibition of free radicals by antioxidants in the samples was compared with the Trolox standard substance (for 0.5, 1.0, 1.5, 2.0 and 3.0 nmol/sample volume). The registered *TEAC* values of each plant organ extract exceeded the calibration curve, therefore the experiment required dilutions of stock solution extracts with methanol (R1), in molar ratios of 1:20, 1:30, 1:50.

The analysis of the obtained results in case of different molar dilution reports highlights the following aspects:

- The hydroalcoholic extract obtained from the vegetable product *Taraxacum* sp., herba, obtained both by cold maceration and by refluxing in ethyl alcohol of 70% concentration evidenced the highest values of antioxidant activity.
- In hydroalcoholic extracts of *Taraxacum* sp., herba and radix, obtained by cold maceration in ethyl alcohol of concentration 70%, a significant total antioxidant capacity was registered for herba sample (14.238 μmoli echiv. Trolox/g tissue) and for radix sample (13.558 μmoli echiv. Trolox/g tissue), both at the maximum dilution of the stock solution in concentration 10% of 1:1; the result indicates that the influence of pedoclimatic conditions is quite small and the antioxidant activity of the extracts has slightly higher values.
- In the case of hydroalcoholic extracts of *Taraxacum* sp., herba and radix, obtained by Soxhlet extraction technique, a significant total antioxidant capacity, compared to extracts obtained by cold maceration, was recorded for herba (13.995 μmol equiv. Trolox/g tissue).

Table 3. Total antioxidant capacity (TEAC) of *Taraxacum* sp. hydroalcoholic extracts stock solution at different dilution in methanol

No.	*Sample Type, Dilution*	*Free radicals Max. Inhibition*	*Quantity Means (TEAC) (μmol equiv. Trolox/g tissue)*
1.	Radix 50%, 1:1	0.977	11.910
2.	Radix 50%, 1:30	0.588	2.032
3.	Radix 50%, 1:50	0.620	2.280
4.	Radix 70%, 1:1	0.994	13.558
5.	Radix 70%, 1:30	0.756	3.783
6.	Radix 70%, 1:50	0.625	2.315
7.	Herba 50%, 1:1	0.900	7.286
8.	Herba 50%, 1:30	0.842	5.451
9.	Herba 50%, 1:50	0.669	2.718
10.	Herba 70%, 1:1	1.000	14.238
11.	Herba 70%, 1:30	0.807	4.667
12.	Herba 70%, 1:50	0.395	1.006
13.	Mix 50%, 1:1	0.994	13.541
14.	Mix 50%, 1:30	0.712	3.186
15.	Mix 50%, 1:50	0.642	2.464
16.	Mix 70%, 1:1	0.998	14.059
17.	Mix 70%, 1:30	0.842	5.452
18.	Mix 70%, 1:50	0.716	3.233
19.	Radix Soxhlet 70%, 1:1	0.994	13.572
20.	Radix Soxhlet 70%, 1:30	0.673	2.758
21.	Radix Soxhlet 70%, 1:50	0.558	1.825
22.	Herba Soxhlet 70%, 1:1	0.998	13.995
23.	Herba Soxhlet 70%, 1:30	0.998	13.984
24.	Herba Soxhlet 70%, 1:50	0.704	3.096

- The comparative study of the total polyphenols content and total antioxidant capacity shows an almost linear dependence of these parameters (Fig. 5). The obtained results show that even at a high degree of dilution (1:50) of the initial stock solutions, *Taraxacum* sp. vegetal extracts emphasize a valuable antioxidant activity.

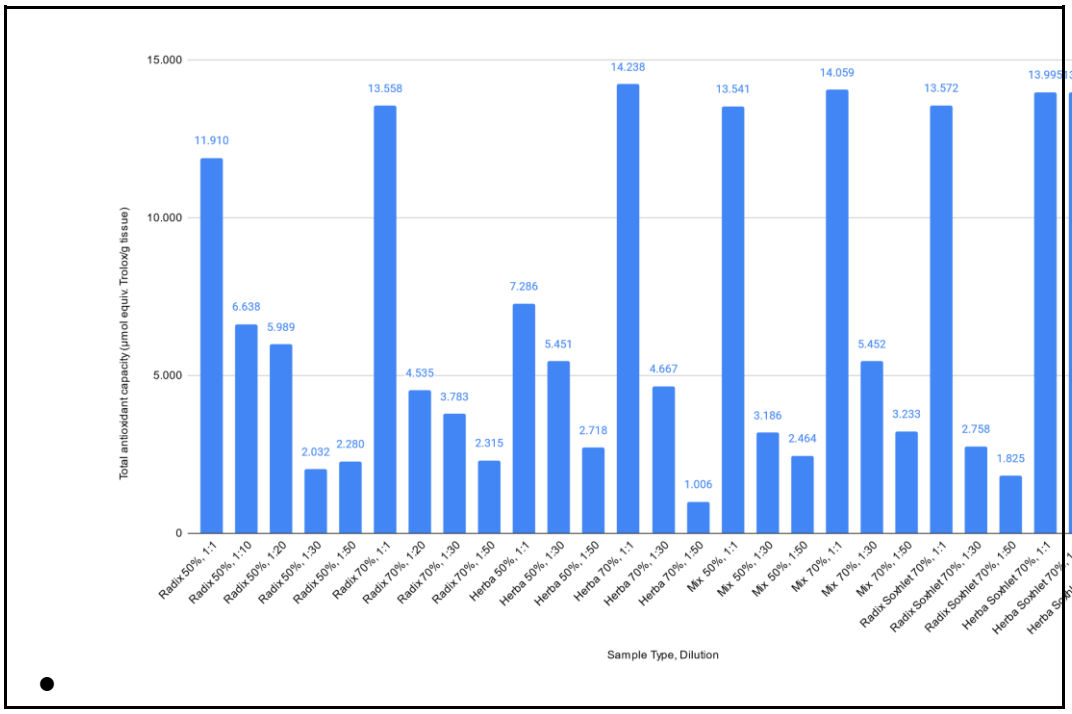

Fig. 5. Total antioxidant capacity (µmoli equiv. Trolox/g tissue) of 70% ethyl alcohol extracts of *Taraxacum* sp. samples at different molar ratio dilution

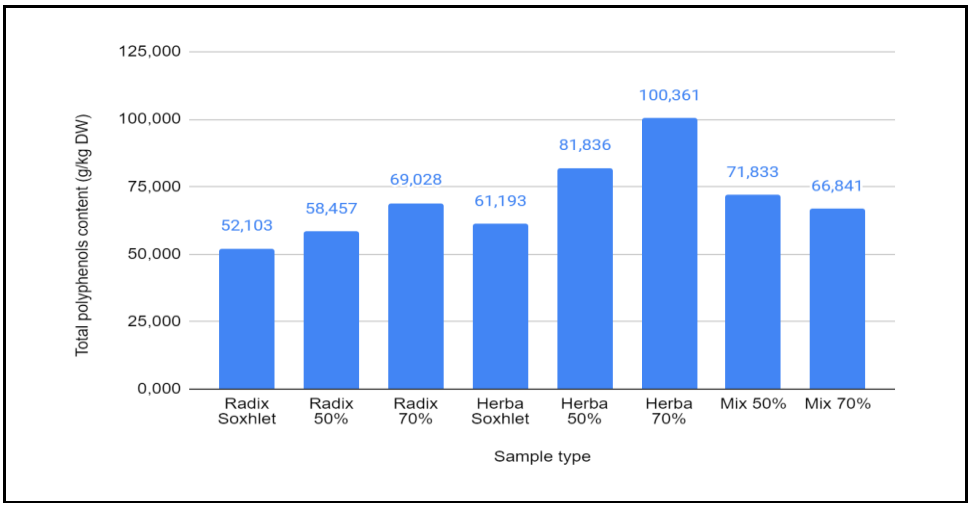

Fig 6. Total polyphenols content (g/kg DW) in different sample of 50% and 70% ethyl alcohol extracts of *Taraxacum* sp.

- The highest total polyphenols content (100.361 mg/kg DW) was registered for the cold maceration hydroalcoholic extract of 70% concentration, obtained from the

vegetable product *Taraxacum* herba, which correlates with the highest antioxidant capacity recorded (14.238 µmoli equiv. Trolox/g tissue sample).

● The highest total carotenoids content was determined for cold macerated hydroalcoholic extracts of concentration 70% *Taraxacum* sp. mix (134.441 mg/kg DW), respectively *Taraxacum* sp. herba (104.009 mg//kg DW) (Fig. 7).

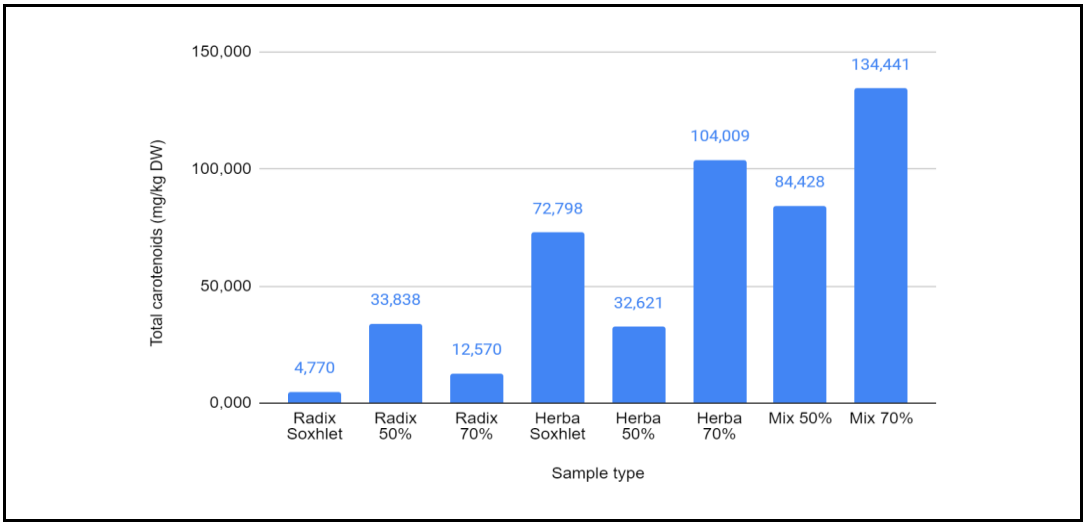

Fig 7. Total carotenoids (mg/kg DW) content in different hydroalcoholic extracts of *Taraxacum* sp.

● The flavonoids content showed higher values in the cold macerated hydroalcoholic extract *Taraxacum* herba of concentration 70% (36.514 mg//kg DW), respectively in the hydroalcoholic extract *Taraxacum* radix 70% (28.167 mg/kg DW) (Fig. 8).

●

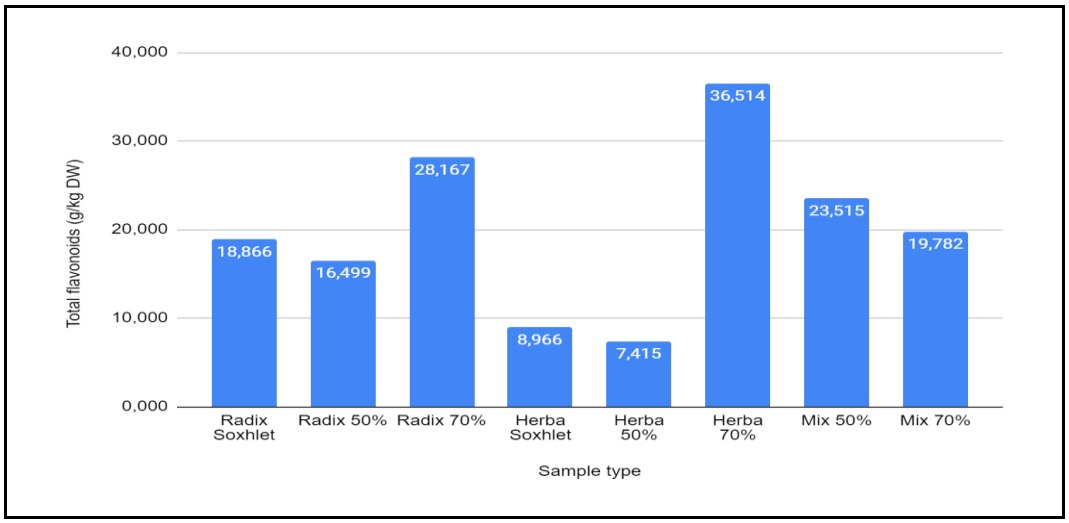

Fig 8. Total flavonoids (g/kg DW) content in different hydroalcoholic extracts of *Taraxacum* sp.

● Total carotenoids and flavonoids concentration, respectively polyphenols content, were increased in 70% ethanol vegetal extracts, for the two applied extraction methods. Total antioxidant capacity (*TEAC*) presented variable values, more increased for 70% ethanol extracts of both vegetal products, *Taraxacum* sp. herba and radix.

Conclusions

In these preliminary determinations, the hydroalcoholic extracts of *Taraxacum* sp. of concentration 10% in ethyl alcohol 50% and 70% studied, emphasize a high values of total antioxidant capacity by photochemiluminescence method, with close values.

Samples of hydroalcoholic extracts obtained from vegetable organs cold macerated in ethyl alcohol of concentration 70% *Taraxacum* herba and mix recorded higher values in carotenoids, and for flavonoids higher values were obtained in the hydroalcoholic extracts cold macerated *Taraxacum* herba and radix.

The highest content of total polyphenols was recorded in the case of the hydroalcoholic extract from the vegetable product *Taraxacum* herba of 70% concentration, obtained by cold maceration, which correlates with the highest value of total antioxidant capacity.

The comparative study of total polyphenol content and total antioxidant capacity shows an almost linear dependence of these parameters for hydroalcoholic extracts obtained by cold maceration and hydroalcoholic extracts obtained by reflux, both in ethyl alcohol of 70% concentration.

Preliminary comparative study of the two extraction methods used for *Taraxacum* sp. vegetal product, showed that cold maceration is the most valuable method and is recommended for the extraction of the analysed bioactive principles, total polyphenols, carotenoids and flavonoids.

Further research is needed to evaluate in detail the composition of bioactive compounds and to confirm and expand the therapeutic potential of the native plant species *Taraxacum* sp.

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