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Comparison of Total Flavonoid and Phenol Contents and Antioxidant Capacities of Three *Hypericum* L. Species Growing in Turkey

Türkiye'de Yetişen üç *Hypericum* L.Türünün Total Flavonoid ve Fenol İçerikleri ile Antioksidan Kapasitelerinin Karşılaştırılması

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Abstract

The first aim of the present study was to investigate the probable antioxidant effect of the total extracts of *Hypericum perforatum* L., *H. empetrifolium* Willd., *H. triquetrifolium* Turra. Secondary aim was to expose total flavonoid and phenol contents of these extracts. The extracts were analysed for the determination of antioxidant activity by phosphomolibdenum spectrophotometric method and total flavonoid content by using the aluminum-chloride method. Total phenol estimation was determined by the colorimetric method modified Mc Donald at 765 nm using the Folin-Ciocalteus Reagent. Our results clearly demonstrate that all extracts have antioxidant capacity. The inventions of antioxidant capacity harmonize with total flavonoid and phenol contents.

Key words: *Hypericum perforatum, Hypericum triquetrifolium, Hypericum empetrifolium,* Total flavonoid, Total phenol, Antioxidant capacity.

Introduction

Hypericum L. species are distributed in Europe, Asia, Northern Africa and USA (Brolis *et al.*, 1998). There are 80 *Hypericum* species growing wildly in Turkey (Robson,1967,1988,2000) Some of them were investigated with phytochemical approach (Şerbetçi,2002).*Hypericum perforatum* L. is well known medicinal plant used in folk-medicine to promote wounds and burns healing and against rheumatism, gout and diarrhea. Recently, antidepressive activity based preparation of *H. perforatum* has been clinically demonstrated (Dukic *et al.*, 1998). Chemical investigations of some *Hypericum* species have been done by several groups of workers and flavonoids, phenols, xanthones and other constituents were reported (Kikuchi *et al.*, 1985). Flavonoids and phenols are the major plant compounds with antioxidant activity (Moure *et al.*, 2001). The aim of our study was to investigate the probable antioxidant effect of the three *Hypericum* species namely *H. perforatum* L; *H. empertrifolium* willd. and *H. triquetrifolium* Turra growing around İzmir. The second aim of the study was to expose total flavonoid and phenol contents of these plants.

Materials and Methods

Chemicals: Chemicals used were as follows; methanol (Lab-Scan C2517), glacial acetic acid, aluminium chloride, ethyl acetate, quercetin, gallic acid, Folin Ciocalteus Reagent (Merck). Other chemicals were of analytical grade and used without further purification.

Plant Material: Fresh plants of *H. perforatum* from wild collections, of Karagöl- Izmir, June 1999; *H. triquetrifolium* of Karaali village- Manisa, October 1999 and *H. empetrifolium* from

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Urla- Izmir, July 1999 were used. Mainly, the aerial parts of the plants carrying a high proportion of buds and flowers were selected. The plants were identified by em. Professor E. Sauer, Saarland University, Institute of Botany, Germany. Voucher specimens of the plants were kept for record in (IZEF) the Herbarium of Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany (Voucher no. 5434, 5435, 5436 resp.).

Extraction : The crude drug was dried in shade and finely powdered by a mill (Brabender OHG, Duisburg). A modified method of Wagner and Bladt was used for the extraction of the powdered plant (Wagner and Bladt, 1994). Plant material (100g) extracted with methanol (750ml) at 80 ^{0}C temparature using a Soxhlete apparatus, and methanol extract was distilled under vacuo. The crude extracts obtained from *H. perforatum, H. empetrifolium,* and *H. triquetrifolium* were 25.94%, 36.2%, 26.36%, respectively. After lyophilization (Labconco lyophilizateur, - 50 ^{O}C) of the extracts they were dissolved in suitable solvents.

Methods: This spectrophotometric assay for the quantative determination of antioxidant capacity was carried out, essentially as described by Prieto *et al.* (Prieto *et al.*, 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The results were expressed as mM α-Tocopherol acetate /g dry mass.

Flavonoid contents were determined spectrophotometrically in the samples according to the German Pharmacopoeia (Deutsches Arzneibuch, 1996) method, measuring the flavonoids in Al_3-complex form of purified ethyl acetate phase obtained after acid hydrolysis. The spectrophotometric assay based on aluminium chloride complex formation which is one of the most commonly analytical procedures applied to flavonoid content determination. The results were expressed as %.

The method used for the determination of total phenols using Folin Ciocalteus Reagent was modified from Mc Donald *et al.* (Mc Donald *et al.*, 2000). A diluted extract (0.5ml of 1:10 v/v) or phenolic standard was mixed with Folin Ciocalteus Reagent (5ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4ml, 1M). Solutions were heated in a 45 °C water bath for 15 minutes and the total phenols were determined colorimetrically at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mgL^{-1} solutions of gallic acid in methanol:water (50:50, v/v). Total phenol values are expressed as gallic acid equivalents (mg g^{-1} dry mass) which is a common reference compound.

Results and Discussion

Table 1 shows the total flavonoid and phenol contents, and total antioxidant capacities of the plant extracts. The results given here are the mean ± SD of 10 separate experiments.

Table 1. Total flavonoid and phenol contents, and total antioxidant capacities of the plant extracts.

Extracts	Total Flavonoid (%)	Total Phenol (mg g^{-1} dry mass)	Total Antioxidant Capacity (mM α-Tocopherol acetate /g dry mass)
H.perforatum	0.252 ± 0.012	325.00 ± 17.505	4.615 ± 0.283
H. triquetrifolium	0.232 ± 0.015	299.36 ± 7.117	3.222 ± 0.869
H. empetrifolium	0.288 ± 0.009	397.62 ±12.467	5.483 ± 0.232

The most popular *Hypericum* species in the world today is *H. perforatum*, therefore our aim was to compare antioxidant capacity of two other *Hypericum* species widely available in our region with *H. perforatum.* Our results showed that these *Hypericum* species also have antioxidant effect and *H. empetrifolium* has the most antioxidant activity.

Plant materials contain many compounds with antioxidant activity. Several plants have been studied as sources of potentially safe natural antioxidants for the food, pharmaceutical and cosmetic industries; various compounds have been isolated many of them being polyphenols (Moure *et al.*, 2001). The results of the study show that *H. empetrifolium* has the most total phenol and flavonoid contents and antioxidant capacity. Different results were reported on this aspect; whereas some authors found correlation between the polyphenol and flavonoid content and the antioxidant activity, others found no such relationships (Andarwullan *et al.*, 1999; Maillard and Berset, 1995). We founda correlation between the polyphenol and flavonoid contents and the antioxidant activity.

Özet

Bu çalışmanın öncelikli amacı *Hypericum perforatum* L., *H. empetrifolium* Willd., *H. triquetrifolium* Turra'nın total ekstrelerine ait olası antioksidan etkilerinin incelenmesidir. İkinci amacımız bu ekstrelerin total flavonoid ve fenol içeriklerini ortaya koymaktır. Ekstrelerin antioksidan aktiviteleri spektrofotometrik fosfomolibden yöntemiyle ile total flavonoid içerikleri alüminyum-klorid yöntemiyle incelenmiştir. Total fenol içeriği, Mc Donald yönteminin modifikasyonu sonucu 765 nm'de Folin-Ciocalteus belirtecinin kullanılması ile kolorimetrik olarak ölçülmüştür. Sonuçlarımız tüm ekstrelerin antioksidan kapasiteye sahip olduğunu göstermiştir. Antioksidan kapasite bulguları total flavonoid ve fenol içerikleri ile uyum göstermektedir.

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