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Determination of Antioxidant activity of *Hypericum perforatum*

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Abstract

Hypericum perforatum has been used by physicians since ancient times to treat certain diseases. Nowadays, phytotherapy has an important place for people to turn to natural treatments. It is significant to examine the bioactivity of *H. perforatum* which is of pharmacological importance and used as a traditional medicine. *H. perforatum* plant was harvested in mountainous area in Celikhan region of Adiyaman. This plant was subjected to extraction process and the antioxidant capacity of the obtained extract was examined. *H. Perforatum* was found to have remarkable activity when evaluated for DPPH free radical scavenging activity and FRAP reducing power activity.

Keywords: *Hypericum perforatum*, Antioxidant Capacity, Free radical

Hypericum perforatum antik çağlardan beri bazı hastalıkları tedavi etmek için hekimler tarafından kullanılmıştır. Günümüzde insanların doğal tedavilere yönelmesi açısından fitoterapi önemli bir yere sahiptir. Farmakolojik önem taşıyan ve geleneksel ilaç olarak kullanılan *H. perforatum* bitkisinin biyoaktivitelerinin incelenmesi önem arz etmektedir. *H. perforatum* bitkisi Adıyaman Çelikhan bölgesinde dağlık alanda hasat edilmiştir. Bu bitki ekstraksiyon işlemine tabi tutulup, elde edilen ekraktın antioksidan kapasiteleri incelenmiştir. *H. perforatum* bitkisi DPPH serbest radikal giderme aktivitesi ve FRAP indirgeme gücü aktivitesi açısından değerlendirildiğinde önemli bir aktiviteye sahip olduğu görülmüştür.

Anahtar Kelimeler: *Hypericum perforatum*, Antioksidan kapasite, Serbest radikal

Introduction

The *Hypericum* genus belongs to the *Clusiaceae* family and *Hypericaceae* subfamily and covers more than 400 species, and 106 species have been identified under 20 sections in Turkey (Başköse & Savran, 2018; Curtis & Lersten, 1990). The use of *Hypericum perforatum* as a medicine dates back to ancient Greek history. At that time, doctors used *H. perforatum* as diuretic, wound healing and also for the treatment of menstrual disorders and intestinal worms (Klemow et al., 2011; Redvers et al., 2001). *H. perforatum*, which is more in demand today, is considered important for its antidepressant and wound healing properties (Chrea et al., 2014; Miller, 1998). *H. perforatum* is also used in the remedy of cancer, (Schempp et al., 2002) diabetes, chronic rheumatism, gastrointestinal diseases, diuretic sedative, jaundice, bronchitis, diarrhoea, dysentery,(Duke, 1990) as well as sore throat infections, (Tümen & Sekendiz, 1989) colds, worm lowering, antiseptic (Duke, 1990) and burn wounds (Baytop, 1999). In addition *H. Perforatum* is used as an anti-inflammatory agent (Di Carlo et al., 2001) and a universal antidote (Holtom & Hylton, 1979). *H. Perforatum* has many secondary metabolites, including naphthodianthrones (hypericin, pseudo-hypericin, etc.), phloroglucinols derivatives (hyperforin, adhiperforin, etc.), flavonoids (hyperositis, routine, quercetin, etc.), biflavones (biapigenin, amentoflavone), phenolic acids (ferulic acid, caffeic acid etc.), organic acids, essential oils, amino acids, xanthones , tannins, procyanidins and other water-soluble components (Cracchiolo, 1998; Greeson et al., 2001; Nahrstedt & Butterweck, 1997). The main responsible components of the pharmacological effect of *H. perforatum* extracts are the active constituent of naphthodianthrone pigments is hypericin and pseudohypericin, phloroglucinol derivative hyperforin, flavonoids and essential oils (V Butterweck et al., 1997; Hışıl et al., 2005; Medina et al., 2006; Patocka, 2003). It shows that hypericin is the main component in antidepressant effect of *H. perforatum* used in mental disorders (Briskin, 2000; Veronika Butterweck et al., 1998). In a screening study of *Calendula officinalis, Hypericum perforatum, Plantago lanceolata* and *Glycyrrhiza glabra*, *H. perforatum* L. was determined to be the most potent antioxidant effect of plants in scavenging free radicals (Herold et al., 2003).

Free radicals are substances of atomic or molecular structure containing one or more unpaired electrons. Radicals are chemically very active due to their unpaired electrons and attack other biological molecules in the environment, destroying their biological structures. The presence of free radicals in living systems is normal and forms the basis for many other metabolic functions, such as electron transfer, biosignal production and destruction of bacteria in macrophages (Akkuş, 1995; B. Halliwell & Cross, 1994; B. Halliwell & Gutteridge, 1984). However, the presence of high levels of these radicals and reagents in living organisms causes harmful processes such as tissue damage, cell death, premature aging, cancer, cardiovascular diseases and neurological disorders as a result of oxidation of biomolecules. These radical and reactive products can oxidize nucleic acids, proteins and lipids, disrupt their chemical structure and produce negative results in their metabolism (Sen et al., 2000).

There are many defense mechanisms to eliminate the formation of reactive oxygen species (ROS) and their damage. These mechanisms are known as antioxidants. Antioxidants eliminate these radical and reactive products and inhibit their negative effects (Barry Halliwell, 1994, 1996).

Antioxidants can be synthesized in the body or taken from the diet. The antioxidant defense system in living organisms is divided into two main groups. These; are produced in the metabolism (endogenous) and external diet (exogenous) antioxidant systems. The endogenous antioxidant system is formed of enzymes such as antioxidant enzymes, proteases and phospholipase to remove damaged molecules, systems for synthesizing new compounds, glutathione, uric acid and various metal binders. Exogenous antioxidants are available in both synthetic and natural forms. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiarybutylhydroquinone (TBHQ), propyl gallate (PG) and Trolox are members of the synthetic antioxidants. Phenols, flavones, tocopherols, carotenes and catechins belong to the group of natural antioxidants (Akkuş, 1995; Bursal et al., 2013).

MATERIALS AND METHODS

General experimental procedures

BHT, BHA, Trolox, trichloroacetic acid (TCA), ABTS, $CuCl_2$, neocuprin, ammonium acetate (NH_4Ac), DPPH·, Potasium ferricyanide ($K_3Fe(CN)_6$), potassium persulfate ($K_2S_2O_8$) were used for antioxidant assays and all solvents and chemical compounds supplied from chemical company E. Merck.

Plant Material

H. perforatum plant was collected in the mountainous area from Çelikhan district of Adıyaman (38°09'12.7"N 38°12'55.0"E), the southeast Anatolia region of Turkey during the flowering period in June and identified by Prof. Dr. Murad Aydın Şanda. We brought to the laboratory for the bioactivity of the *H. perforatum* plant which was dried in a way that would not see the sun.

Extraction

For ethanol extraction, 5 g of the sample was pulverized in a mill and mixed with 50 mL of ethanol. The material was vortexed and immersed in ultrasonic bath for 30 minutes and extracted for 5 days. The resulting extract was filtered on filter paper. The extract was placed in falcon tubes and was used for antioxidant activity analysis.

Evaluation of Antioxidant Activities

Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

The reducing powers of *H. perforatum* extract was carried out by modified Oyaizu method (Elmastaş et al., 2006; Oyaizu, 1986). To the *H. perforatum* extract solutions (10, 20, 40 µg/mL) in

1 mL of methanol was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixtures were incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to each mixture and they were centrifuged (at 3,000 rpm for 10 min). The upper layers of solutions (2.5 mL) were mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and absorbances were measured at 700 nm. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

DPPH$^{\bullet}$ Free Radical Scavenging Activity

DPPH$^{\bullet}$ free radical scavenging activity of *H. perforatum* extract and standards were determined by the method of Blois (Blois, 1958). 0.1 mM solution of DPPH$^{\bullet}$ in ethanol was prepared and 1 mL of this solution was added to 3 mL of the samples solution in ethanol at different concentrations (10, 20, 40 µg/mL). These solutions were vortexed thoroughly and kept in the darkness for 30 min. The absorbance was measured at 517 nm by a spectrophotometer and the lower absorbance of the reaction mixture revealed the higher free radical scavenging activity. The capability to scavenge DPPH$^{\bullet}$ radical was calculated using the following equation:

DPPH$^{\bullet}$ scavenging effect (%) = (Absorbance of control - Absorbance of sample) / Absorbance of control x 100

ABTS$^{\bullet+}$ Radical Cation Scavenging Assay

ABTS$^{\bullet+}$scavenging activity was performed according to Re method (Re et al., 1999). The process of ABTS$^{\bullet+}$ (2.0 mM) in water with potassium persulfate ($K_2S_2O_8$) (2.45 mM) at room temperature in dark for 4 h gave the ABTS cation radical. Dilution of ABTS$^{\bullet+}$ was applied with phosphate buff er (0.1 mol/L, pH 7.4) to measure the absorbance at 734 nm. The reactions of ABTS$^{\bullet+}$solution (1.0 mL) with samples solution in ethanol (3.0 mL) at different concentrations (10, 20, 40 µg/mL) were performed. The inhibition was calculated at 734 nm for each concentration. The scavenging ability of ABTS$^{\bullet+}$ was calculated as the following equation:

ABTS$^{\bullet+}$ scavenging activity (%) = [(Ac – As) /Ac] x 100 where Ac is the initial concentration of ABTS$^{\bullet+}$and as is the absorbance of the remaining concentration of ABTS$^{\bullet+}$in the samples.

Cu $^{++}$ Ion Reducing Capacity (CUPRAC)

The Cuprak method is an antioxidant activity test based on the reduction of Cu(II)-Nc to Cu(I)-Nc chelate (Apak et al., 2004). To the test tube was added 1 mL of a solution of $CuCl_2$ (0.01 M) to 1 mL of neocuprin (2,9-dimethyl-1,10-phenanthroline) (7.5×10^{-3} M alcohol) and 1mL of ammonium acetate (NH_4Ac) buffer solution and mixed well. Then different concentrations (10, 20, 40 µg/mL) of extracts was added and the total volume was adjusted to 4 mL with water. After incubation for 30 minutes at room temperature, the absorbance was recorded at 450 nm.

Using the Trolox calibration curve, the results were calculated as mmol Trolox equivalent/g *H. perforatum* (R^2 = 0,9992).

Statistical analysis

The results of the study were performed by taking the average ± SD of at least three independent measurements.

RESULTS AND DISCUSSION

Antioxidant activities

Antioxidant properties of secondary metabolites in plants are important for pharmacokinetic. In this work, the antioxidant activity of ethanol extract of *H. perforatum* was compared to standard antioxidants (BHT, BHA and Trolox). The antioxidant activity of the *H. perforatum* was high in the determination of reducing power activity (Figure 1).

DPPH

DPPH• free radical scavenging activity after the antioxidant compound reacts with DPPH, it is made by measuring the changing color of the DPPH radical at 517 nm. Antioxidants stabilize by donating hydrogen to the DPPH radical (Soare et al., 1997). *H. perforatum* showed a higher free radical scavenging activity than the standard antioxidant BHA. Based on these data, the *H. perforatum* plant can be a free radical inhibitor.

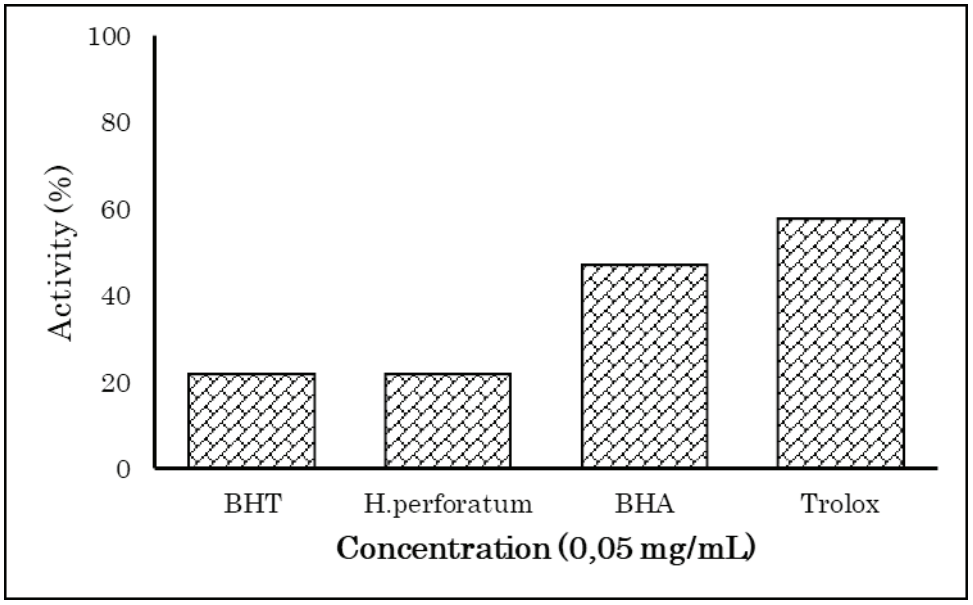

Figure 1. DPPH free radical scavenging activity of *H. perforatum* and standard antioxidants.

FRAP

The reducing capacity in a compound is an important measure of antioxidant activity (Meir et al., 1995). Figure 2 shows the reducing power of *H. perforatum* compared to BHA, BHT and Trolox at the same concentrations. *H. perforatum showed a higher reducing capacity than the standard antioxidant BHA*. The reducing of *H. perforatum* and the power of standard antioxidants was carried out in the following order: Trolox>BHT> *H. perforatum* > BHA

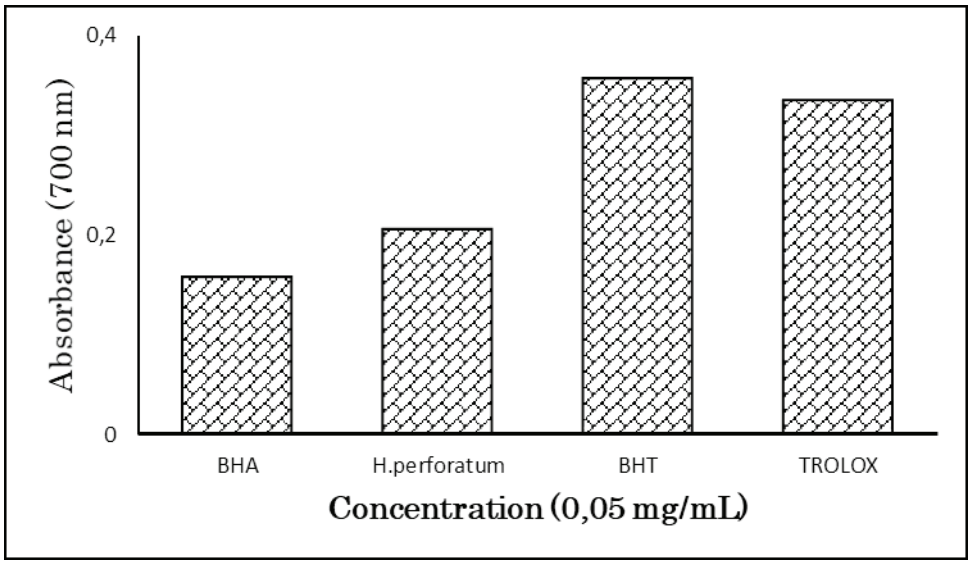

Figure 2. FRAP reducing power activity of *H. perforatum* and standard antioxidants.

ABTS

The antioxidant ability of the *H. perforatum* plant to scavenge the ABTS radical cation was determined according to the radical scavenging ability of BHA, BHT and Trolox. *H. perforatum* showed relatively low activity compared to standard antioxidants.

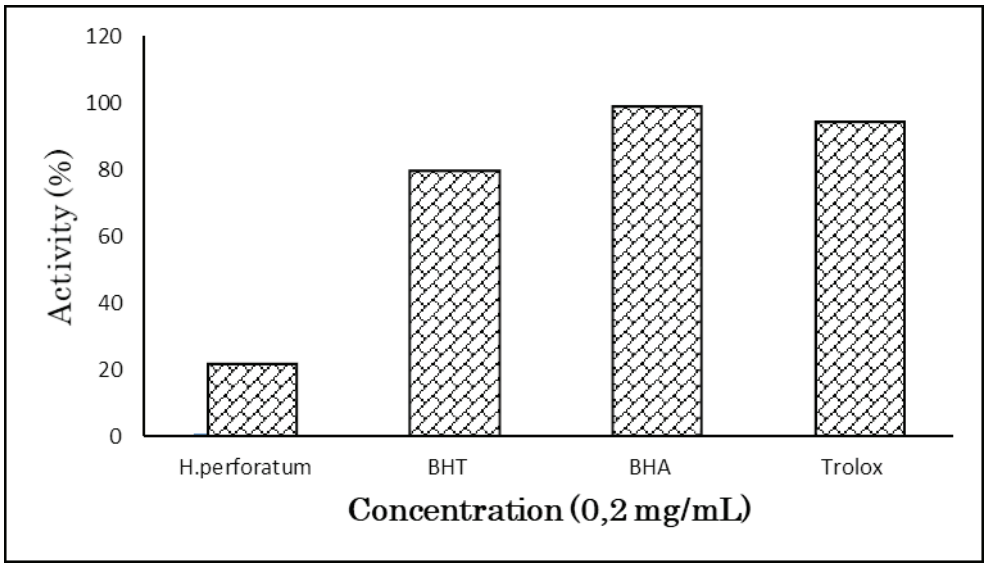

Figure 3. ABTS cation radical scavenging activity of *H. perforatum* and standard antioxidants.

CUPRAC

Antioxidants are converted to quinone structures by CUPRAC redox reaction and Cu (I)-Nc chelate produced by this redox reaction gives maximum absorbance at 450 nm (Apak et al., 2004). *H. perforatum* showed relatively low Cu^{++} ion reducing capacity compared to standard antioxidants.

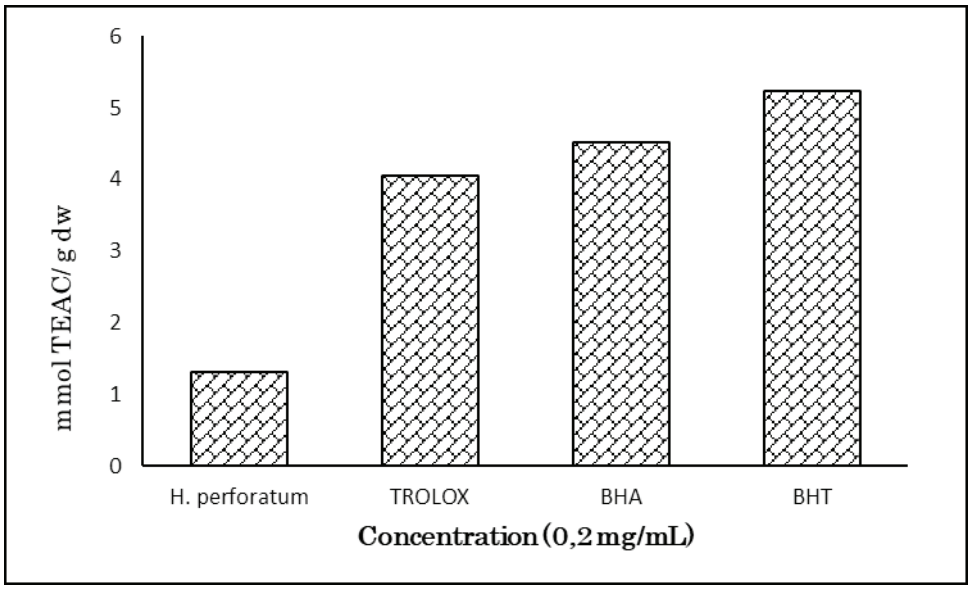

Figure 4. Cu $^{++}$ Ion Reducing Capacity of *H. perforatum* and standard antioxidants.

CONCLUSION

In this study, when *H. perforatum* was found to have significant activity when evaluated for DPPH and FRAP. As can be seen from the previous studies, the presence of functional compound confirms the antioxidant activity of *H. perforatum* plant (Cracchiolo, 1998; Nahrstedt & Butterweck, 1997).

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