Journal of Integrative and Anatolian Medicine
Bütünleyici ve Anadolu Tıbbı Dergisi

Cilt/ Volume: 2 Sayı / Issue: 2
Yıl/Year: 2021

Yayıncı / Publisher
Sağlık Bilimleri Üniversitesi
University of Health Sciences Turkey

Araştırma Makalesi / Research Article, 2(2): 3 - 9, 2021

Determination of Phenolic Compound Composition of Water and Ethanol Extracts of Horsetail (*Equisetum arvense*)

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ABSTRACT

Equisetum arvense, commonly known as horsetail, is generally used in traditional medicine as a therapeutic and pain reliever in wound, rheumatism, tuberculosis, skin diseases, hair loss, prostate, asthma, and urinary system diseases. *Equisetum arvense* contains many phenolic components. These components show high antioxidant activity.

The aim of this study is to determine the phenolic compound composition of water and ethanol extracts of *Equisetum arvense* by High Performance Liquid Chromatography Equipped with Diode Array Detector (HPLC-DAD) which is sold by herbalists at the local markets and used for treatment in folk medicine.

As a result of our study, the phenolic components of horsetail are found as 4-hydroxybenzoic acid (1.535 µg/kg DW), rutin (14.383 µg/kg DW), salicylic acid (9.639 µg/kg DW), vanillic acid (2.32 µg/kg DW) and chicoric acid (21.313 µg/kg DW) in water extract. Ethanol extract contains rutin (6.23695 µg/kg DW), quercetin (32.9995 µg/kg DW), gallic acid (0.95 µg/kg DW.), epicatechin (24.97 µg/kg DW), *p*-coumaric acid (5.97495 µg/kg DW), chicoric acid (39.98495 µg/kg DW), and cinnamic acid (1.7695 µg/kg DW).

Key words: *Equisetum arvense*, Horsetail, Phenolic compound, Traditional medicine

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At Kuyruğu (*Equisetum arvense*) Bitkisinin Su ve Etanol Ekstrelerinin Fenolik Bileşik İçeriğinin Belirlenmesi

ÖZET

Equisetum arvense, halk arasında bilinen adıyla atkuyruğu, geleneksel tıpta genellikle, yara, romatizma, tüberküloz, deri hastalıkları, saç dökülmesi, prostat, astım ve üriner sistem hastalıklarında tedavi edici ve ağrı kesici olarak kullanılır. *Equisetum arvense* yapısında birçok fenolik bileşen içerir bu bileşenler yüksek antioksidan aktivite gösterir.

Bu çalışmanın amacı aktardan satın alınan atkuyruğu bitkisinin etanol ve su ekstrelerinin Diyot Array Detektörlü Yüksek Performans Sıvı Kromatografisi (HPLC-DAD) ile bireysel fenolik bileşik kompozisyonunun incelenmesidir.

Çalışmamızın sonucunda atkuyruğunun halk arasında kullanılan şekli olan su ekstresinde fenolik bileşik miktarları kilogram kuru bitki başına; 4-hidroksibenzoik asit (1.535 µg/kg), rutin (14.383 µg/kg), salisilik asit (9.639 µg/kg,) vanilik asit (2.32 µg/kg) ve chicoric asit (21.313 µg/kg) olarak bulunurken etanol ekstresinde rutin (6.23695 µg/kg), quercetin (32.9995 µg/kg), gallik asit (0.95 µg/kg), epikatesin (24.97 µg/kg), *p*-kumarik asit (5.97495 µg/kg), chicoric asit (39.98495 µg/kg) ve sinamik asit (1.7695 µg/kg) olarak bulunmuştur.

Anahtar kelimeler: *Equisetum arvense,* Atkuyruğu, Fenolik bileşik, Geleneksel tıp

1. INTRODUCTION

Equisetum arvense, commonly known as horsetail, is a perennial herb that usually grows in moist areas. It grows widely in the northern hemisphere (Jinous Asgarpanah, 2012). The plant is often used in the treatment of tuberculosis, urinary system diseases, rheumatic diseases, wound healing, ulcers, and hair loss as folk medicine. In Anatolian Medicine 2-3 cups a day are drunk as a 1-2% infusion as a diuretic, wound healing and stopping internal bleeding (Baytop, 1984). In addition, it is used in the treatment of asthma, liver obstruction, and prostate (Carneiro et al., 2013; Kelimeler, 2014; Melikoğlu et al., 2015). *E. arvense* contains alkaloids, carbohydrates, proteins and amino acids, phytosterols, saponins, sterols, ascorbic acid, silicic acid, phenol, tannin, flavonoids, and triterpenoids (Al-Snafi, 2017).

It has been reported that the antioxidant components of *E. arvense* are caffeic acid, chlorogenic acid, ferulic acid, campherol, quercetin, isoquercetin, apigenin, and luteolin (Garcia et al., 2012). In a study on ethanolic extract of *E. arvense*, it was reported that the main phenolic components were campherol, quercetin, genkawin, hydrocinnamic acid derivatives, mono and caffeoyl tartaric acid (Gründemann et al., 2014). In another study on ethanolic extract of *E. arvense*, it was determined that the main phenolic components were apigenin, luteolin, luteolin-5-*O*-β-*D*-glucopyranoside, isoquercetin, apigenin-5-*O*-glucoside, ursolic acid and oleanolic acid (Zhang et al., 2015). The hydroalcoholic extract of *E. arvense*, camphero-3-*O*-rutinoside-7-*O*-glucoside, campherol dihexocide, quercetin and caffeic acid phenolic compounds were found in a previous report (Garcia et al., 2011). The main phenolic compound of the aqueous extract of *Equisetium arvense* was reported as di-E-caffeoyl-meso-tartaric acid (Mimica-Dukic et al., 2008). In addition, one study found that the ethanolic extract of *E. arvense* contains more phenolic compounds than the aqueous extract (Nagai et al., 2005). Phenolic compounds and antioxidant activity may differ in different *E. arvense* extracts (Cetojević-Simin et al., 2010). The aim of this study is to determine the phenolic compound composition of water and ethanol extracts of *Equisetum arvense* which is sold by herbalists at the local markets in İstanbul and used for treatment in folk medicine.

2. EXPERIMENTAL

2.1. Plant materials

Equisetum arvense plant was purchased from local markets in Eminönü district in Istanbul, Turkey. The horsetail plant that we have purchased is the product of a company that has been approved by the Ministry of Agriculture and Forestry for its production and sale and that produces commercially standardized products in accordance with the Turkish Food Codex. The plant scientific name was written on the package as *Equisetum arvense*.

2.2. Extraction

For preparation of the ethanol and water extracts, 5 grams of dry and crushed *E. arvense* were weighed, 100 mL of ethanol added for ethanolic extract and for water extraction, infusion was prepared by adding 5g of horsetail to 100 mL of boiled water. After incubation in a shaker for 2 hours, both were kept in an ultrasonic bath for 15 minutes. The solvent of the ethanolic extract was removed using a rotary evaporator under lower pressure. Removed of water in the infusion was caried out by a freeze dryer.

2.3. HPLC Analysis

Shimadzu Nexera-i LC-2040C 3D Plus brand HPLC device was used for quantitative analysis. DAD detector (scanning at 254 nm) was used as detector, Phenylhexyl 4.6 x 150 mm, 3 µm (UP) (GL Sciences InterSustain Made in Japan) C18 reverse phase filler column was used for discrimination. Pump program was given at Table 1. Solvent A is contained 0.1% formic acid in water. Solvent B is acetonitrile (Merck, HPLC

grade) as mobile phase. During the analysis, the mobile phase flow rate was set at 1 mL/min. The samples and standards injection volume are adjusted as 10 µL. The column temperature is set to 30 °C. Stock solutions were prepared at 2 mg/mL for each extract and for standards stock solutions were prepared at 1 mg/mL concentration.

Table 1. Pump Program

Steps	Flow rate (mL/min.)	Time (min.)	Solvent B (%)	Solvent A (%)
Step 1	1.00	0.01	5	95
Step 2	1.00	7	9.5	90.5
Step 3	1.00	20	17	83
Step 4	1.00	35	40	60
Step 5	1.00	40	0	100
Step 6	1.00	40.01	Stop	

3. RESULTS and DISCUSSION

In this study, individual phenolic compounds of water and ethanol extracts of horsetail plant were quantitatively analysed by HPLC. In our study, using the standards of 15 phenolic compounds given in Table 3, the amount of individual phenolic compounds in the extracts was determined according to the calibration graph obtained in different concentrations by HPLC-DAD. Extraction yield of both extracts were given in Table 2.

The HPLC chromatogram of phenolic compounds was used external standards given in Figure 1.

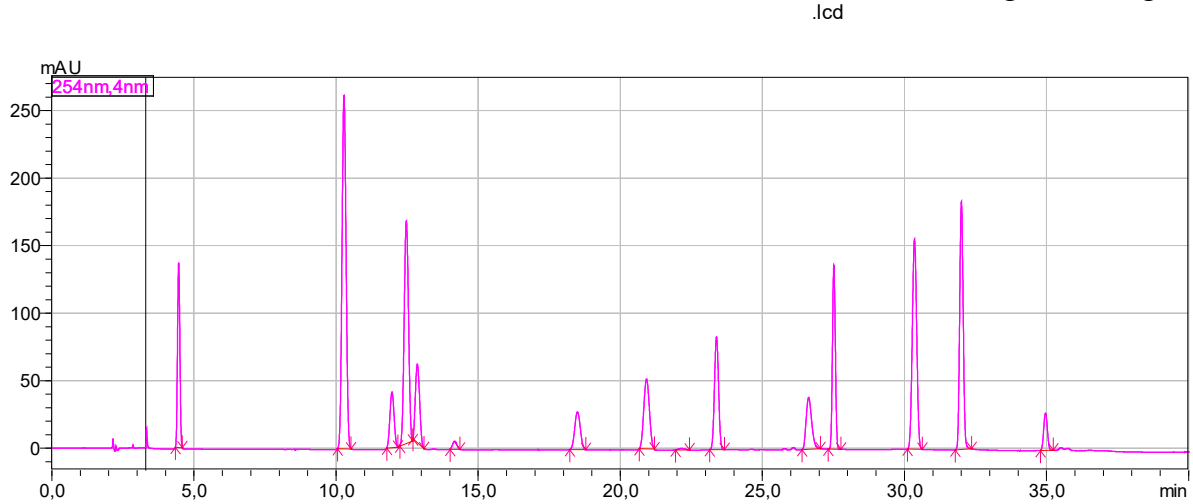

Figure 1. Chromatogram of phenolic compounds used as standards.

While 7 phenolic compounds (Gallic acid, Epicatechin, *p*-Coumaric acid, Rutin, Chicoric acid, Cinnamic acid, and Quercetin) are detected in ethanol extract, 5 phenolic compounds (4-Hydroxybenzoic acid, Vanillic acid, Salicylic acid, Rutin, and Chicoric acid) are detected in water extract. Rutin and Cichoric acids were found in both extracts, but their amounts were

determined differently. While Rutin amount (14.38 µg/kg DW) was found high in water extract, Chicoric acid (39.98 µg/kg DW) was found high in ethanol extract (Table 3).

Table 2. *Equisetum arvense* extraction yield of both extracts

Extraction method	**Extraction yield (%)**
Ethanol extraction	8.4
Water extraction	5.6

Table 3. Calibration curves of fifteen phenolic compounds used as standards in HPLC analysis and concentrations in both extracts.

Phenolic Compounds	Phenolic Compound Concentration of Ethanol Extract (µg/kg dry weight)	Phenolic Compound Concentration of Water Extract (µg/kg dry weight)	Retention time (min.)	Equation of the calibration curve*	Correlation factor R^2
Gallic acid	0.95	ND	4.352	y=29799.9x+6494.60	0.999
4-Hydroxybenzoic acid	ND	1.535	10.217	y=40036.7x+1238.33	0.999
Chlorogenic acid	ND	ND	12.073	y=28066.0x+25870.2	0.999
Vanillic acid	ND	2.32	12.437	y=48654.4x-26981.1	0.999
Caffeic acid	ND	ND	12.850	y=16914.9x-1409.46	0.997
Epicatechin	24.97	ND	14.150	y=4788.08x+789.457	0.998
p-Coumaric acid	5.97	ND	18.486	y=64013.4x-19190.2	0..996
Ferulic acid	ND	ND	20.971	y=46665.0x-14606.2	0.996
Salicylic acid	ND	9.63	21.929	y=23472.2x+25113.1	0.999
Rutin	6.236	14.38	23.494	y=17392.1x-5957.13	0.995
Chicoric acid	39.98	21.31	27.011	y=7443.82x+72438.6	0.988
Apigenin 7-*O*-glucoside	ND	ND	27.574	y=39321.9x+1685.18	0.999
Cinnamic acid	1.76	ND	30.234	y=75026.0x-11276.0	0.998
Quercetin	32.99	ND	32.008	y=26403.4x+1558.71	0.999
Naringenin	ND	ND	34.939	y=24207.0x+2212.93	0.992

*The calibration curves were plotted in linear regression analysis of the integrated peak area (y) versus concentration (x). ND: not detected.

The phenolics found in both extracts were rutin and chicoric acid. The main peak in both extracts was chicoric acid. The results of our study are agreement with some previous studies (Badole. 2014; Garcia et al. 2012). Čanadanović-Brunet and colleagues were found protocatechuic acid, caffeic acid, syringic acid, ferulic acid, and rutin in their studies (Čanadanović-Brunet et al. 2009). The results of Čanadanović-Brunet and colleagues was disagreement our results. In our results gallic acid, epicatechin, *p*-coumaric acid, rutin hydrate, chicoric acid, cinnamic acid, and quercetin were detected in ethanol extract. In previous studies campherol, quercetin, genkawin, hydroxy cinnamic acid derivatives, apigenin, isoquercetin, campherol dihexocide, and caffeic acid were found as phenolic compounds in ethanol and hydroalcoholic extracts of *E. arvense* (Badole. 2014; Garcia et al.. 2012; Pallag et al. 2018; Zhang et al.. 2015).

When the results obtained in this study and the previous studies on *E. arvense* were evaluated, it was concluded that further research should be done on the plant. We think that this important herb, which is used for medical purposes in the public, will gain new information to the literature by bioassay guided isolation studies.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Acknowledgements

This study was financially supported by University of Health Sciences Turkey, Scientific Research Projects Coordinator. The authors thank the University of Health Sciences Turkey for their financial support (project number 2020/086).

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