Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 12, pp.43735-43738, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY AND PHENOLIC PROFILES WITH HPLC OF *CONSOLIDA ORIENTALIS*

[1]Seyda Akkaya, [2]Merve Badem, [2]SilaOzlem Sener, [1]Nuriye Korkmaz and [1,*]Rezzan Aliyazicioglu

[1]Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey
[2]Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

ARTICLE INFO

Article History:
Received 14th September, 2016
Received in revised form
10th October, 2016
Accepted 28th November, 2016
Published online 30th December, 2016

Key words:

Consolida orientalis,
Antioxidant,
HPLC,
Phenolics.

ABSTRACT

Background and Objectives: *Consolida* genus belongs to Ranunculaceae and includes about 52 species world-wide. The aim of our study was to investigate in terms of phenolic contents and antioxidant capacity of *Consolida orientalis.*

Materials and Methods: Reverse phase-high performance liquid chromatography (RP-HPLC) was used for the identification of the phenolic compounds of *C.orientalis*. Antioxidant activity of methanolic extract was investigated by two methods, namely ferric reducing antioxidant power (FRAP), and 2,2-diphenylpicrylhydrazyl radical scavenging (DPPH) activity.

Results: Chlorogenic acid, *p*-hydroxy benzoic acid, caffeic acid, *p*-coumaric acid, and sinapic acid were determined as main phenolic compounds in the methanolic extract. The IC_{50} value for DPPH assay has been found as 0.4178 ± 0.0113 (mg/mL), FRAP value is 200 ± 1.732 (μMTrolox/g sample), and total phenolic content value is 6.6 ± 0.153 mg gallic acid per gram sample in methanolic extract of the aerial parts of *C.orientalis*.

Conclusion: The methanolic extract of *C.orientalis* might be used as raw material by pharmaceutical and food industries for the preparation of natural drugs.

Citation: Seyda Akkaya, Merve Badem, SilaOzlem Sener, Nuriye Korkmaz and Rezzan Aliyazicioglu, 2016. "Antioxidant activity and phenolic profiles with hplc of Consolida orientalis", *International Journal of Current Research*, 8, (12), 43735-43738.

INTRODUCTION

The fact that natural products can serve as medicinal agents has been known for several millennia. Rapid advances in science and technology mean that many drugs with fewer side-effects can now be derived from natural products (Gupta and Raina, 1998). Numerous novel compounds are produced from traditional medicinal or herbal products on an annual basis (Elumalai *et al.,* 2011). The compounds isolated from herbal plants and their components are of particular significance to the pharmaceutical industry (Hostettmann *et al.,* 1998; Balandrin *et al.,* 1985). Free radicals give rise to the oxidation of biomolecules, resulting in cell injury and death (Brand-Williams *et al.,* 1995). Additionally, the oxidative stress resulting from disequilibrium between the production of free radicals and their elimination by the antioxidant system is implicated in numerous human diseases, including aging, cancer and neurodegenerative conditions such as Alzheimer's, Parkinson's and Huntington's diseases (Nabavi *et al.,* 2008). *Consolida orientalis* Rech. f. (Ranunculaceae) is indigenous to parts of southern and south-western Europe, central Asia and north Africa. It is generally found as a weed of winter crops, particularly winter wheat, and more rarely in winter oil seed rape and winter barley. *C. orientalis*is more homogenous in regions where it is well naturalized and which contain higher proportions of winter crops. Lack of effective control allows this weed species to propagate easily in crop stands. The purpose of this study was to evaluate methanolic extract of *C. orientalis*as new potential sources of natural antioxidants.

****Corresponding author: Rezzan Aliyazicioglu***
Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey.

MATERIALS AND METHODSS

Chemicals and Instrumentation

The phenolic standards employed were all of HPLC grade. Gallic acid, protocathechuic acid, proto-catechuic aldehyde, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, syring aldehyde, *p*-coumaric acid, ferulic acid, sinapic acid, and benzoic acid were obtained from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany). Acetic acid, acetonitrile, methanol, trolox (6-hydroxy–2,5,7,8-tetramethylchroman–2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), and Folin-Ciocalteu's phenol reagent were obtained purchased from Merck (Darmstadt, Germany) and FlukaChemie GmbH (Buchs, Switzerland). Sartorius (Goettingen, Germany) supplied the polytetrafluoroethylene membranes (porosity 0.45 μm) used

for extract filtration. High performance liquid chromatography (HPLC) (Agilent 1100, DAD 1200 Agilent Technologies, Waldbronn, Germany) was used to analyze phenolic compounds. The experimental set-up included a reverse-phase waters spherisorp ODS2-C18 column (4.6×250 mm, 5 µm) on a gradient program with a two-solvents system (A: 2% acetic acid in water; B: 0.5% acetic acid in acetonitrile: water [1:1]) at an uninterrupted solvent flow rate of 1.2 $mL.min^{-1}$. The injection volume was set at 20 µL. Signals were identified at 232, 246, 260, 272, 280, 290, 308 and 328 nm by means of DAD and at 280 nm via UV detection. The column temperature was fixed at 25°C.

Preparation of extract for phenolic and antioxidant analysis

C. orientalis specimens were collected from Gumushane in Turkey between May and June, 2014. Biological authentication was performed by Professor UfukOzgen. One gram of desiccated powder obtained from aerial parts of *C. orientalis* was extracted with 20 mL methanol in a glass jar linked to the condenser inside a sonicator apparatus at 60 ºC over the course of 3 h. Antioxidant activities were investigated by taking 10 mL from each extract. The remaining methanol extracts were subsequently evaporated to dry state before concentration at 50 ºC with the assistance of a rotary evaporator. The resulting crude extract was next dissolved in 10 mL distilled water prior to liquid-liquid extractions. Extraction was performed three times using 5 mL diethyl ether and 5 mL ethyl acetate. After the organic moiety had been picked up in the same flask it was evaporated to dry state under lowered pressure using a rotary evaporator at 40 °C. Finally, the resulting residue was weighed and dissolved in methanol for HPLC assay.

Determination of Antioxidant Capacity

Total phenolic contents (TPC) were identified using the technique previously described by Folin-Ciocalteau with gallic acid as standard. In summary, 0.1 mL of differing concentrations of gallic acid and methanolic specimens (1 $mg.mL^{-1}$) were diluted with 5.0 mL distilled water. In the next stage, we added 0.5 mL of 0.2 N Folin-Ciocalteu reagent, after which they were subjected to vortexing. In the subsequent phase, 1.5 mL of Na_2CO_3 (2%) solution was added after a 3-min period of incubation. The resulting mixture was then incubated, with periodic shaking, for 2 h at 20 ^{0}C. Absorbance was calculated at 760 nm at the termination of the incubation period. TPC concentrations were expressed as mg of gallic acid equivalents per gram of 100 g sample using a standard chart. We employed the ferric-reducing/antioxidant power (FRAP) method previously described by Benzie and Strain (1996), with various minor modifications, to measure antioxidant activities in methanolic specimens. FRAP reagent was obtained by combining 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mMHCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Next, 100 µL of sample was mixed with 3 mL of the freshly produced FRAP reagent. This mixture was next incubated at 37 ^{0}C for 4 min. Absorbance was calculated at 593 nm against a blank obtained using distilled water and incubated for 1 h, rather than 4 min. We used a calibration curve with Trolox concentrations between 100 and 1000 µM. Trolox® was also tested under identical conditions as a standard antioxidant compound for purposes of comparison. FRAP values were expressed as µM Trolox equivalent of g sample. Butylated hydroxytoluene (BHT) served as a reference antioxidant substance during the DPPH assay (Molyneux, 2004). Varying concentrations of 0.75 mL of methanolic extract of *C. orientalis* were mixed together with 0.75 mL of 0.1 mM of DPPH in methanol. Radical scavenging activities are given in the form of IC_{50} (mg sample per mL), indicating the concentration of samples giving rise to 50% scavenging of DPPH radicals. All absorbances were calculated by means of a Spectro UV-Vis Double PC–8 auto cell spectrophotometer (Labomed Inc., California, U.S.A.). Solutions were prepared using deionized water purified in an Elgacan® C114 Ultra Pure Water System Deionizer device (The Elga Group, Buckinghamshire, England). Evaporation took place with the assistance of an IKA® RV 05 Basic (IKA®, Werke, USA) rotary evaporator system, while extraction involved a Heidolph Promax 2020 (Heidolph Instruments GmbH & Co., Schwabach, Germany) shaker. Use was also made of a Heidolph Reax top vortex (Heidolph Instruments GmbH & Co., Schwabach, Germany) and an Elma® Transsonic Digital ultrasonic water bath (Singen/Htw, Germany) in the course of all dissolution procedures. A Hanna (HI 110 series) instruments microprocessor pH meter (Hanna Instruments Inc., Rhode Island, U.S.A.) was used to measure pH and a Mettler Toledo (Mettler-Toledo GmbH., Gießen, Germany) scale for all weight calculations, expressed in grams.

RESULTS AND DISCUSSION

There has recently been growing interest on the part of the public and the medical profession in the use of indigenous drugs for therapeutic purposes (Siddique *et al.,* 2010). Plant foods (fruits, grains and vegetables) have attracted particular interest due to the nutrients and bioactive components (phytochemicals) they contain. Polyphenols are micronutrients found in considerable quantities in the human diet and also in a wide range of medicinal plants (Manach *et al.,* 2004). The antioxidant properties of phenolics found in functional foods are the result of direct free radical scavenging and reducing activities and of the indirect effect of chelation of metal ions (Mustafa *et al.,* 2010; de Oliveira *et al.,* 2009). The possible therapeutic applications of a great many traditional medicinal plants are thus in large part due to their phenolic contents (Aliyazicioglu *et al.,* 2015). Various studies (Stanojevic *et al.*, 2009; Siddique *et al.*, 2010; Aliyazicioglu *et al.,* 2013) have reported positive correlation between phenolic compound contents and their antioxidant properties. Variations in antioxidant activities may thus be ascribed more to the nature of the phenolic compounds in question, from phenolic acids to flavonoids, than to their contents (Kahkonen*et al.,* 2009; Mhamdi *et al.,* 2010). For instance, the radical scavenging activities exhibited by phenolic acids and by their derivatives, including esters, as well as flavonoids in plants may be attributed to the number of hydroxyl groups within the molecules (Soobrattee *et al.*, 2005; Siddique *et al.,* 2010). Some extracts exhibit low levels of antioxidant activities despite containing high phenol/flavonoid concentrations. Others, meanwhile, exhibit high levels of antioxidant activity but very low phenol/flavonoid concentrations. The differing reactions displayed by phenolic compounds to the Folin–Ciocalteu reagent derive from the numbers of their phenolic hydroxyl groups (Wong *et al.,* 2006). Total phenol compound, as determined by the Folin Ciocalteau method, was reported as gallic acide quivalents. TPC value of *C. orientalis* extract was calculated at 6.6 ± 0.153 mg GAE/100 g dry weight (DW) (Table 1).

Table 1. The antioxidant activities of the methanolic extract (MeOH) of *C.orientalis*

Test compounds	TPC[1]	FRAP[2]	DPPH[3]
MeOH	6.6± 0.153	200±1.732	0.4178±0.0113
BHT			0.0099 ± 0.0002

[1]Total phenolic content expressed in mg of gallic acid equivalent (GAE) per gram of dry plant weight, [2]Expressed as µM trolox equivalents (TE) per gram of dry plant weight, [3]Concentration of test sample (mg/mL) required to produce 50 % inhibition of the DPPH radical.

Table 2. Phenolic composition of the methanolicextract of *C.orientalis*

Phenolic compound assignment	Retention time (min)	Amount (mg/100 g)
Gallic acid	7.582	5.81
Protocatechuic acid	10.860	25.41
Protocatechuic aldehyde	14.103	4.80
p-OH-benzoic acid	15.935	138.51
Chlorogenic acid	16.890	147.58
Vanillic acid	18.561	15.13
Caffeic acid	19.575	1455
Vanillin	24.198	6.11
Syringaldehyde	26.621	10.87
p-Coumaric acid	28.531	372.17
Ferulic acid	31.920	12.64
Sinapic acid	32.726	107.85
Benzoic acid	35.168	5.90

The IC_{50} value for DPPH assay has been found as 987.11±28.66 (mg/mL), and total phenolic content value is 38.83 ± 2.09 mg gallic acid per gram sample in ethylacetate extract of the aerial parts of *C. orientalis* (Dehpour, 2016). Various biologically active compounds in plants defend against a range of different physical and chemical hazards, including diseases, parasites and bacteria (Kolayli *et al.,* 2010; Aliyazicioglu *et al.,* 2013). Their phenolic contents also mean that they are capable of exhibiting bioactive characteristics. Specimens will almost always exhibit a range of different phenolic compounds, meaning that it may be difficult to measure these on an individual basis. RP-HPLC was employed for the analysis of 13 phenolic acids, including gallic acid, proto-catechuic acid, proto-catechuic aldehyde, p-hydroxy benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, syringaldehyde, *p*-coumaric acid, ferulic acid, sinapic acid, and benzoic acid. The RP-HPLC chromatograms derived from the standard phenolic compounds in the current research are presented in Fig. 1.Comparison of the individual phenolic compounds contents identified caffeic acid as the main phenolic component in aerial parts of *Consolida orientalis.* Also, chlorogenicacid, *p*-OH-benzoicacid, *p*-coumaricacid, and sinapic acid was identified from aerial parts of *Consolida orientalis* (Fig. 2 and Table 2). The stable DPPH radical scavenging model is a technique commonly to assess the free radical scavenging properties of different specimens (Lee *et al.,* 2003). DPPH is a stable nitrogen-centered free radical that changes from violet to yellow in color following reduction by means of hydrogen or electron donation. Substances capable of carrying out this reaction may be regarded as antioxidants and consequently as radical scavengers (Brand-Williams *et al.,* 1995). The radical scavenging activity of the extract was observed to increase in a concentration-dependent manner. This plant's high total phenol contents may account for its excellent DPPH scavenging properties. Hodzic *et al.* (2009) reported that the FRAP assay can be employed to evaluate antioxidant activity because it can be carried out easily and quickly. Additionally, the reaction in question is reproducible and correlated with the molar concentration of antioxidants in a linear manner.

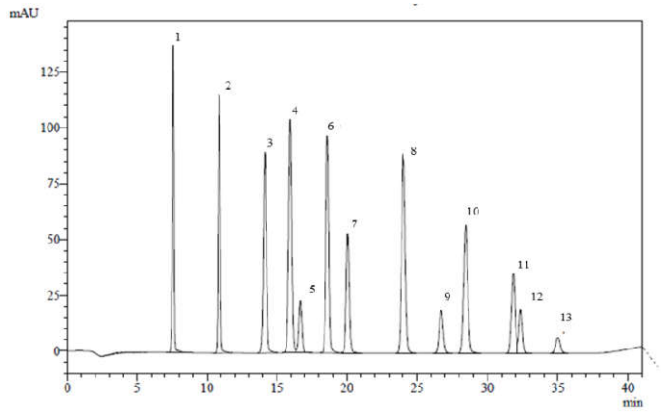

Figure 1. RP-HPLC chromatogram of phenolic standards (25 µM) searched in *Consolida orientalis* samples. Waters spherisorp ODS2 -C18 column (4.6×250 mm, 5 µm), gradient eluent acetic acid/ acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (1) gallic acid, (2) *proto*-catechuic acid, (3) *proto*-catechuic aldehyde, (4) *p*-OH benzoic acid, (5) chlorogenic acid, (6) vanillic acid, (7) caffeic acid, (8) vanillin, (9) syring aldehyde, (10) *p*-coumaric acid, (11) ferulic acid, (12) sinapic acid, (13) benzoic acid.

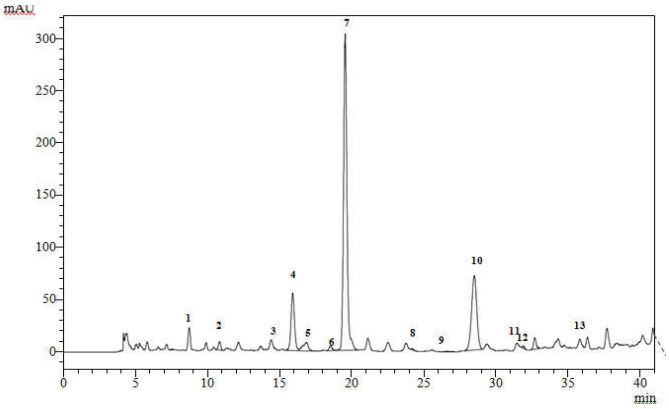

Figure 2. RP-HPLC DAD chromatogram of *C.orientalis* pH 7 methanol extract (50 mg/mL) at 280 nm. Waters spherisorp ODS2 -C18 column (4.6×250 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (1) gallic acid, (2) *proto*-catechuic acid, (3) *proto*-catechuic aldehyde, (4) *p*-OH benzoic acid, (5) chlorogenic acid, (6)vanillic acid, (7) caffeic acid, (8) vanillin, (9) syring aldehyde, (10) *p*-coumaric acid, (11) ferulic acid, (12)sinapic acid, (13) benzoic acid.

However, there are also a number of disadvantages to this technique, since the assay does not exhibit rapid reaction with various antioxidants, including glutathione (Guo*et al.,* 2003). Schafer and Buettner *et al*. (2001) recommended that the FRAP method is nevertheless suitable for use in evaluating antioxidant activity in plant materials, since humans absorb only very low levels of glutathione. Higher FRAP values indicate a greater antioxidant capacity since they are based on the reduction of ferric ion, in which antioxidants constitute the reducing agent. Antioxidants are able to donate a single electron or hydrogen atom for reduction.

Conclusions

In conclusion, several antioxidant assays, and HPLC method were utilized in order to evaluate the biological properties of *C.orientalis* extract. The methanolic extract exhibited powerful antioxidant activity. Especially, chlorogenic acid, *p*-OH-benzoic acid, *p*-coumaric acid, and sinapic acid were main phenolic compounds present in methanolic extract of *C.orientalis* in high amounts and may be assessed as possible

markers for the botanical classification of *Consolida* genus. In addition, *C.orientalis* extract may be an attractive source of nutraceuticals and medicinal ingredients.

Conflict of interest statement: The authors inform that there was no conflict of interest.

Acknowledgements: The authors thank UfukOzgen for her assistance in plant authentication.

REFERENCES

Aliyazicioglu R, Sahin H, Ulusoy E, Erturk O, Kolayli S. 2013. Properties of the phenolic composition and biological activity of the propolis from Turkey.*Int. J. Food Prop*., 16: 277-287.

Aliyazicioglu R, Yildiz O, Sahin H, Eyupoglu OE, Ozkan MT, Alpay Karaoglu S, Kolayli S. 2015. Phenolic Components And Antioxidant Activity of *Prunusspinosa* from Gumushane, Turkey. *Chem. Nat. Comp.,* 51: 346-349.

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. 1985. Natural plantchemicals: sources of industrial and medicinalmaterials. *Science*, 228 (4704):1154–1160.

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal.Biochem*., 239: 70-76.

Brand-Williams W, Cuvelier M, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *FoodSci. Technol.,* 28: 25–30.

Dehpour AA. Antioxidant Activity and Isolation of β-Sitosterol from Ethyl Acetate Extract of Aerial Parts of *Consolida orientalis.* Preprints., 2016. 2016090037 (doi: 10.20944/preprints201609.0037.v1).

Elumalai E, Ramachandran M, Thirumalai T, and Vinothkumar P. 2011. Antibacterial activity of various leaf extracts of Merremiaemarginata. *Asian Pac J Trop Biomed,* 1 (5):406–408.

Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. 2003. Antioxidant activities of peel, pulp, and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, 23 (12): 1719–1726.

Gupta LM. and Raina R. 1998. Side effects of some medicinal plants. *Current Science,* 75: 897-900.

Hodzic Z, Pasalic H, Memisevic A, Scrabovic M, Saletovic M, Poljakovic M. 2009. The influence of total phenolscontent on antioxidant capacity in the whole grain extracts. *European Journal of Scientific Research*, 28:471–477.

Hostettmann K, Potterat O, and Wolfender J-L. 1998. The Potential of Higher Plants as a Source of New Drugs. *CHIMIA International Journal for Chemistry*, 52(1–2):10–17.

Kahkonen MP, Hopia A, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*., 47: 3954-3962.

Kolayli S, Kara M, Tezcan F, Erim FB, Sahin H, Ulusoy E, Aliyazicioglu R. 2010. Comparative study of chemical and biochemical properties of different melon cultivars: Standard, hybrid, and grafted melons. *J. Agric. Food Chem.,* 58: 9764-9769.

Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. 2003. Screening of medicinal plant extracts for antioxidant activity. *Life Sci.,*73:167–179.

Manach C, Scalbert A, Morand C, Rémésy C, Jimenez L. 2004. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.,* 79: 727-747.

Mhamdi B, AidiWannes W, Sriti J, Jellali I, Ksouri R, Marzouk B. 2010. Effect of harvesting time on phenolic compounds and antiradical scavenging activity of Boragoofficinalis seed extracts. *Ind. Crop. Prod.*, 31: 1-4.

Molyneux P. 2004.The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity.*Songklanakarin. J. Sci. Technol.,* 26: 211-219.

Mustafa RA, Abdul Hamid A, Mohamed S, Abubakar F. 2010. Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants*. J. Food Sci.,* 75: 28-35.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Jafari M. 2008. Free radicals cavenging activity and antioxidant capacity of Eryngium caucasicum Trautv and Froripiasubpinata. *Pharmacologyonline,*3:19-25.

Oliveira AC, Valentim IB, Silva CA, Bechara EJH, de Barros MP, Mano CM. 2009. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chem.*, 115: 469-475.

Schafer FQ, Buettner GR. 2001. Redox environment of the cell as viewed through the redoxstate of the gluthathionedisulfide/gluthathione couple. *Free Radical Biology Medicinal*, 30(11): 1191-1212.

Siddique NA, Mujeeb M, Najmi AK, Akram M. 2010. Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of Aeglemarmelos. *Afr. J. Plant. Sci.,* 4: 1-5.

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. 2005.Phenolics as potential antioxidant therapeutic agents: *Mechanism and actions. Mut. Res. Fund. Mol. Med.,* 579: 200-213.

Stanojevic L, Stankovic M, Nikolic V, Nikolic L, Ristic D, Canadanovic-Brunet J, Tumbas V. 2009. Antioxidant activity and total phenolic and flavonoid contents of *Hieraciumpilosella* L. extracts. *Sensors,* 9: 5702-5714.

Wong SP, Leong LP, Koh JHW. 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.*, 99: 775-783.

****