

ANTIOXIDANT ACTIVITY OF *HYPERICUM TRIQUETRIFOLIUM* TURRA METHANOL EXTRACT

Khulood W. Abbood¹, Ruqaia M. Ibraheem¹ and Ali H. Ad'hiah^{2*}

¹Biotechnology Department, College of Science, Al-Nahrain University, Baghdad, Iraq.

²Tropical-Biological Research Unit, College of Science, University of Baghdad,
Baghdad, Iraq.

Article Received on
05 Nov 2014,

Revised on 30 Nov 2014,
Accepted on 25 Dec 2014

***Correspondence for
Author**

Dr. Ali H. Ad'hiah
Tropical-Biological
Research Unit, College of
Science, University of
Baghdad, Baghdad, Iraq.

ABSTRACT

Hypericum triquetrifolium is a promising medicinal plant from Eastern Europe and the Mediterranean area. It is traditionally used for its sedative, anti-helminthic, anti-inflammatory, and anti-septic effects. In addition, several studies have reported the potential use of its crude extracts as therapeutic substances, mainly in the treatment of burns and gastroenteritis, and as anti-nociceptive and anti-oxidant drug. Therefore, it was aimed to determine the total flavonoid content and reductive ability of the plant methanol extract, in addition to assessment of anti-oxidant activity on the basis of the radical scavenging effect of the stable DPPH free radical. Results revealed that the extract contained 115.73 ± 5.65 µg/ml flavonoids and showed a

high reductive ability and anti-oxidant activity. Accordingly, it was concluded that *H. triquetrifolium* is a rich source of flavonoids that lend the plant anti-oxidant and radical scavenging activities.

KEYWORDS: *Hypericum triquetrifolium*, flavonoids, reductive ability, anti-oxidant activity.

INTRODUCTION

The vast majority of people on this planet still rely on their traditional *materia medica* (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs, and according to the World Health Organization (WHO), 80% of the world's population, primarily those of developing

countries, rely on plant-derived medicines for their healthcare.^[1] This is reasoned by the fact that medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health, and accordingly, they have been subjected to an intensive investigation to reveal their pharmaceutical potentials.^[2] One of these potentials is anti-oxidant activity and one of the medicinal plants is *Hypericum triquetrifolium* Turra, which has been used in traditional Arab herbal medicine to treat various diseases. The most common names of the plant are Dathi, Nabtat Yohanna, tangled hypericum, wavy leaf St. John's wort and curled leaved St. John's wort.^[3,4] The plant is native to Eastern Europe and the Mediterranean area and has been traditionally used for its sedative, anti-helminthic, anti-inflammatory and anti-septic effects.^[5] In addition, several studies have reported the potential use of its crude extracts as therapeutic substances; for instance, treatment of burns and gastroenteritis, as well as its anti-inflammatory and anti-oxidant potentials have been suggested.^[6,7]

The human body has a complex system of natural enzymatic and non-enzymatic anti-oxidant defenses, which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, aging and atherosclerosis.^[8] Protection against free radicals can be enhanced by ample intake of dietary anti-oxidants, and there is a substantial evidence indicates that nutrients containing anti-oxidants and medicinal plants or their secondary metabolite are of a major importance in disease prevention; therefore, anti-oxidants are of a great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases due to oxidative stress.^[9]

One of the important anti-oxidants is flavonoids, which represent a range of polyphenolic compounds naturally occurring in plants; including *H. triquetrifolium*.^[10] Flavonoids are potentially involved in cardiovascular prevention mainly by decreasing oxidative stress and increasing NO bioavailability; therefore the estimation of flavonoid content in plant pay important roles in providing protection against reactive oxygen species (ROS).^[11] Accordingly, the present investigation was planned with the aims to determine the total flavonoid content and reductive ability of *H. triquetrifolium* methanol extract, in addition to assessment of anti-oxidant activity.

MATERIALS AND METHODS

Preparation of Plant Extract

The aerial parts of *H. triquetrifolium* (leaves) were supplied as powdered dried material. It was collected from mountain regions in Tasloga (Sulaymaniyah); a city 330 Km north the capital Baghdad. Methanol extract of *H. triquetrifolium* was prepared according to Fua *et al.*^[12] Fifty grams of the plant powder were extracted with 80% methanol (250 ml) at 65°C for 3 hours using the soxhlet apparatus. The extract solution was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried crude extract, which was frozen at -20°C until use to prepare the required concentrations.

Determination of Total Flavonoids

Total flavonoids content was spectrophotometrically determined in the methanol extract as rutin (flavonoids standard) equivalent by aluminium chloride colorimetric method as described by Sakanaka *et al.*^[13] Briefly, the methanol extract (3.2 mg) was dissolved in 5 ml of 50% methanol, followed by addition of 1 ml of 5% (w/v) sodium nitrite solution. After 6 minutes, 1 ml of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 minutes before 10 ml of a 10% (w/v) NaOH solution was added. The mixture was made up to 50 ml with distilled water and mixed well. Then the absorbance was measured at 450nm with a spectrometer after 15 minutes. A similar procedure was applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg) of rutin.

Reductive Ability

The method described by Fua *et al.*^[12] was adopted to evaluate the reductive ability, in which 1 ml of each concentration of the plant extract (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) was mixed with 1 ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide, and incubated at 50°C for 20 minutes. Then, 1ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged for 10 minutes at 3000 rpm, and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 0.1% Ferric chloride. After that, the absorbance was measured at 700nm. The same procedure was applied to Trolox solutions (standards). All tests were done in triplicates

Determination of Anti-Oxidant Activity

The antioxidant activity of plant methanol extract and standard (vitamin C) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the method of

Sanja *et al.* was followed.^[14] An aliquot of 0.1 ml of the extract or standard (0.625, 0.125, 0.250 and 0.500 mg/ml) was added to 3.9 ml of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance of each solution was determined at 517nm using spectrophotometer. All measurements were made in triplicates. The ability to scavenge DPPH radical was calculated by the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \right) \times 100$$

Statistical Analysis

Data were given as mean $\pm$ standard deviation (SD), and significant differences between means were assessed by ANOVA (analysis of variance) followed by LSD (least significant difference) or Duncan test, in which $P \leq 0.05$ was considered significant. The SPSS version 13.0 (statistical package for social sciences) was employed to carried such analyses.

RESULTS

Determination of Total Flavonoids

Total flavonoids content was spectrophotometrically determined in methanol extract of *H. triquetrifolium* as rutin equivalent. The extract was found to contain 115.73 ± 5.65 $\mu\text{g/ml}$ flavonoids.

Reductive Ability

At all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), the absorbance of *H. triquetrifolium* methanol extract was significantly higher ($P \leq 0.001$) than trolox (vitamin E), and such findings suggest that the plant extract is more effective than trolox in the reductive ability, which was concentration-dependent. It was 0.193 ± 0.010 at the concentration 0.02 mg/ml of the methanol extract, and increased significantly ($P \leq 0.05$) to 0.743 at the concentration 0.64 mg/ml (Table 1).

Table 1: Reductive ability of *H. triquetrifolium* methanol extract and trolox (vitamin E).

Concentration (mg/ml)	Reductive Ability Absorbance (Mean $\pm$ SD)		P $\leq$
	<i>H. triquetrifolium</i> Extract	Trolox (Vitamin E)	
0.02	0.193 ± 0.010^E	0.100 ± 0.001^D	0.001
0.04	0.292 ± 0.007^D	0.101 ± 0.001^{CD}	0.001
0.08	0.451 ± 0.007^C	0.108 ± 0.001^{CD}	0.001
0.16	0.660 ± 0.013^B	0.114 ± 0.004^C	0.001
0.32	0.668 ± 0.016^B	0.132 ± 0.007^B	0.001
0.64	0.743 ± 0.013^A	0.211 ± 0.015^A	0.001

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

P: Probability of difference between means of rows.

Anti-Oxidant Activity

Methanol extract of *H. triquetrifolium* was significantly more effective in DPPH radical scavenging activity than vitamin C at the four concentrations tested (0.625, 0.125, 0.250 and 0.500 mg/ml). The concentrations 0.250 and 0.500 mg/ml of plant extract shared an approximated radical scavenging activity (80.00 ± 2.00 and $80.66 \pm 1.15\%$, respectively), but it was significantly higher ($P \leq 0.05$) than at the concentrations 0.620 and 0.125 mg/ml (60.66 ± 5.77 and $63.66 \pm 3.51\%$, respectively). Vitamin C also showed variations between the four concentrations but the difference was not significant (Table 2).

Table 2: DPPH radical scavenging activity of *H. triquetrifolium* methanol extract and vitamin C.

Concentration (mg/ml)	DPPH Radical Scavenging Activity (Mean $\pm$ SD; %)		P $\leq$
	<i>H. triquetrifolium</i> Extract	Vitamin C	
0.620	60.66 ± 5.77^B	39.66 ± 2.52^A	0.001
0.125	63.66 ± 3.51^B	41.33 ± 10.01^A	0.001
0.250	80.00 ± 2.00^A	48.33 ± 8.50^A	0.001
0.500	80.66 ± 1.15^A	53.00 ± 10.53^A	0.001

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

P: Probability of difference between means of rows.

DISCUSSION

Flavonoids have attracted considerable interest as dietary constituents and the results of clinical studies have indicated their possible role in preventing cardiovascular diseases and several kinds of cancers.^[15] Furthermore, varieties of flavonoids including apigenin, luteolin and quercetin were found to inhibit NO production through downregulating iNOS induction.^[16] Further studies revealed that natural products such as flavonoids and phenolics have been observed to be efficient free radical scavengers and lipid peroxidation inhibitors,^[17,18] and probably, the best described and most useful property of almost every group of flavonoids is their capacity to act as antioxidants; protecting the body against reactive oxygen species (ROS).^[19,20] Accordingly, there is increasing interest in the potential health benefits of dietary flavonoids, and the present study shared such interest in investigating the anti-oxidant and radical scavenging activity of *H. triquetrifolium* methanol extract, because of its richness in flavonoids.

The increasing in the reductive ability of *H. triquetrifolium* and DPPH radical scavenging activity in comparison with controls of each one (vitamins E and C, respectively) can be attributed to its high flavonoid content, These results similar to the result of methanolic extract of 13 *Hypericum* species growing in Bulgaria. All the species showed a high antioxidant activity and DPPH radical scavenging activity, which was probably due to the presence of high content of tannins, flavonoids, xanthenes and benzophenones,^[21] and these constituents have been suggested to act as anti-oxidants.^[22] DPPH radical scavenging activity has been reported to be increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. These phenolic components possess many hydroxyl groups including o-dihydroxy group, which have a very strong radical scavenging effect and anti-oxidant power.^[23] It has also been known that a variety of plant extracts has antioxidant activities to scavenge free radicals that cause diseases via lipid peroxidation, protein peroxidation and DNA damage.^[24] Phenolics are found in large quantities in the plant kingdom, and they have been proposed to have multiple biological functions, including anti-oxidant activity.^[25] Phenolics, such as flavonoids, phenolic acids, stilbenes, lignans, lignin and tannins that are especially common in leaves, flower tissues, and woody parts such as stems and barks have strong antioxidant activity.^[26] An *in vivo* study revealed differential protective advantages of flavonoid in cancer prevention by either confer direct anti-oxidant protection to cells, or induce enzymes that protect cells against oxidative and other insults ("indirect anti-oxidants") and others appear to be protective by both mechanisms.^[27] Different aromatic plants (for instance, *Origanum dictamnus*, *Nepeta melissifolia*, *Thymus vulgaris* and *Rosmarinus officinalis*) also have antioxidant ability because they possess a good amount of total proanthocyanidin, flavonoids and polyphenols.^[28] Accordingly, there has been an increasing interest in the natural anti-oxidants, namely phenols, present in medicinal and dietary plants, that may help prevent oxidative damage.

Reducing power is normally associated with anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity. Compounds with reducing power indicate that they are electron-donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants.^[29] In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of methanol extract of *H. triquetrifolium* leaves. Presence of reducers causes the conversion of the Fe^{+3} /ferricyanide complex to ferrous form. Reduction of Fe^{+3} is often used as an indicator of electron-donating activity, which is an important mechanism of

phenolic anti-oxidant action, and can be strongly correlated with other anti-oxidant properties. Increase in absorbance of the reaction mixture indicated increase in reducing power of the extract.^[23,30] The reducing power assay showed greater reduction capacity compared with the standard ascorbic acid; hence the methanol extract of *H. triquetrifolium* leaves proved to be potential for the anti-oxidant phyto-constituents, and such finding matches with *H. hookerianum*, which showed high anti-oxidant and DPPH radical scavenging activity.^[31,32]

In conclusion, the methanol extract of *H. triquetrifolium* can be considered as a rich source of flavonoids that lend the plant to be a medicinal plant with a wide range of medicinal applications and have a strong anti-oxidant activity.

REFERENCES

1. Gurib-Fakim, A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med J.*, 2006; 27: 91-93.
2. Ye, Y., Li, X.Q., Tang, C.P. and Yao, S. Natural products chemistry research: progress in China in 2011. *Chin J Nat Med.*, 2013; 11: 97-109.
3. Clark, D.L. and Wilson, M.V. Fire, mowing, and hand-removal of woody species in restoring a native wetland prairie in the Willamette Valley of Oregon. *Wetlands*, 2001; 21: 135-144.
4. Said, O., Khalil, K., Fulder, S. and Azaizeh, H. Ethnobotanical survey of medicinal herbs of the Middle Eastern region. *J Ethnopharmacol.*, 2002; 83: 251-265.
5. Rouis, Z., Abid, N., Koudja, S., Yangui, T., Elaissi, A., Cioni, P.L., Flamini, G. and Aouni, M. Evaluation of the cytotoxic effect and antibacterial, antifungal, and antiviral activities of *Hypericum triquetrifolium* Turra essential oils from Tunisia. *BMC Complement Altern Med.*, 2013; 13: 24.
6. Saad, B., Abouatta, B.S., Basha, W., Hmade, A., Kmail, A., Khasib, S. and Said, O. *Hypericum triquetrifolium*-derived factors downregulate the production levels of LPS-induced nitric oxide and tumor necrosis factor- α in THP-1 Cells. *Evid Based Complement Alternat Med.*, 2011; 2011: 1-7.
7. Özkan, E.E. and Mat, A. An overview on *Hypericum* species of Turkey. *J Pharmagonosy Phytopher.*, 2013; 5: 38-46.
8. Chatterjee, S., Chatterjee, S., Dey, K.K. and Dutta, S. Study of antioxidant activity and immune stimulating potency of the ethnomedicinal plant, *Cassia alata* (L.) Roxb. *Med Aromat Plants.*, 2013; 2: 13.

9. Zhu, X.X., Yang, L., Li, Y.J., Zhang, D., Chen, Y., Kostecká, P., Kmoníčková, E. and Zidek, Z. Effects of sesquiterpene, flavonoid and coumarin types of compounds from *Artemisia annua* L. on production of mediators of angiogenesis. *Pharmacol Rep.*, 2013; 65: 410-420.
10. Çirak, C., Radušien, J., Janulis, V., Ivanauskas, L., Çamaş, N. and Kemal, A. Phenolic constituents of *Hypericum triquetrifolium* Turra (Guttiferae) growing in Turkey: variation among populations and plant parts. *Ayan Turk J Biol.*, 2011; 35: 449-456.
11. Grassi, D., Desideri, G. and Ferri, C. Flavonoids: antioxidants against atherosclerosis. *Nutrients*, 2010; 2: 889-902.
12. Fua, W., Chena, J., Caia, Y., Leia, Y., Chenb, L., Peic, L., Zhoua, D., Lianga, X. and Ruana, J. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. *J Ethnopharmacol.*, 2010; 130: 521-528.
13. Sakanaka, S., Tachibana, Y. and Okada, Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinohacha). *Food Chem.*, 2005; 89: 569-575.
14. Sanja, S.D., Sheth, N.R., Patel, N.K., Dhaval, P. and Biraju, P. Characterization and evaluation of antioxidant activity of *portulaca oleracea*. *Int. J. Pharm. Pharm. Sci.*, 2009; 1: 74-84.
15. Chu, Y.H., Chang, C.L. and Hsu, H.F. Flavonoid content of several vegetables and their antioxidant activity. *J Sci Food Agric.*, 2000; 80: 561-566.
16. Kim, H.P., Son, K.H., Chang, H.W. and Kang, S.S. Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci.*, 2004; 96: 229-245.
17. Galleano, M., Verstraeten, S.V., Oteiza, P.I. and Fraga, C.G. Antioxidant actions of flavonoids: thermodynamic and kinetic analysis. *Arch Biochem Biophys.*, 2010; 501: 23-30.
18. Egert, S. and Rimbach, G. Which sources of flavonoids: complex diets or dietary supplements. *Adv Nutr.*, 2011; 2: 8-14.
19. Lu, M.F., Xiao, Z.T. and Zhang, H.Y. Where do health benefits of flavonoids come from? Insights from flavonoid targets and their evolutionary history. *Biochem Biophys Res Commun.*, 2013; 434: 701-704.
20. Xiao, J., Chen, T. and Cao, H. Flavonoid glycosylation and biological benefits. *Biotechnol Adv.* (In press), 2014.

21. Dimitrova, D.Z., Nedialkov, P. and Kitanov, G. Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria. *Pharmacogn Mag.*, 2010; 6: 74-78.
22. Albayrak, S., Aksoy, A., Yurtseven, L. and Yaşar, A. A comparative study on phenolic components and biological activity of some *Senecio* species in Turkey. *J Pharm Pharmacol.* (In press), 2014.
23. Berber, A., Zengin, G., Aktumsek, A., Sanda, M.A. and Uysal, T. Antioxidant capacity and fatty acid composition of different parts of *Adenocarpus complicatus* (Fabaceae) from Turkey. *Rev Biol Trop.*, 2014; 62: 337-346.
24. Hsu, C.Y., Chan, Y.P. and Chang, J. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol Res.*, 2007; 40: 13-21.
25. Franco, M.N., Galeano-Díaz, T., López, O., Fernández-Bolaños, J.G., Sánchez, J., De Miguel, C., Gil, M.V. and Martín-Vertedor, D. Phenolic compounds and antioxidant capacity of virgin olive oil. *Food Chem.*, 2014; 163: 289-298.
26. El-dib, R.A., Soliman, H.S., Hussein, M.H. and Attia, H.G. Two new flavonoids and biological activity of *Astragalus abyssinicus* (Hochst.) Steud. ex A. rich. aerial parts. *Drug Res.*, (Stuttg). (In press), 2014.
27. Tsuji, P.A., Stephenson, K.K., Wade, K.L., Liu, H. and Fahey, J.W. Structure-activity analysis of flavonoids: direct and indirect antioxidant, and antiinflammatory potencies and toxicities. *Nutr Cancer.*, 2013; 65: 1014-1025.
28. Chrpoval, D., Kouřimsk ál, L., Gordon, M. H., Heřmanová1, V., Roubíčková1, I. and Pánek1, J. Antioxidant activity of selected phenols and herbs used in diets for medical conditions. *Czech J. Food Sci.*, 2010; 28: 317-325.
29. Manach, C., Scalbert, A., Morand, C., Remes, C. and Jimenez, L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr.*, 2004; 79: 727-747.
30. Dorman, H. J. D., Peltoketo, A., Hiltunen, R. and Tikkanen, M. J. Characterization of the antioxidant properties of de odourised aqueous extracts from selected Lamiaceae herbs. *Food Chem.*, 2003; 83: 255-26.
31. Kirmizibekmez, H., Bassarello, C., Piacente, S., Celep, E., Atay, I., Mercanoğlu, G. and Yeşilada, E. Phenolic compounds from *Hypericum calycinum* and their antioxidant activity. *Nat Prod Commun.*, 2009; 4: 531-534.
32. Lu, Y.L., Liu, Y.H., Chyuan, J.H., Cheng, K.T., Liang, W.L. and Hou, W.C. Antioxidant activities of different wild bitter gourd (*Momordica charantia* L. var. abbreviata Seringe cultivars). *Botanical Studies*, 2012; 53: 207-214.