**Research Article**

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# Bioactivities of *Hypericum perforatum* L. and *Equisetum arvense* L. fractions obtained with different solvents

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**ABSTRACT**

In this study, anticholinesterase and antioxidant effects of HPL aqueous extracts (HPLAE), HPL methanol extracts (HPLME), EAL aqueous extracts (EALAE) and EAL methanol extracts (EALME) obtained from *Hypericum perforatum* L. (HPL) and *Equisetum arvense* L. (EAL) were investigated. The HPLME fraction on acetylcholinesterase (AChE) showed an inhibitory effect, while others showed no inhibitory effect. Antioxidant activities of different fractions of HPL and EAL were determined using different in vitro methods including $Fe^{3+}$- $Fe^{2+}$ reduction capacity, $ABTS^{\cdot+}$ radical scavenging capacity, $DPPH^{\cdot}$ free radical reduction capacity, and CUPRAC methods. In the study, the fractions were compared with the standard antioxidant BHT, BHA, and Trolox. The fractions obtained from these plants have 52% radical scavenger activity close to standards, and moderate metal reduction activity. As a result, different fractions of these medicinal plants used to treat many diseases caused by oxidative stress have varying bioactivities.

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## Introduction

Acetylcholinesterase (AChE), important for neurodegenerative diseases, is found at erythrocytes, serum, and cholinergic brain synapses. AChE inhibitors, which inhibit or slow the hydrolysis of acetylcholine (ACh), play an important role in the treatment of many diseases including Alzheimer's disease (AD), myasthenia gravis, and ataxia [1-3]. Furthermore, the AChE inhibitors used in the symptomatic treatment of AD are known to be effective in eliminating the neurotoxic effect of β-amyloid (Aß) on disease development, protecting cells from oxidative damage and producing cellular antioxidants [4-6]. In light of this information, it is thought that the formation of oxidative damage with an excessive increase of AChE activity may be due to the formation of free radicals. The free radicals have an atomic or molecular structure containing unincorporated electrons. The structures, which easily exchange electrons with different molecules, are called " reactive oxygen species (ROS)". Oxidative stress occurs with excessive increase

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of ROS. This causes significant biomolecular damage in organisms [7]. Antioxidants have importance for reducing oxidative stress. Antioxidants have recently become one of the topics investigated by scientists due to their ability to scavenging free radicals. Today, it has been reported that many synthetic antioxidants are used especially in food additives and they negatively affect human health because they are not natural [8]. Phenolic compounds have been reported to be beneficial to health due to their antioxidant properties [9-10]. Many plant species used medically today are known to have rich phenolic content.

Hypericum species are extremely important plants that have been popularly used because of their various pharmacological effects. *Hypericum perforatum* L (HPL), a species of Hypericum known in Turkey as yellow cantaron and blood grass, is commonly known as "Saint John's Wort" around the world. It has been classified as a natural source of food sweeteners (Class 5) by the Council of Europe. Moreover, it has been reported that the plant can be used in the treatment of moderate depression. Furthermore, the studies have reported that HPL can be as effective as antidepressant used in traditional medical treatment [11,12]. It has been reported by the German Ministry of Health that HPL extract is used for the treatment of psychovegetative disorders, depressive disorders, anxiety and/or agitation situations and adjustment disorders [13]. In previous studies, HPL extracts have been reported to show a significant scavenging capacity for free radicals produced by the xanthine oxidase / xanthine system [14]. It has been reported in the literature that the important pharmacological effects of the plant originate from naftodiantron compounds (hypericin, psodohipericin etc.), fluoroglusinols (hyperforin, adhiperforin etc.), flavonoids (hyperositis, routine, quercetin etc.), biflavones (biapigenin, amentoflavones) phenolic acids, (ferulic acid, caffeic acid, etc.), proanthocyanidins, and essential oils [15]. *Equisetum arvense* L (EAL) is considered to be a living fossil that has been used for medicinal purposes since time immemorial and has been protected in many countries [16,17]. In traditional medicine, it has been used in urinary and prostatic diseases, symptoms of the urinary system, repairing lung tissue, lung tuberculosis, hormonal or metabolic edema, rheumatism, and wounds [18]. EAL contains caffeic acid derivatives, saponins, flavonoids, silica, and alkaloids, which have anti-inflammatory and antioxidant effects. EAL has various pharmacological properties as it contains various secondary metabolites such as phenolics, phytosterols, alkaloids, minerals [19-23]. It is

important to know the bioactivities of medicinal plants traditionally used to reduce oxidative stress caused by free radicals. In this study, anticholinesterase and antioxidant effects of HPL aqueous extracts (HPLAE), HPL methanol extracts (HPLME), EAL aqueous extracts (EALAE) and EAL methanol extracts (EALE) obtained from *Hypericum perforatum* L. (HPL) and *Equisetum arvense* L. (EAL) were investigated.

## Materials and Methods

### Determination of antioxidant activity

Ferric cyanide ($Fe^{3+}$) reducing antioxidant test (FRAP) was performed by modifying the method reported by Oyaizu (1986). When ferric ions ($Fe^{3+}$) are reduced to ferrous ions ($Fe^{2+}$) at 700 nm, the complex is formed and is a method of spectrophotometric measurement of this complex. Reduction capacity ($Cu^{2+}$) for Cupric ions was determined by the cupric ions reduction assay (CUPRAC) in previous studies [24-26].

### DPPH and ABTS radical scavenging activity

DPPH scavenging activities of methanol and water fractions of HPL and EAL were determined according to the method performed by Blois (1958). In the method, the stable DPPH radical is removed by the free radical removal activity of the sample. Extract of 10, 20 and 40 µg/mL was prepared from the samples then the volume with ethanol was adjusted to 1 mL. Then the prepared DPPH solution (1 mL, 0.1 m) was added and left in the dark for 30 minutes. DPPH removal activity of the sample after incubation was measured spectrophotometrically [1,27]. In this method, a sample is added to a pre-prepared ABTS· solution and after 30 minutes, the remaining cationic ABTS radical was measured spectrophotometrically at 734 nm [28]. Then, 1 mL of cationic ABTS radical solution was added to the sample at different concentrations. Absorbance was determined 30 minutes after mixing and for each concentration, radical removal percentage and $IC_{50}$ values were calculated [29].

### Determination of enzyme activity

The inhibitory effects on AChE of different HPL and EAL fractions were tested by Ellman's spectrophotometric method [30]. Reaction solution containing 50 µl AChE ($5.32x10^{-3}$ U), 100 µl of Tris–HCl solution (1 M, pH 8.0) and 50 µl 5,5'-dithio-bis(2-nitro-benzoic)acid compound (DTNB) was mixed and incubated at 30 °C for 15 minutes. Then, the reaction was started by adding 50 µl acetylthiocholine iodide (AChI) that was used as a substrate, and was performed spectrophotometric measurement at 412 nm [31].

## Result and Discussion

Inhibition of acetylcholinesterase (AChE) that hydrolyzes acetylcholine (ACh), is a basic approach in the symptomatic treatment of diseases such as ataxia, Alzheimer's disease (AD), myasthenia gravis, senile and dementia. Inhibition of AChE is important for increasing ACh levels in the synaptic cavity [1,6,32]. The use of AChE inhibitors such as galantamine, rivastigmine, and donepezil used for the treatment of AD in recent years has been limited due to side effects such as hepatotoxicity, abdominal pain, novelization, nausea, vomiting, and diarrhea. Therefore, it is important to provide potential source of AChE inhibitors from plants that are abundant in nature [33,34]. In a study on the inhibitory effect of different extracts on AChE (*in vitro*), the results showed that methanolic fractions had a more active effect than water fractions. The $IC_{50}$ values obtained for methanolic plant fractions included Nardostachys jatamansi (rhizome), Tinospora cordifolia (stem), Withania somnifera (root), Ficus religiosa (stem bark) Embelia ribes (Root) and Semecarpus anacardium (stem bark) were found in the range of 16.74-73.69 μg/ml [35]. In another study, inhibitory effects on AChE and BChE of rosmarinic acid were investigated. Rosmarinic acid was found to have an 85.8% inhibition effect on AChE at 1.0 mg / mL [36]. In our results, *Hypericum Perforatum* L. methanol fraction (HPLME) showed inhibitory effect on AChE with $IC_{50}$ values of 0.262 ± 0.03 mg/ml, while no inhibitory effect was observed in others (**Table 1**). *Hypericum Perforatum* L. water extract (HPLAE) showed no significant AChE inhibitory potential. The inhibition effect of methanol extract is consistent with the higher inhibitory potential in methanolic extracts of plants in previous studies.

Hypericum species often have good antioxidant effects due to the large number of different phenolic compounds they contain. According to the results of a study in which the free radical scavenging effect of *H. perforatum* was investigated in vitro, the antioxidant effect of the extract obtained was found to be directly proportional to concentration. Plant extract is strongly hydroxyl and superoxide anion- scavenging and prevents lipid peroxidation [37]. Plant fractions may have the potential to control or prevent the formation of reactive oxygen species (ROS) due to their volatile compound and phenolic content [38]. Phenolic compounds that can readily give hydrogen from hydroxyl groups along the aromatic ring prevent the harmful effects of ROS and free

radical oxidation [39]. The antioxidant/anticholinesterase activities and total phenolic content of HPLA, HPLME, EALAE, and EALME are shown in Table 1 in this study.

**Table 1** The anticholinesterase and radical removal activity of HPL and EAL fractions in different concentrations

| **Extracts** | DPPH[b] [0.2 mg/ml] | ABTS[b] [0.2 mg/ml] | Total phenolic content (μg GAE $mg^{-1}$ extract) | AChE $IC_{50}$ (mg/mL) |
|---|---|---|---|---|
| EALAE | 5.12 ± 0.3 | 4.63 ± 0.4 | 32.27 ± 3.6 | inactive |
| EALME | 52.41 ± 4.2 | 30.65 ± 2.8 | 30. 3 ± 3.9 | inactive |
| HPLAE | 48.64 ± 4.8 | 60.74 ± 4.6 | 16.04 ± 1.2 | inactive |
| HPLME | 25.44 ± 2.6 | 6.04 ± 0.4 | 21.46 ± 2.3 | 0.262 ± 0.03 |
| BHT[a] | 52.54 ± 5.5 | 58.06 ± 5.3 | - | - |
| BHA[a] | 80.59 ± 6.7 | 93.62 ± 6.2 | - | - |
| Trolox[a] | 90.56 ± 6.4 | 89.99 ± 5.9 | - | - |

Data mean ± standard deviation,

[a]standard antioxidant

[b]The percent (%) of ABTS and DPPH radical scavenging activity

The study by Dragana D et al. reported that the total phenolic compound was 79.52 ± 3.97 mg/g for EALME and was 25.4 ± 1.19 mg / g for EALAE [40]. In the study by Takeshi Nagai et al., head and stem parts of *Equisetum arvense* were studied and the results reported 12.8 mg/g in water extract for head part, 12.3 mg/g in ethanol extract, 7.98 mg / g in water extract for trunk part, and 23.9 mg / g in ethanol extract for head part [41]. The study by Annamaria Pallag et al. reported a total phenolic amount for EALME as 82.63 ± 0.06 mg / GAE and total flavonoid amount as 71.23 ± 4.33 mg / QE [42]. In addition, in FRAP, DPPH and CUPRAC assay reported results as 84.160 ± 0.078 μm Trolox equivalent/g, 87.30 ± 0.039, 49.2± 0.104 μm Trolox equivalent / G, respectively. The study by Bruno A Silva et al. identified 7 different fractions. *Hypericum perforatum* ethanol extract reported free radical-scavenger activity ($IC_{50}$) value of 21 lg dwb/ml [43]. In this study, metal reduction capacity was examined as FRAP and CUPRAC. When the

results of metal reduction capacity at 0.2 mg/mL were examined, from large to small HPLAE, EALME, HPLME and EALAE were found. From these extracts, HPLAE and EALME metal reduction capacity was found to be higher than HPLME and EALAE (Fig. 1a-b).

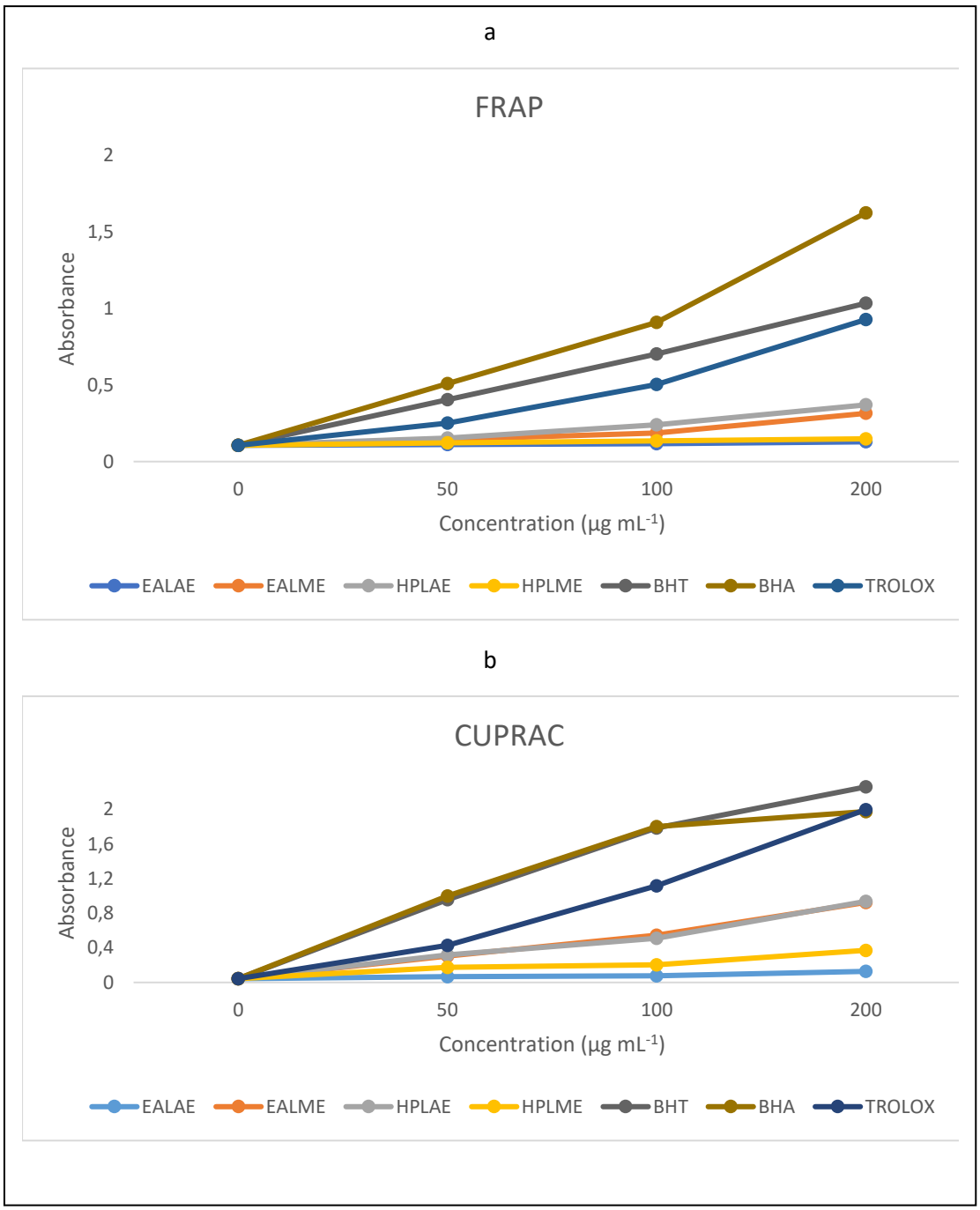


**Fig 1** Metal-reducing capacity with FRAP (a) and CUPRAC (b) assays of HPL and EAL fractions in different concentrations

DPPH and ABTS are well-known radical scavenging methods for measuring antioxidant activity. In the present study, EALME showed DPPH radical scavenging of about 52%, HPLAE 49%, HPLME 26%. HPLAE showed ABTS radical scavenging activity of approximately 61% and EALME 31% (Table 1). Total phenolic compound quantities were also found to be approximately 32 (µg GAE $mg^{-1}$ extract) for EALAE, 31 (µg GAE $mg^{-1}$ extract) for EALME, 21 (µg GAE $mg^{-1}$ extract) for HPLME, and 16 (µg GAE mg-1 extract) for HPLAE. The study by Dragana D et al. reported the total phenolic content for EALAE as 25.4 ± 1.19 mg/g. In this study, the total phenolic content for EALAE was found to be 32.269 ± 3.6 µg GAE $mg^{-1}$ extract. The results of our study and previous studies are consistent and supportive of each other. DPPH is a useful reagent for investigating the free radical scavenging effect of phenolic compounds [44]. The reduction of DPPH absorption is an indication of the capacity of extracts to free radicals scavenging. The best free radical scavenging activity was reported for EALME. The lowest activity was observed for the EALAE fraction. The fractions have shown radical scavenging activity close to standard antioxidants. Reactive oxygen species (ROS) are known to damage central nervous systems [45]. For this reason, antioxidants have important radical removal activities to eliminate these harmful effects. As a result, HPLAE and EALME have high antioxidant activities. The incorporation of certain HPL and EAL fractions into food products or pharmaceutical preparations is important in terms of the health-beneficial antioxidant content. It appears that the use of *H. perforatum* fractions does not cause any significant side effects evident in most consumers [46].

As it is known in the literature, it is important to determine the bioactivity of natural products recently. It has been found that some of the methanol and water fractions of HPL and EAL have radical scavenging and anticholinergic effects. HPL and EAL are thought to be used in the treatment of many diseases that develop oxidative stress due to this bioactivity property.

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