Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014-15; 6(4); 889-893

ISSN: 0975-4873

Research Article

Polyphenols Content and Antioxidant Activities of *Taraxacum officinale* F.H. Wigg (Dandelion) Leaves

*Ivan Georgiev Ivanov

Department of Organic Chemistry, University of Food Technologies, 26 Maritza Blvd., 4002, Plovdiv, Bulgaria

Available Online: 22nd November, 2014

ABSTRACT

Plant of genus Taraxacum known as dandelion have long been used in tradition medicinal. Extracts from dandelion possess anti-influenza virus, anti-fertility and anti-retrovirus activity, antioxidant and hepatoprotective effects. Chicoric acid also known as dicaffeyltartaric acid and belongs to phenylpropanoids is one of the main constituent of *T. officinale*. The current investigation was conducted to determinate the total polyphenols and total dihydroxycinnamic derivatives contents, and to evaluate the antioxidant capacities in aqueous and ethanol-water extracts prepared from *T. officinale* leaves. Total phenolic content was determined by using the Folin-Ciocalteau method. The antioxidant capacities in the forms of DPPH, FRAP and CuPRAC were evaluated by spectrophotometric methods. The results indicated that TPC, chicoric acid concentration, DPPH, FRAP and CuPRAC values were higher in 50% ethanol extract of *T. officinale* leaves: 33.90 ± 0.57 mg GAE/ g DW, 3.1 g/ 100g DW, 136.3 mM TE/ g DW (DPPH method), 131.5 mM TE / g DW (FRAP method) and 407.8 mM TE/ g DW (CuPRAC method). The results clearly demonstrated the *Taraxacum officinale* F.H. Wigg leaves are rich source of polyphenols possess high antioxidant properties. The high yield of cichoric acid make this plant valuable source of commercial production.

Keywords: *Taraxacum officinale*, chicoric acid, HPLC, antioxidants

INTRODUCTION

Dandelion *Taraxacum officinale* (L.) Weber ex F.H. Wigg have been used as medicinal infusions and decoctions of root and herb (1). In Turkish folk medicine, the herb is used as a laxative, diuretic and potent anti-diabetic medicine (2). The traditional Chinese medicine uses *Taraxacum officinale* to enhance immune response to the upper respiratory tract infections, and bronchitis or pneumonia (1). In combination with other herbs, *Taraxacum officinale* is used to treat hepatitis. Their extracts contain lipotropic substances which can improve functionality of hepatocytes (3). Other several health-promoting benefits including anti-rheumatic, anti-inflammatory, anti-carcinogenic and hypoglycaemic activities have been attributed to the use of dandelion extracts or the plant itself (4,5) *Taraxacum officinale* flower extract, particularly the ethyl acetate fraction, possesses bioactive phytochemicals which have the ability to scavenge reactive oxygen species (ROS) and prevent DNA from ROS-induced damage *in vitro* (6). Dandelion can protect against cholecystokinin octapeptide induced acute pancreatitis in rats (7). In addition, leaves from *Taraxacum officinale* ingested by ruminants during pregnancy strengthen the liver and can help prevent preeclampsia, which manifests as high blood pressure with edema. Leaves help prevent anemia, because they are rich in iron and helps the fetus to develop a strong liver of its own (8). Also, the ethanolic extract of *Taraxacum officinale* increases the frequency and excretion ratio of fluids in healthy humans (9). Extracts from dandelion possess anti-influenza virus (10), anti-fertility (11) and showed strong anti-HIV–1 retrovirus activity (12), antioxidant and hepatoprotective effects (13).

Chicoric acid is one of the main constituent of *T. officinale*. It is also known as dicaffeyltartaric acid and belongs to phenylpropanoids. Chicoric acid occurs naturally in large number of medicinal plants that were being used in folk medicine since time immemorial (14). Its anti-angiogenic, anti-inflamatory, antibacterial, anti-nociceptive activities and inhibit HIV integrase are well documented (12, 15, 16, 17).

MATERIALS AND METHODS

Plant material: Aerial part (leaves) of *Taraxacum officinale* was collected from Plovdiv Bulgaria.

Extraction procedure: Samples of herbs desiccated in the lab at room temperature were pulverized in a laboratory crusher. The powder of ground sample (1.0 g) were carefully extracted with 100 ml of ethanol-water (1:1 v/v), 96 % ethanol and water in a water bath at 80°C for 1 h, stirred for 1 h at room temperature and then filtered.

HPLC analysis: The HPLC analysis of phenolics acids were performed by Waters HPLC systems (Milford, MA, USA) equipped with binary pump (Waters 11525), a UV-VIS detector (Waters 2487) and Breeze 3.30 SPA software. Detailed conditions of HPLC analyses are previously reported by Marchev et al (18).

Author for correspondence

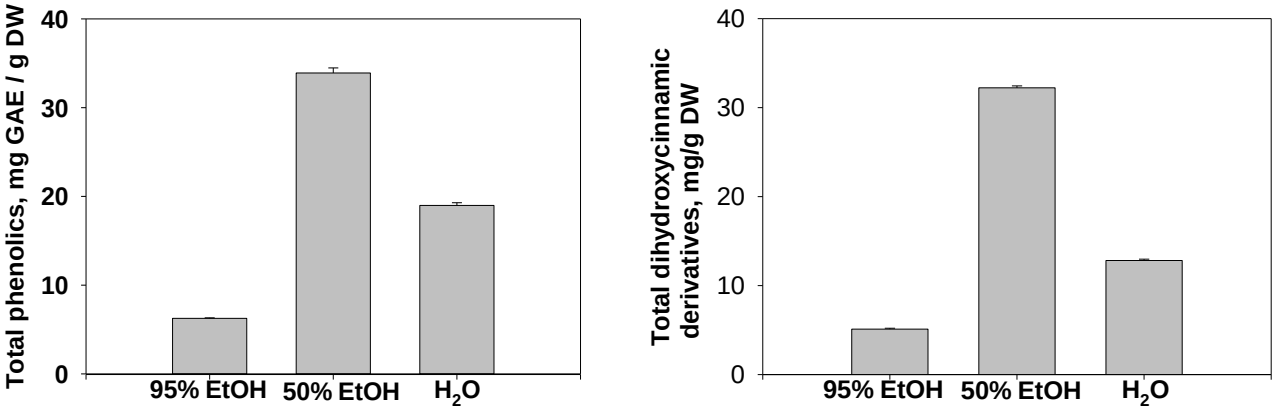

Fig.1: Total phenolics and total dihidrixycinnamic derivatives in different extracts from T. officinale leaves (EtOH – ethanol).

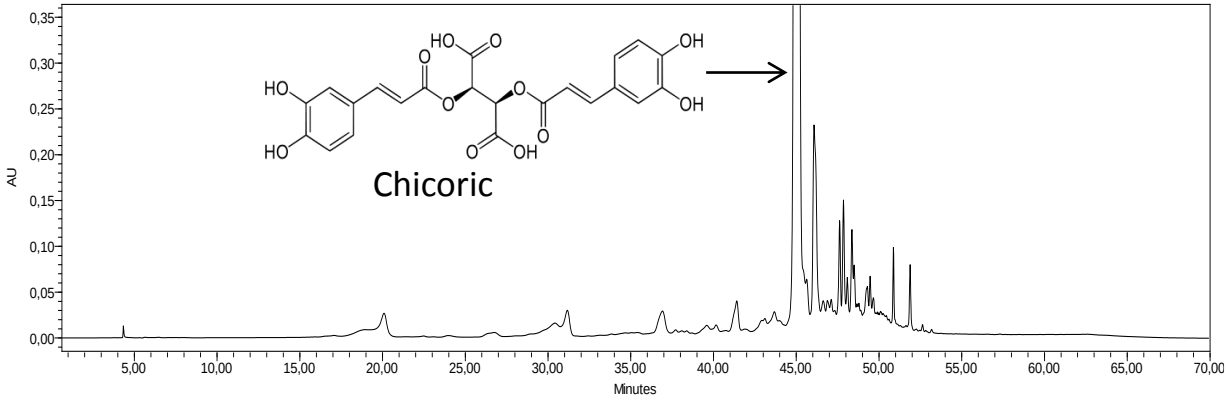

Fig. 2: HPLC chromatogram of extract obtain from T. officinale leaves with 50% ethanol.

Determination of total dihydroxycinnamic derivatives: Total dihydroxycinnamic acid contents (including caffeoyl derivatives) were expressed as chlorogenic acid as previously described in the European Pharmacopoeia (6th ed. 2008). The extract (1 ml) was added to 2 ml 0.5 M HCl, 2 ml Arnow's reagent (10 g sodium nitrite and 10 g sodium moiybdate made up to 100 ml with distilled water), 2 ml NaOH (at a concentration of 2.125 M) and 3 ml of water. Each solution was compared with the same mixture without Arnow's reagent. Absorbance was read at 525 nm. The content of each plant was calculated and expressed as mg chlorogenic acid derivates per g DW (19).

Determination of total polyphenolic compounds: The total phenolic contents were measured using a Folin-Ciocalteu assay according to the procedure described by Stintzing et al. (20) with some modifications. Folin-Ciocalteu reagent (1mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5 % Na_2CO_3. The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve; build in range of 0.02 - 0.10 mg gallic acid used as a standard.

Antioxidant activity (AOA): *DPPH radical scavenging activity*: Investigated extract (150 µl) were mixed with 2850 µl freshly prepared DPPH solution (0.1 mM in methanol). The mixtures were incubated for 15 min at 37 °C in darkness and the reduction in absorbance at 517 nm was measured by spectrophotometer. A standard curve was created with Trolox in concentration between 0.005 and 1.0 mM. The results are expressed in mM Trolox® equivalents (TE) per g dry weight (DW).

Ferris-reducing antioxidant power assay (FRAP): FRAP assay was carried out by method described by Banzie and Strain (21) with some modifications. The reagent solution was freshly prepared by mixing acetate buffer (300 mM) 2,4,6-trypyridyl-s-triazine (TPTZ) solution (10 mM) : $FeCl_3x6H_2O$ (20 mM) (in proportions of 10:1:1; v/v/v). 100 µl of investigated extracts were added to 3000 µl of reagent solution and allowed to react for 10 min at 37 °C. The absorbances of the formed colored product were measured at 593 nm. A standard curve was created with Trolox in concentrations between 0.05 and 1.0 mM. The results are expressed in mM Trolox® (TE) per g DW.

Cupric ion reducing antioxidant capacity: The reaction was started by mixing of 1 ml $CuCl_2xH_2O$ (10 mM in dd H_2O), 1 ml Neocuproine (7.5 mM in methanol), 1 ml ammonium acetate buffer (0.1 M; pH 7.0), 100 µl of investigated extract and 1 ml dd H_2O. The reaction time was 20 min at 50 °C. After cooling, the absorbance (450 nm) was read against a reagent blank, developed on the same way but the extract was replaced with methanol. A standard curve was created with Trolox. The results are expressed in mM Trolox® (TE) per g DW.

Table 1: HPLC profile of polyphenols content in different extracts from T. officinale, mg/100g DW.

Phenolic acids	Extraction with 95% ethanol	Extraction with 50% ethanol	Extraction with water
Chlorogenic acid	18	37	N.D.
Caffeic acid	15	22	N.D.
p-Coumaric acid	12	88	N.D.
Sinapic acid	24	141	26
Ferulic acid	N.D.	97	12
Cichoric acid	484	3148	1195

N.D. – not detected

RESULTS AND DISCUSSION

The extraction yield is a measure of the solvent efficiency to extract specific components from the original materials. In the case of *T. officinale*, it will give an idea about the extractability of total phenolics including the caffeic acid derivatives under different extraction conditions. Therefore, in the present study, only the traditional solvent extraction was used to extract the phytochemicals in dandelion leaves. The established total polyphenolic compounds and total caffeic acid derivatives of *T. officinale* leaves were significantly affected by the etanol concentration. The higest total polyphenolic concentration and total total dihidrixycinnamic derivatives (33.90 ± 0.57 mg GAE/ g DW and 32.22 ± 0.22 mg CAE/ g DW, respectively) ware registered by extraction with 50 % ethanol (Figure 1). The contents of total phenolics, individual and total caffeic acid derivatives in extracts were also affected by the ethanol concentrations (Table 1). These results are related to solvent polarity and solubility of compounds in tested solvents (22). In this study, when the ethanol volume percentage was lower than 50%, the contents of total phenols, cichoric acid, and total caffeic acid derivatives were increased with the increases of ethanol concentrations. However, the total phenols and total caffeic acid derivatives contents in extracts were decreasing when the ethanol concentration was 50%. This result is in agreement with the report of Tsai et al (23), that 50% ethanol is favorable for extracting individual caffeic acid derivatives from *E. purpurea*.

Phenolic acids are secondary metabolites that are commonly found in plant-derived foods. They have attracted considerable interest due to their many potential health benefits, which are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, antiinflammatory, and vasodilatory actions (24).

The chemical composition of the phenolic acids of *T. officinale* in different extracts was determined by HPLC (Figure 2). As shown in Table 1, six components were identified (chlorogenic, caffeic, *p*-coumaric, sinapic, ferulic and cichoric acid). The most abundant phenolics acid in investigated samples were cichoric acid (484, 1195 and 3148 mg/100g DW) and sinapic acid (between 24-141 mg/100g DW) in various extracts of *T. officinale*.

Echinacea purpurea root and *Ocimum basilicum* L. have been reported to be a major human dietary sources of chicoric acid (25, 26). The yield of chicoreic acid in thus plants between 1.0 g/100g DW – 2.0 g/100g DW by *E. purpurea* (25, 27), and *O. basilicum* between 52-88 mg/100g DW (26). *T. officinale* biosynthesized about 2 times more chicoric acid (3.1 g/100g DW) (Table 1) than *E. purpurea*, similar results for yield of chicoreic acid obtain Fraisse et al. (19) – 3.4 g/100g DW. Amount of extracted cichoreic acid from dandelion leaves with 50% ethanol was up to 89 % from total yield of extracted phenolics (Table 1.). This results show that next purification and crystallization of chicoric acid from 50% ethanol extracts would be easily than extracts obtain from *E. purpurea*.

In recent years, interest in plant-derived food additives has grown. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically. Plant-derived food additives, especially polyphenolic compounds, have been ascribed health-promoting properties (28).

In our study, we decided to evaluate antioxidant activities of ethanol extracts of *T. officinale* by applying two methods, based on mixed [hydrogen atom transfer (HAT) and single electron transfer (SET)] mechanisms (DPPH) and two methods, based only on SET mechanism (FRAP and CUPRAC) (Table 2). Antioxidant activities of plant extracts were usually explained with the presence of phenolic acids in them (29). Thus, the 50 % ethanol extracts of *T. officinale* had the highest antioxidant activities and the highest total phenolics. Relatively high concentrations of cichoreic acid and sinapic acid in extracts of investigated plant were the most probable reason for observed high antioxidant activities in this plant. Table 2. presents data on the antioxidant activity of the extracts from *T. officinale* determined in vitro by DPPH radical scavenging activity and metal reducing activity. These results are expressed as mM Trolox® (TE) per g DW of total antioxidant capacity for each extract. Species that have the highest antioxidant activities are the richest in polyphenols and chicoric acid, while the least active species are less rich in polyphenols (Figure 1, Table 2). On the whole, the antioxidant activities of the extracts compared to their different polyphenolic levels showed good correlations between total phenolics or total dihydroxycinnamic derivatives and plant antioxidant capacity, i.e. $R^2 = 0.8951$ and $R^2 = 0.9977$, respectively, and a relatively very good correlation between chicoreic acid determined by HPLC and antioxidant activity, at $R^2 = 0.9992$ (DPPH method), $R^2 = 0.9867$ (FRAP method) and $R^2 = 0.9999$ (CuPRAC method). The established relationships it can be conducted that the presence of the cichoreic acid seemed to be the main factor dictating free radical scavenging and metal reducing activity of the extracts.

CONCLUSION

Table 2: Antioxidant activity in different extracts from *T. officinale*.

Extracts	Radical scavenging activity		Metal reducing activity	
	DPPH	EC $_{50}$, mg/ml	FRAP	CuPRAC
95% ethanol extract	28.1 ± 0.8*	17.2	26.1 ± 0.2	97.1 ± 0.1
50% ethanol extract	136.3 ± 4.7	3.5	131.5 ± 2.2	407.8 ± 7.5
Water extract	54.1 ± 1.1	9.0	42.5 ± 4.7	180.1 ± 1.5
Standart				
Chlorogenic acid		0.15		

*- *mM TE/ g DW*

The current report is the first comprehensive study that presented detailed information for phenolic, content and antioxidant activity of edible dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg.) grown in Bulgaria. The antioxidant potential of extracts positively correlated with their phenolic contents and, respectively. Dandelion is important source of cichoric acid with potential application as radical scavengers and metal reducing activity. Therefore, this complex of biologically active substance offers many future applications in field of herbal medicine and nutrition for production of healthy food with well-pronounced healthy effect

REFERENCES

1. Schütz, K., Carle, R., Schieber, K. Taraxacum – a review on its phytochemical and pharmacological profile. *Journal of Ethnopharmacopogy*, 2006, 107: 313-323
2. Önal, S., Timur, S., Okutucu, B., Zihnioglu, F. Inhibition of L-glucosidase by aqueous extract of some potent antidiabetic medicinal herbs. *Preparative Biochemistry and Biotechnology* 2005, 35: 29–36.
3. Dorsch, W. Clinical application of extracts of *Echinacea purpurea* or Echinacea pallid. Critical evaluation of controlled clinical studies. *Zeitschrift für-ärztliche Fortbildung* 1996, 90: 117–122.
4. Shidoji, Y, Ogawa, H. Natural occurrence of cancer-preventive geranylgeranoic acid in medicinal herbs. The Journal of Lipid Research 2004, 45: 1092–1103.
5. Koh, Y-j., Cha, D-S., Ko, J-S., Park, H-J., Choi, H-E Anti-inflammatory effects of *Taraxacum officinale* leaves on lipopolysaccharide-induced inflammatory reslonses in RAW 264.7 cells. *Journal of Medicinal Food*, 2010, 13(4): 870-878
6. Hu, C., Kitts, D.D. Dandelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. *Phytomedicine*. 2005, 12: 588–597.
7. Wan, S.S., Koo, H.N., An, H.J., Kwon, K.B., Lim, B.C., Seo, E.A., ***et al.*** *Taraxacum officinale* protects against cholecystokinin-induced acute pancreatitis in rats. *World Journal of Gastroenterology* 2005, 11: 597–599.
8. Lans, C., Turner, N., Khan, T., Brauer, G., Boepple, W. Ethnoveterinary medicines used for ruminants in British Columbia, Canada. *Journal of Ethnobiology and Ethnomedicin*e 2007, 3: 1–22.
9. Clare, B.A., Conroy, R.S., Spelman, K. The diuretic effect in human subjects of an extract of *Taraxacum officinale* folium over a single day. *Journal of Alternative and Complementary Medicine*, 2009, 15: 929–934.
10. He, W., Han, H., Wang, W., Gao, B. Anti-influenza virus effect of aqueous extracts from dandelion. *Virology Journal*, 2011, 8: 538
11. Tahtamouni, L. H., Alqurna, N. M., Al-Hudhud, M. Y., Al-Hajj, H. A. Dandelion (*Taraxacum officinale*) decrease male rat fertility in vivo. *Journal of Ethnopharmacopogy*, 2011, 135: 102-109
12. Han, H., He, W., Wang, W., Gao B. Inhibitory effect of aqueous dandelion extract on HIV-1 replication and reverse transcriptase activity. *BMC Complementary and Alternative Medicine*, 2011, 11: 112
13. Colle, D., Arantes, L. P., Gubert, P., Almeida da Luz, S. C., Athayde, M. L., Rocha, J. B. T., Soares, F. A. A. Antiohidant properties of *Taraxacum officinale* leaf extract are involved in the protective effects against hepatoxicity induced by acetaminophen in mice. *Journal of Medicinal Food*, 2012, 15(6): 549-556
14. Chkhikvishvili, I.D., Kharebava, G.I. Chicoric and chlorogenic acid in plant species from Georgia. *Applied Biocemistry and Microbiolog*, 2001, 37(2): 188-191
15. Jeon, H.J., Kang, H.J., Jung, H., Kang, Y.S., Lim, C.M., Kim, Y.M. Anti-inflammatory activity of *Taraxacum officinale*. *Journal of Ethnopharmacology* 2008, 115: 82–88.
16. Kaur, K., Bani, S. Chicoric acid regulates behavior and biochemical alterations induced by chronic stress in experimental Swiss albino mice. *Pharmacology, Biochemistry and Bihavior*, 2011, 99: 342-348
17. Sohail M., Iqbal Z., Afzal M., Afzal A., Ur Rahman I., Shad S., ***et al.*** 2014 In vitro antibacterial study of *Taraxacum officinale* leaves extracts against different bacterial pathogenic strains, *Journal of Pharmacognosy and Phytochemistry*, 3(2): 15-17
18. Marchev, A., Georgiev, V., Ivanov, I., Badjakov, I. and Pavlov, A. Two-phase temporary immersion system for *Agrobacterium rhizogenes* genetic transformation of sage (*Salvia tomentosa* Mill.). *Biotechnol Lett*. 2011, 33 (9): 1873-1878.
19. Fraisse D., Felgines C., Texier O., Lamaison J-L. Caffeoyl derivatives: Major antioxidant compounds of some wild herbs of the Asteraceae family, *Food and Nutrition Sciences*, 2011, 2: 181-192
20. Stintzing, F.C., Nerbach, K.M., Mosshammer, M., Carle, R., Yi, W., Sellappan, S., Acoh, C.C., Bunch, R., Felker, P. Color, betalain pattern, and antioxidant

properties of cactus pear (*Opuntia* spp.) clones. *J. Agric Food Chem*. 2005, 53 (2): 442-451.
21. Benzie, I.F. and Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem*. 1996, 239 (1): 70-76.
22. Xi, J., Shen, D., Zhao, S., Lu, B., Li, Y., Zhang, R. Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. *International Journal of Pharmaceutics*, 2009, 382: 139-143.
23. Tsai Y-L., Chiou S-Y., Chan K-C., Sung J-M., Lin S-D. Caffeic acid derivatives, total phenols, antioxidant and antimutagenic activities of Echinacea purpurea flower extracts, *LWT - Food Science and Technology*, 2012, 46: 169-176
24. Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T., Polyphenols and antioxidant capacity of Bulgarian medicinal plants, *Journal of Ethnopharmacology*, 2005, 96: 145–150
25. Luo X-B., Chen B., Yao S-Z., Zeng J-G. Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of *Echinacea purpurea* by high-performance liquid chromatography-photodiode array detection-electrospray mass specctometry, *Journal of Chromatography A*, 2003, 986: 73-81
26. Lee J., Scagel C.F. Chicoric acid found in basil (*Ocimum basilicum* L.) leaves, *Food Chemistry*, 2009, 115: 650-656
27. Zolgharnein J., Niazi A., Afiuni-Zadeh S., Zamani K. Determination of cichoreic acid as a biomarker in Echinacea purpurea cultivated in Iran using high performance liquid chromatography, *Chinese Medicine*, 2010, 1: 23-27
28. Hinneburg I., DormanH.J.D. Hiltunen antiohidant activities of extracts from selected culinary herb and spices. *Food Chemistry*, 2006, 97: 122-129
29. Ivanov I.G., Vrancheva R.Z., Marchev A.S., Petkova N.T., Aneva I.Y., Denev P.P., Georgiev V.G., Pavlov A.I. Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species, *Int.J.Curr.Microbiol.App.Sci*, 2014, 3(2): 296-306