https://doi.org/10.31925/farmacia.2022.1.7 **ORIGINAL ARTICLE**

POLYPHENOLIC COMPOUNDS, ANTIOXIDANT ACTIVITY AND NEPHROPROTECTIVE PROPERTIES OF ROMANIAN *TARAXACUM OFFICINALE*

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Manuscript received: July 2021

Abstract

Taraxacum officinale (L.) Weber ex.F.H.Wigg. (dandelion) is a medicinal herb from the *Asteraceae* family, with a complex chemical composition. The purpose of this research was to determine the polyphenolic profile of *Taraxaci herba* tincture and to evaluate the antioxidant capacity and the nephroprotective activity. The polyphenolic compounds: total polyphenols content (TPC), total flavonoids content (TFC) and total caffeic acid derivates content (TCAD) were analysed by spectrophotometric and chromatographic methods. Some polyphenols were identified and quantified by HPLC-MS method, with cichoric acid as major compound. The evaluation of the *in vitro* antioxidant activity was performed using the DPPH• and FRAP methods. The nephroprotective activity of *T. herba* tincture was evaluated on a rat model with acute kidney injury (AKI) induced by gentamicin. Serum oxidative parameters (total oxidative stress – TOS, oxidative stress index – OSI, total antioxidant capacity – TAC, total nitrites and nitrates – NOx, malondyaldehide – MDA and total thiols – SH), kidney functional parameters (serum creatinine – SCr, serum urea – SU, urinary creatinine – Ucr, urinary urea – UU and creatinine clearance – ClCr) and the transcription factor (Nuclear Factor Kappa B) NF-kB were determined. Although the *in vitro* test results were modest, *in vivo* experiments showed a pronounced antioxidant capacity, associated with an important nephroprotective activity.

Rezumat

Taraxacum officinale L. (păpădie) este o plantă medicinală din familia *Asteraceae*, cu o compoziție chimică foarte complexă. Scopul prezentei cercetări a fost determinarea profilului polifenolic al tincturii obținută din *Taraxaci herba*, precum și evaluarea capacității antioxidante și a activității nefroprotectoare. Compușii polifenolici – conținutul de polifenoli totali (TPC), conținutul de flavonoide totale (TFC) și conținutul de derivați de acid cafeic (TCAD) au fost analizați prin metode spectrofotometrice și cromatografice. Prin HPLC-MS s-au identificat și cuantificat compuși polifenolici, acidul cicoric fiind compusul majoritar. Evaluarea activității antioxidante *in vitro* s-a realizat prin metodele DPPH• și FRAP. Activitatea nefroprotectoare a tincturii de *T. herba* s-a evaluat pe șobolani de laborator cu insuficiență renală acută (AKI) indusă cu gentamicină. S-au evaluat parametri ai stresului oxidativ (status oxidativ total – TOS, index al stresului oxidativ – OSI, capacitatea antioxidantă totală – TAC, nivelul de nitriți și nitrați – NOx, malondialdehida – MDA, tioli – SH), parametri funcționali renali (creatinina serică – SCr, urea serică – SU, creatinina urinară – UCr, urea urinară – UU și clearance-ul creatininic – ClCr) și factorul de transcripție NF-kB. Deși rezultatele testărilor *in vitro* au fost modeste, experimentele *in vivo* au arătat o acțiune antioxidantă bună, asociată cu o importantă activitate nefroprotectoare.

Keywords: *Taraxacum officinale*, polyphenols, antioxidant capacity, nephroprotective activity

Introduction

Taraxacum officinale L. is a perennial herbaceous plant belonging to the *Asteraceae* family, *Cichorioideae* subfamily, with a wide distribution in the Northern Hemisphere [32]. It grows spontaneous in wild population all across Romania, from the grasslands to the mountain regions [9]. In Transylvania, the leaves of the plant were used as a diuretic in traditional medicine, for counteracting water retention, as well as in kidney related diseases [11]. The active compounds that determine the pharmacological properties are present in both aerial parts and roots [39]. Generally, the active principles of the roots are active towards liver

and gallbladder illnesses, while the compounds from aerial parts exhibit diuretic properties, antioxidant, anti-inflammatory, anti-carcinogenic, analgesic, anti-coagulant and anti-hyperglycaemic activities [37, 39]. Recent studies have shown new properties such as neuroprotective and anti-depressive [20, 26]**.** The composition of *T. officinale* consists mainly in polyphenols, terpenoids, together with polysaccharides (inulin), vitamins and minerals. Polyphenols are largely distributed in all parts of the plant with higher quantities in aerial parts [48]. The major polyphenolic compounds from the entire plant are hydroxycinnamic acid derivates, such as cichoric acid (2,3-dicaffeoyl-L-tartaric acid), monocaffeoyl-tartaric acid and chlorogenic acid. Flavonoids were detected only in aerial parts [48]. The most abundant are luteolin 7-glucoside, luteolin 7-rutinoside, apigenin 7-glucoside, quercetin 7-O-glucoside, isorhamnetin 3-glucoside [21, 48]. The terpenes identified in the dandelion are di- or tri-terpenes (taraxacin, taraxacerin, taraxerol, taraxasterol, beta-amyrin) and sterols (sitosterol, stigmasterol) [40, 48]. The diuretic activity of dandelion extracts tested on mice and rats was comparable to furosemide [13, 28]. In addition, due to the high content of potassium, *T. officinale* extract is able to replace potassium lost through diuresis, being an important agent in restoring the electrolytic imbalance [24]. Thus, the focus of this research was to evaluate the nephroprotective activity of *T. herba* tincture on rats, by determining several parameters involved in the oxidative stress and inflammatory processes, which may be responsible of some kidney diseases.

Materials and Methods

Plant material: *T. officinale* aerial parts (*T. herba*) were harvested from Sibiu County, Lat. 45.714966 / Long. 24.321213 Romania, during blooming stage, in May 2018, from wild populations. The plant material was identified by botany Prof. Mircea Tămaș. A sample of the vegetable material is available in the herbarium of Pharmacognosy Department (voucher number 115), Faculty of Pharmacy, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The vegetable material was air-dried and grinded; the powder was used for the extracts preparation.

Extraction procedure: The vegetable powder was extracted with dichloromethane in Soxhlet apparatus [17] and then the tincture (THT; 1:10 g/g w/w) was obtained, accordingly to the Romanian Pharmacopoeia: 50 g of herbal powder and 500 g 70% ethanol, maceration at room temperature, 7 days [50].

For the assessment of the nephroprotective activity, *T. herba* tincture (THT) was diluted with distilled water as follows: THT 1:1 (corresponding to 0.5 mg dry weight (d.w.) plant material/10 mL) and THT 1:3 (corresponding to 0.25 mg d.w. plant material/10 mL).

The total polyphenols content (TPC) was determined using the Folin-Ciocâlteu spectrophotometric method and the results were expressed as mg gallic acid equivalents (GAE)/100 g d.w. plant material (R^2 = 0.999) [36].

The total flavonoids content (TFC) was spectrophotometrically determined using $AlCl_3$ as a colouring reagent. The results were expressed as mg rutin equivalents (RE)/100 g d.w. plant material (R^2 = 0.999) [22].

The total caffeic acid derivates content (TCADC): a spectrophotometric method was used, based on the reaction with the Arnow reagent. The results were expressed as mg cichoric acid equivalents (CAE)/100 g dry plant material (R^2 = 0.994) [35].

The HPLC analysis of polyphenolic compounds was undertaken using an Agilent 1100 HPLC Series system equipped with degasser, binary gradient pump, column thermostat and autosampler. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap SL). The analysis was performed using the conditions previously described [34]. The polyphenol-carboxylic acids were UV detected at 330 nm, and the flavonoids at 370 nm. The polyphenolic compounds were identified based on their retention time and MS spectra compared to standards (different polyphenolic compounds). Calibration curves in the 0.5 - 50 µL/mL concentration range (R2 > 0.999) were used [29]. Cichoric acid was quantified using a newly optimised method of LC-MS previously described [10]. Cichoric acid was identified based on the retention time and the MS spectra compared to the standard. The calibration curve was prepared in the range of 0.75 - 15 µg/mL concentrations, with R2 > 0.999. All determinations were performed in triplicate.

The antioxidant capacity was assessed *in vitro*, with the DPPH• and FRAP methods.

The DPPH• (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method evaluate the antioxidant capacity of the tincture based on an electron-transfer reaction. The results were defined as IC50 value. The half maximal inhibitory concentration representing the concentration of the sample that can scavenge 50% of DPPH free radical. The IC50 value is inversely proportional to the free radical scavenging activity/antioxidant property of the sample) [18].

The FRAP method evaluate the reduction of the ferric ion to the ferrous ion, by forming the complex of iron with the radical 2,4,6-tripyridyl-s-triazine. The results were expressed as mM Trolox equivalents/ 100 mL extract (R^2 = 0.992) The calibration curve was obtained with 10 - 40 mg/L Trolox standard [41]. All the quantitative determinations were performed in triplicate.

Nephroprotective activity evaluation

The experiments were performed on adult male Winstar Albino rats that were bred in the "Iuliu Hațieganu"

University of Medicine and Pharmacy Animal Facility, Cluj-Napoca, Romania, and held under strict controlled conditions. At the end of the study, the animals were sacrificed by cervical dislocation under general anaesthesia. The experimental design was approved by the Institutional Animal Ethical Committee (IAEC) of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania, and by the National Sanitary Veterinary and Food Safety Agency from Romania (no. 171/13.07.2019).

The animals were divided into 5 groups (n = 5): negative control (Control), gentamicin group (Genta), and three *T. herba* tincture groups. The animals were given orally in a single administration of 5 mL *per* day water by gavage in Control and Genta groups, respectively the three dilutions of extract in the THT groups: THT, THT 1:1 and THT 1:3, during seven days [18]. Excepting the Control group, in the days 7 and 8, animals received gentamicin (s.c. 400 mg/kg b.w./day) for nephrotoxic acute kidney failure induction [12]. The 24 hours urine was collected, between day 8 and 9. In day 9 blood samples were collected by retro-orbital puncture under general anaesthesia (ketamine 70 mg/kg b.w. and xylazine 10 mg/kg b.w.) [47]. Serum was separated and stored at -80ºC until the evaluation of oxidative stress and renal parameters.

Oxidative stress parameters evaluation

TOS was determined using a colorimetric method based on the oxidation of a ferrous ion to a ferric ion in the presence of various oxidant species [5]. The results were expressed in µmol H_2O_2 equiv/L. TAC was assessed using a colorimetric assay and the results were expressed as mmol Trolox equiv./L [19]. OSI was calculated as the ratio between TOS and TAC. MDA parameter representing a lipid peroxidation marker was determined using the thiobarbituric acid assay [15]. MDA serum concentration was expressed as nmol/mL. The serum NO concentration was assessed using the Griess reaction and expressed as nitrite µmol/L (NOx) [33]. Serum total thiols were quantified as mmol GSH/mL and were determined using Ellman's reagent [25]. Serum and urine creatinine were measured according to the manufacturer instructions and CrCL was evaluated accordingly. The NF-kB, an inflammation marker, was evaluated using a NF-kB ELISA KIT, (ER1186, Fine Biotech, and Wuhan, China) according to the manufacturer instructions.

Statistical analyses

The results were expressed as means ± standard deviation (± SD). One way ANOVA test and Bonferroni-Holm post-hoc test were used to compare the data. The correlation between the parameters of the same group was evaluated using Pearson's coefficient (r) correspondingly to the Colton scale. The level of statistical significance was set at $p < 0.05$. The statistical analysis was performed using STATISTICA 12.0 software.

Results and Discussion

The results for the determinations of TPC, TFC, TCADC and the antioxidant capacity are summarized in Table I.

Table I

Polyphenol content and antioxidant activity of *T. herba* tincture

Sample	TPC (mg GAE/g d.w.)	TFC (mg RE/g d.w.)	TCADC (mg cichoric acid /g d.w.)	IC50 (µg/mL)	FRAP (µM TE/ 100 mL extract
THT	13.15 ± 0.81	6.87 ± 0.34	9.7 ± 0.36	234.988 ± 11.74	365.56 ± 14.25

Values are expressed as mean of 3 determinations ± SD. TPC – Total polyphenols content; TFC – Total flavonoids content; TCADC – Total caffeic acid derivates content; IC50 – half maximal inhibitory concentration; FRAP – ferric reducing ability of plasma; GAE – gallic acid equivalents; RE – rutin equivalents; TE – trolox equivalents

Our results show a good level of polyphenols in the tincture, with high concentrations of flavonoids and caffeic acid derivatives. The TPC value was smaller than those obtained by Ivanov (33.90 mg GAE/g d.w., 50% ethanol extract) and by Khan *et al*. (41.47 - 691.6 mg GAE/g d.w., aqueous and hydroalcoholic extracts) [28, 30]. Xue *et al*. reported values of 33.94 mg GAE/g d.w. (50% ethanol extract) and 23.27 mg GAE/g d.w. (80% ethanol extract) [49]. All these results are in agreement with Tsai *et al*. considering that higher concentrations of ethanol determine a possible decrease in the polyphenols extraction [43]. The TFC was lower in *T. herba* tincture than the values reported by other authors: 14.00 mg RE/g d.w. – 50% ethanol and 12.35 mg RE/g d.w. – 80% ethanol [49]. To the best of our knowledge, the TCAD content in *T. herba* tincture was not reported before. Our results show an important concentration of these compounds, expressed in cichoric acid.

The results of the antioxidant activity determination showed a modest antioxidant capacity. Our results were higher than those obtained in similar conditions by Aremu *et al*. (IC50 = 400 µg/mL) and lower than those obtained by Indradi *et al*. (IC50 = 100 µg/mL [6, 27]. The FRAP assay showed better antioxidant capacity than that reported by Aremu *et al*. [6, 28].

The HPLC assay was employed in order to evaluate the qualitative and quantitative polyphenols composition of *T. herba* tincture. The results were summarized in Table II.

Table II

Phenolic compounds identified in *T. herba* tincture by HPLC-MS

Compound	RT ± SD (min)	Concentration in tincture (µg/mL)	Concentration (µg/g d.w.)
Ferulic acid	12.20 ± 0.24	9.253 ± 0.18	92.53 ± 1.11
Cichoric acid	15.96 ± 0.13	716.311 ± 6.44	7163.11 ± 53.64
Rutin	20.20 ± 0.15	1.599 ± 0.02	15.99 ± 0.31
Quercetin	26.8 ± 0.12	0.779 ± 0.02	7.79 ± 0.17
Luteolin	29.10 ± 0.19	25.565 ± 0.31	255.65 ± 2.49
Apigenin	33.10 ± 0.15	0.481 ± 0.04	4.81 ± 0.93

Values are expressed as mean of 3 determinations ± SD. RT – retention time.

Cichoric acid was the predominant polyphenolic compound in *T. herba* tincture, with a substantial quantity compared to other polyphenols. Other authors reported 4840 µg/g cichoric acid d.w. (ethanol 95% extract) and 31480 µg/g cichoric acid d.w. (ethanol 50% extract) [28]. Stylianou *et al*. found cichoric acid concentrations between 3190 µg/g - 5060 µg/g for flower and stems [42].

The identified flavonoids were rutin, quercetin, luteolin and apigenin, data comparable with other findings as those of Schutz *et al*. [40].

Nephroprotective activity evaluation

In order to evaluate the effect of *T. herba* tincture on acute kidney injure (AKI) induced by gentamicin, the functional renal parameters (Table III), the serum oxidative markers and the NF-Kb levels (Table IV) were determined.

Table III

Renal function parameters in rat gentamicin-induced acute kidney injury

Groups	SCr (mg/dL)	UCr (mg/dL)	SU (mg/dL)	UU (mg/dL)	CrCl (mL/min)
Control	0.72 ± 0.03	32.10 ± 1.66	23.23 ± 4.17	173.33 ± 61.28	0.60 ± 0.08
Genta	1.24 ± 0.07	47.46 ± 0.32	79.04 ± 6.55	433.33 ± 0.00	0.28 ± 0.001
THT	1.03 ± 0.14	36.18 ± 7.53	45.48*** ± 4.66	219.56*** ± 20.01	0.42 ± 0.14
THT 1:1	1.01* ± 0.05	49.4 ± 6.60	48.12*** ± 4.40	208.00 *** ± 0.00	0.67* ± 0.10
THT 1:3	1.14 ± 0.08	50.38 ± 2.93	46.73 *** ± 4.40	199.33*** ± 17.33	0.31 ± 0.07

Values are expressed as ± SD (n=5). n.s. = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ *versus* gentamicin group.
SCr – serum creatinine; UCr – urine creatinine; SU – serum urea; UU – urine urea; CrCl – creatinine clearance; Genta – gentamicin; THT – *Taraxaci herba* tincture

Consecutive to the AKI, the renal functional parameters exhibit higher values, and THT pre-treatments reduced AKI consequences. The SCr was significantly reduced only by THT 1:1 ($p < 0.05$). SU and UU were significantly reduced by all extract dilutions ($p < 0.001$). CrCl was increased by THT 1:1 ($p < 0.05$). None of the pre-treatments had any significant activity on UCr ($p > 0.05$).

The tincture pre-treatments lowered the oxidative stress by reducing TOS, OSI and NOx. TOS was lowered in the order THT 1:1 → THT → THT 1:3 ($p < 0.05$), NOx was reduced by THT 1:1 ($p < 0.05$). The THT had no significant effect on TAC, MDA and SH ($p > 0.05$). NF-kB, an inflammation marker, was lowered significantly by all tincture dilutions ($p < 0.001$), mostly by THT. The correlation between the parameters was evaluated with Pearson coefficient referring to Colton's scale. In the Genta group, TOS and OSI were negative correlated with TAC and SH. The same relationship was observed between UCr and SH, while UCr was correlated positive with MDA. The NF-kB parameter correlated positive with TOS and OSI and UCr and negative with SH. In the THT groups, the renal functional parameters were negatively correlated with oxidative stress parameters and CrCl was positive correlated with TAC.

Table IV

Serum oxidative stress markers in the rat gentamicin-induced acute kidney injury

Groups	TOS (µM H_2O_2 equiv/L)	OSI	TAC (mmolTrolox equiv/L)	NOx (µM/L)	MDA (nM/L)	SH (mM/L)	NF-Kb (ng/mL)
Control	5.13 ± 0.84	4.70 ± 0.77	1.09 ± 0.001	32.67 ± 2.38	1.91 ± 0.19	0.52 ± 0.05	2.2 ± 0.22
Genta	7.55 ± 1.43	6.94 ± 1.33	1.09 ± 0.001	51.73 ± 4.25	2.77 ± 0.26	0.48 ± 0.02	5.81 ± 0.10
THT	4.61** ± 0.20	4.24* ± 0.18	1.09 ± 0.001	44.51 ±10.36	2.77 ± 0.30	0.42 ± 0.11	2.39*** ± 0.10
THT 1:1	4.48** ± 1.15	4.11* ± 0.14	1.09 ± 0.001	37.00** ± 2.46	2.76 ± 0.27	0.45 ± 0.04	3.40*** ± 0.43
THT 1:3	4.63 ** ± 0.27	4.26* ± 0.24	1.09 ± 0.001	43.84 ± 7.74	2.38 ± 0.29	0.44 ± 0.06	2.59*** ± 0.98

Values are expressed as ± SD (n = 5). n.s. = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ *versus* gentamicin group. TOS – total oxidative status; TAC – total antioxidant capacity; OSI – oxidative stress index; NOx – nitrites and nitrates; MDA – malondialdehyde; SH – total thiols; NF-kB – nuclear factor kappa-light-chain-enhancer of activated B cells; THT – *Taraxaci herba* tincture

Gentamicin has been showed to accumulate in the renal proximal tubules in larger quantities than in the serum inducing acute kidney failure in a dose dependant manner [16]. Nephrotoxicity studies have shown the generation of hydrogen peroxide, enhancing generation of the superoxide anion and hydroxyl radical in renal mitochondria, and promoting pro-inflammatory responses as possible mechanism [3, 45]. The AKI was in accordance to the increased levels of SCr, SU, Ucr, UU and ClCr [44]. The increased value of NF-kB supported the hypothesis of inflammation [31] and the escalation of serum oxidative parameters promoted the involvement of oxidative stress [3]. These findings show the need of an approach on anti-inflammatory therapy based on the antioxidant effect. Our results showed that in the AKI group, THT reduced the serum oxidative parameters TOS, OSI, NOx and NF-kB, THT 1:1 reduced the most TOS and NOx and the undiluted tincture was the most effective in lowering NF-Kb. SU and UU were lowered by the THT, in a reversed dose dependent manner, the lower concentrations being the optimum inhibitor. In addition, SCr and CrCl were lowered by THT 1:1. A possible explanation of the higher *in vivo* antioxidant activity for the lower concentration of extract is the fact that some anti-oxidants may have pro-oxidant effect at higher levels [7]. Our findings are consistent with the results documented by other researchers that flavonoids (as luteolin, rutin, quercetol, apigenin and their glycosides) can act as nephroprotective agents through an antioxidant mechanism, in AKI induced by several chemical compounds in an animal model [2, 4, 23, 38]. In a previous study, the role of cichoric acid as a major polyphenolic compound of *Cichorium intybus*, in re-establishing renal parameters post AKI induced on a rat model, was highlighted [18]. Also, the nephro-protective effect of cichoric acid was studied by Sanna *et al*. revealing a mechanism based on anti-oxidant activity and the inhibition of NF-kB pro-inflammatory pathway [1].

Recent studies have shown the presence of poly-phenolic compounds as cichoric acid, quercetin and luteolin as well as active metabolites, in special 4'-O-methyl-luteolin-3'-O-β-D-glucuronide and luteolin-3'-O-β-D-glucuronide in the kidney tissue. These findings also support the pharmacological activity on kidney and the role in nephroprotection [8, 14, 46].

Conclusions

Our study confirmed that *T. officinale* is a valuable source of polyphenols, with an important concentration of cichoric acid. Our findings reveal a good *in vivo* antioxidant activity of *T. herba* tincture, by evaluating serum oxidative parameters and kidney functional parameters, although the *in vitro* results were modest. *T. officinale* tincture has nephroprotective effect in gentamicin induced AKI, due to the antioxidant and anti-inflammatory properties. Further studies may extend the knowledge about the nephroprotective mechanism and other possible benefits in the prevention or the treatment of various kidney disorders.

Conflict of interest

The authors declare no conflict of interest.

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