Original Article

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Free radical scavenging activity and lipid peroxidation inhibition of *Hypericum helianthemoides* (spach) Boiss

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Abstract

Antioxidants are compounds that obstruct the oxidation of macromolecules in the body. In general, there are two categories of antioxidants, natural and synthetic. Recently, interest has been increased considerably for obtaining new natural antioxidants. In this study, the scavenging of free radicals such as DPPH, NO and OH by *Hypericum helianthemoides* extract was evaluated. Also, the antioxidant properties of this extract were evaluated by FRAP, FTC methods and determination phenolic compounds. The plant was collected from north of Fars Province and plant extraction was obtained using ethanol. In DPPH radical scavenging, different concentrations of the *Hypericum* extract were added to DPPH radical. In hydroxyl radical scavenging, Fenton reaction mixture, TCA and TBA were mixed with *Hypericum* extract. In nitric radical scavenging, nitropruside was mixed with *Hypericum* extract and then sulphanilic acid, naphthylene diamine were added. In determination of phenolic compounds, Folin-ciocalteu and sodium carbonate were added to *Hypericum* extract. In DPPH radical scavenging, the IC_{50} of *Hypericum* extract (309.35±6.5µg/ml) was higher than the antioxidant standards, BHT (IC_{50}=81.9±2.6 µg/ml) and quercetin (IC_{50}=60.04±6.48 µg/ml). The highest scavenging of hydroxyl radicals was observed in *Hypericum* extract (70.3±0.8%, 125 µg/ml). In gallic acid it was (73.8±3.3%). In 200 µg/ml of *Hypericum* extract scavenged NO radical (85.2±2.7%). In FRAP method, the IC_{50} of this extract was 109.7±10.5 µg/ml. In FTC method, the inhibition of lipid peroxidation by *Hypericum* extract, BHT and ascorbic acid were 59.2±2.2, 66.9±0.15, 64.06±0.02 respectively. Total phenol of the plant extract was 3±0.4 mg/g.

Keywords: Hypericum helianthemoides extract; Radical scavenging; Lipid peroxidation; Total phenols

1. Introduction

WHO advises the consumption of natural antioxidants that can retard or delay lipids or other macromolecules oxidation (1). Herbs which consumed in traditional medicine are considered for current therapeutics. World Health Organization (WHO) reported that in developing countries most of the people use medicinal plants. Because of the side effects of some allopathic drugs and resistance to existing drugs, we looked into the nature as a good resource for therapeutics (1). It is well known that the reactive oxygen species possess a significant role in the progress of various diseases, like neurodegenerative disorder (2). Medicinal plants which contain antioxidant compounds can neutralize the effects of reactive oxygen species.
Species of the genus *Hypericum* (450 species) are famous for their consumption in folk medicine. On the basis of our knowledge, studies on the usage of this plant in folk medicine have not been showed so far, although native population described its

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therapeutic capabilities (3).

In the present study, the antioxidant potentials of *Hypericum helianthemoides* (Hh) extract were investigated by DPPH, OH, NO radicals scavenging, inhibition of lipid peroxidation and FRAP (ferric-reducing antioxidant power) method. Also, in this research total phenols (antioxidant compounds) were determined.

2. Materials and methods

2.1. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), quercetin, gallic acid and Folin-ciocalteau reagent were obtained from Sigma Chemical Co., St Louis, MO. All other reagents were obtained from Merck Chem.

2.2. Plant material

Aerial parts of Hh were collected from Sepidan, Fars province, and identified by Dr. Ahmadreza Khosravi (Department of biology Shiraz University, Shiraz, Iran; Voucher NO 519). The voucher deposited in Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences (519).

The aerial parts of Hh were macerated in ethanol (80%, 200 ml) for 48 h. The extracts concentrated in 40 °C and diluted by methanol.

2.3. DPPH radical scavenging

For DPPH radical scavenging, DPPH (100 mM) was mixed with various concentrations (12.5-400 µg/ml) of the extract and incubated at room temperature for 30 min in the dark. The DPPH radical inhibition was measured at 490 nm. The IC_{50} of each sample, concentration of extract scavenges 50% DPPH radicals, was calculated (4).

2.4. FRAP method

In FRAP assay: FRAP reagent contains TPTZ (2, 4, 6-tripyridyl-S-triazine, 10mm/l), FeCl3 (20 mmol/l) and acetate buffer (0.3 mol/l, pH 3.6) were mixed before usage. Twenty µl of each sample and 180 µl of FRAP reagent were mixed in a 96 well microplate then the absorbance of the sample was read at 593 nm (5).

In nitric oxide (NO) scavenging method (6,7): 50 µl of 10 mM sodium nitroprusside was mixed with 50 µl of each sample and incubated at 27 °C for 150 min. After incubation, 100 µl of griss reagent was added to each sample and the absorbance was measured at 542 nm (6).

2.5. Hydroxyl radical scavenging

For hydroxyl radical scavenging ability, the reaction mixture containing 15.62-1000 µg/ml of Hh, 200 µl of 15 mM deoxyribose, 100 µl of 10 mM H_2O_2, 100 µl of 10 mM ascorbic acid, 1 ml of 1% (w/v) TBA and 1 ml of 2.8% TCA was mixed and heated in a water bath at 100 °C for 20 min. The absorbance of the samples was measured at 532 nm (8).

2.5. Inhibition of lipid peroxidation

The ferric thiocynate method or inhibition of lipid peroxidation, a mixture (8) containing 4 mg/ml of the sample, 4.1 ml of 2.5% linoleic acid, 8 ml of 0.02 M phosphate buffer (pH 7) and 3.9 ml of water was prepared. To 0.1 ml of this mixture, 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate, 0.1 ml of 0.02 M ferrous chloride were added and the absorbance was measured at 500 nm. The absorbance was measured at this wavelength every 24 hours until the day after that the absorbance of the control reached its maximum value (6).

2.6. Determination of phenolic compounds

For determination of total phenolic compounds (9), calibration curve was prepared as follows: 0.5 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml gallic acid were mixed with 2.5 ml Folin-ciocalteu reagent (diluted ten-fold) and 2 ml of 76 g/l sodium carbonate. The absorption was read after 30 min at 20 °C, in 765 nm and the calibration curve was drawn. This procedure is also done for 1 ml of the Hh extract (10 g/l) and after 1 hour the absorption was measured. Total phenolic compounds of Hh extract in gallic acid equivalents (GAE) were calculated.

2.7. Statistical analysis

The data were analyzed for statistical significance using one way ANOVA followed by tukey post-test. P value less than 0.05 was considered significant.

3. Results and discussion

In this research, six methods are used for evaluation the antioxidant activities of Hh extract. The results show that DPPH radical scavenging by Hh extract is less than BHT and quercetin (Figure

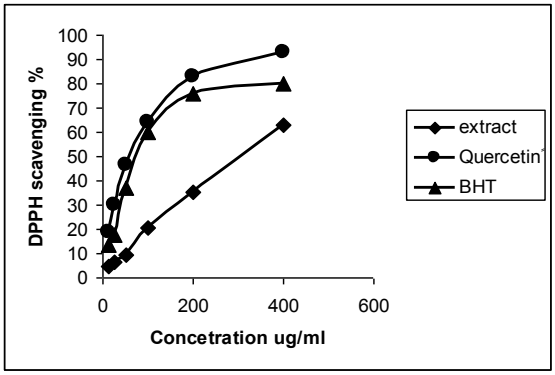

Figure 1. DPPH radical scavenging of *Hypericum helianthemoides* extract was compared with quercetin and BHT as standards.

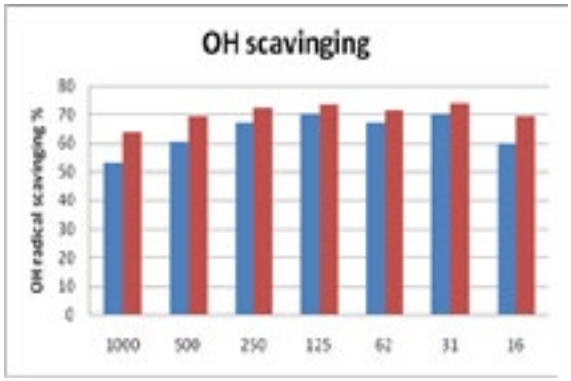

Figure 2. Hydroxyl radical scavenging of *Hypericum helianthemoides* extract (blue column) was compared with gallic acid (red column) as standard

1, Table 1, $p<0.001$). In DPPH radical scavenging, purple color of DPPH converts to yellow color. In other words, more yellow color demonstrates more antioxidant properties.

The extract of Hh in 200 mg/ml possesses the highest NO radical scavenging, (85.2±2.7%, Table 1) which is higher than BHT (42±0.8%, $p<0.0001$). In this method, chromophore is formed from products of nitrite, sulphanilamide and naphthyl ethylene diamine. Antioxidants could prevent from chromophore formation and its absorbance will be reduced. Thus, the absorbance of this chromophore is an indicator of NO scavenging activity (6).

The results also (Table 1) demonstrated that the antioxidant potential of Hh extract in FRAP assay is (IC_{50}=109.7±10.5 µg/ml). In this method, the antioxidant compounds cause reduction in the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex and converts this complex to blue ferrous (Fe^{2+}-TPTZ) compounds (6).

The amount of phenolic compounds of Hh extract is 3±0.4 mg/g in gallic acid equivalents (Table 1). In the present study, the highest OH radical scavenging is observed in 125 µg/ml of Hh (70.3%) and gallic acid (73.8%), respectively ($p<0.05$, Figure 2, Table 1).

In this investigation, lipid peroxidation increased for ten days. The highest inhibition of lipid peroxidation was observed by Hh extract (59.2%), Vit C (64.06%) and BHT (66.9%) in the tenth day (Table 1, Figure 3).

In the present study, the correlation between phenols and OH radical scavenging is (R=0.1). This coefficient between total phenols and NO radical scavenging is (R=0.98). A moderate correlation (R=0.052) was also found between total phenols and DPPH radical scavenging. Also, this moderate correlation is reported in other research (10). On the other hand, a correlation (R=0.507) exists between total phenols and

Table 1.Antioxidant effects of *Hypericum helianthemoides* extract by different methods in comparison withantioxidant standards.

Samples	**DPPH radical scavenging (IC_{50} µg/ml)**	**Nitric oxide scavenging ability %(200 µg/ml)**	**FRAP (IC_{50} µg/ml)**	**FTC**	**OH scavenging 125 µg/ml**	**Total phenols mg/g**
Hypericum helianthemoides extact	309.35±6.5	85.2±2.7	109.7±10.5	59.2±2.2	70.3±0.8	3±0.4
Gallic acid					73.8±3.3	
BHT	63.04±1.2	42±0.8		66.9±0.15		
Quercetin	98.14±0.3					
Ascorbic acid				64.06±0.02		

Results are given as mean ± SD values.

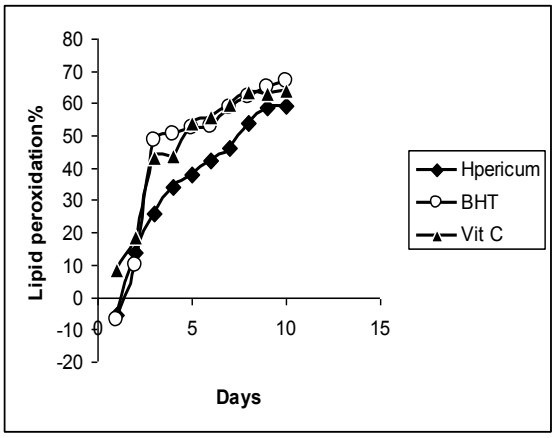

Figure 3. Inhibition of lipid peroxidation by *Hypericum helianthemoides* extract was compared with Vit C and BHT as standards.

inhibition of lipid peroxidation. This relationship between total phenols and antioxidant potential by FRAP method is R=1. In another research, a relationship between antioxidant activities and total phenolics in the *Hypericum* extracts was also observed (11).

4. Conclusion

The extract of *Hypericum helianthemoides* shows antioxidant activities by different methods such as FRAP and free radicals scavenging. This extract inhibits lipid peroxidation until the tenth day and can be used for retardation of oxidation of fatty acids.

5. Acknowledgments

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Conflict of interest

The authors declared that they have not any conflict of interests.

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