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Microwave-Assisted Extraction of Polyphenolics from Some Selected Medicinal Herbs Grown in Turkey

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Abstract: The effect of microwave-assisted extraction (MAE) process on the antioxidant capacity/activity of three medicinal herbs from Turkey was investigated by electrochemical differential pulse voltammetric (DPV)-CUPric Reducing Antioxidant Capacity (CUPRAC) assay. The optimal extraction time, temperature and solvent type were 6 min, 80 °C and 80% (v/v) methanol (MeOH), respectively. Microwave-assisted extracts of herbs (*Hypericum scabrum* L., *Papaver fugax* Poiret var. *platydiscus* Cullen, and *Achillea vermicularis* Trin.) were screened for total antioxidant capacity (TAC), total phenolic content (TPC) and ROS scavenging activities by employing different *in vitro* spectrophotometric assays. A positive correlation was observed between TAC-CUPRAC and TPC (R^2 = 0.972). Similarly, a positive correlation was observed between TAC-CUPRAC and free radical scavenging (FRS) activity (R^2 = 0.977). The order of FRS activities of tested samples was as follows: *Hypericum scabrum* L. > *Achillea vermicularis* Trin. > *Papaver fugax* Poiret var. *platydiscus* Cullen. These results suggest that these medicinal herbs provide promising antioxidant potentials as potential natural preservative agents in pharmaceutical industries.

Keywords: *Hypericum scabrum* L.; *Papaver fugax* Poiret var. *platydiscus* Cullen; *Achillea vermicularis* Trin.; microwave-assisted extraction; DPV-CUPRAC assay.

1. Introduction

Plants are a good source of phenolic compounds usually known as polyphenolics [1]. Polyphenolics have been found to act as antioxidants by inhibition of oxidative enzymes and scavenging reactive oxygen/nitrogen species (ROS/RNS), and many have therapeutic potential for free radicals related diseases [2]. ROS/RNS is involved in the pathology of several human diseases (*i.e.*, including atherosclerosis, malaria, cancer, neurodegenerative diseases, and rheumatoid arthritis) [3]. Recently, there has been growing interest in the therapeutic potentials of herbs as antioxidants in diminishing ROS/RNS induced tissue injury [4]. Therefore, it is important to assess antioxidant properties of the plants used in the herbal medicine either to elucidate the mechanism of their biological activity [5] or to provide information on antioxidant capacity/activity of these herbal plants.

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Papaver fugax Poiret var. *platydiscus* Cullen (endemic) occurs widely in the East Anatolia region of Turkey [6] and the buds and roots of this plant has been used by local people as calmative and analgesic. *Achillea vermicularis* Trin. is grown wild in different parts of East Anatolia region of Turkey [7] and this plant has been used in treatment of ulcer, head and neck pain. *Achillea vermicularis* Trin. contains sesquiterpenes, flavonoids, and diterpenes. It has been reported that this *Achillea* specie possess numerous biological activities including anti-inflammatory, antibacterial, antioxidant, and antispasmodic [8]. *Hypericum scabrum* L. spreads in other regions of Turkey except Marmara, Aegean and West Black Sea regions [9] and this medicinal herbal plant possess remarkable antibacterial, wound healing and anti-inflammatory activities. *H. scabrum* L. contains several compounds (*i.e*., including flavonoids, xanthine, tannins, phloroglucinol, and naphthodianthrones).

A variety of techniques are available for extraction of polyphenolics from medicinal plants and choice of suitable technique depends on the nature of the analyzed plant. Recently, microwave-assisted extraction (MAE) has drawn significant research attention in medicinal plant research due to its inherent advantages. It was also demonstrated that MAE is a more effective technique compared to the conventional solvent liquid extraction techniques. The extraction time was reduced, better extraction efficiency was observed, and less solvent was used [10]. Its higher yield is originally due to the heating generated *via* microwave which is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation leading to an increase in solvent temperature and the solubility of target analytes [11]. In this study, an effective and simple microwave–assisted method for extracting polyphenolics from selected medicinal herbs (*Achillea vermicularis* Trin., *Papaver fugax* Poiret var. *platydiscus* Cullen, and *Hypericum scabrum* L.) was optimized, their antioxidant properties were determined for the first time with the *in vitro* spectrophotometric antioxidant assays, and their polyphenolic constituents were identified and quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The antioxidant/antiradical activities of the medicinal herb extracts were determined by different antioxidant procedures, including electrochemical based total antioxidant capacity (TAC) assay (namely DPV based-CUPRAC method), spectrophotometric TAC assays (CUPRAC and ABTS methods), and the total phenolics content (TPC) of extracts were determined by the Folin-Ciocalteu (FC) procedure.

2. Materials and Methods

2.1. Standards, Samples, and Reagents

The following chemical substances of analytical reagent grade were supplied from the corresponding sources: Neocuproine (Nc) (2,9-dimethyl-1,10-phenanthroline), DPPH (2,2-diphenyl-1-picrylhydrazyl), catalase from bovine liver (1340 U mg^{-1} solid), methanol (MeOH), ethanol (EtOH), acetic acid, and the FC reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA); Potassium sodium tartrate tetrahydrate, copper(II) sulphate, copper(II) chloride dihydrate, iron(II) chloride tetrahydrate, hydrogen peroxide (30%, by wt.), $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, ammonium acetate (NH_4Ac), sodium hydroxide, and potassium persulfate were purchased from E. Merck (Darmstadt, Germany); ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt], disodium–EDTA, and sodium salicylate were purchased from Fluka (Buchs, Switzerland).

The medicinal herbs used as collected in varying periods of the year 2013, pressed, dried according to herbarium techniques, and identified by biologist Dr. Fevzi Özgökçe with respect to Flora of Turkey.

Papaver fugax Poiret var. *platydiscus* Cullen: B9 Van; Muradiye, Karavul Village, Kürk side, steppe, July 14, 2013, F14108, 38S0386765 E, 4333232 N, 2234 m.

Achillea vermicularis Trin.: B9 Van; Muradiye, Morgedik Village, Gola Pire side, rocky, June 9, 2013, 38S0382148 E, 4335710 N, F13985, 2234 m.

Hypericum scabrum L.: B9 Van; Muradiye, Güllüçimen Village, steppe, June 22, 2013, 38S0386542 E, 4334841 N, F14091, 2237 m.

The herbs were lyophilized (Telstar, LyoQuest, Terresa, Spain) and kept at +4 ^{o}C in hermetically vacuum-sealed plastic bags prior to analysis.

2.2. Sample Preparation

The well-dried medicinal herb samples were extracted by a microwave-assisted extraction system (Milestone ETHOS ONE, Sheldon, CT, USA). Microwave-assisted extraction (MAE) was employed in Teflon closed vessels with an automatic fiber optic temperature control system, sample (0.5 g) was extracted with 10 mL water/methanol mixture (20:80, v/v) at 80 °C for 3 min after 3 min temperature balancing time, and the power of microwave (0-1500 W) was modified spontaneously in accordance with temperature. The achieved methanolic extracts were filtered through a filter paper, then 0.45 µm PTFE syringe filters (Whatman), and kept at +4 °C until use. All the electrochemical and spectrophotochemical assays relating to extracts were carried out in *N*=5 replicates.

2.3. Antioxidant Efficiency Analysis

TPC of the medicinal herb extracts were evaluated by using the FC method [12]. Antioxidant capacity was done according to the CUPRAC method [13]. This spectrophotometric CUPRAC method is based on the reduction of Cu(II)-Nc by an antioxidant compound to the yellow-orange colored cuprous chelate (Cu(I)-Nc). TAC of the herbs were also determined by the electrochemical DPV-based CUPRAC method [14]. This electroanalytical method based on the reduction of $Cu(Nc)_2^{2+}$ to $Cu(Nc)_2^{+}$ by antioxidants and electrochemical detection of the remaining Cu(II)-Nc, the difference being related to TAC of the samples. The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to previously described method of ABTS-TEAC [15]. Radical scavenging abilities of the samples were evaluated using DPPH assay as described previously [16]. HRS activity (% inhibition) was measured using salicylate as a probe by the method of HRS-CUPRAC. The hydroxyl radicals ($^{\bullet}OH$) in aqueous solution were generated by the reaction of iron(II)-EDTA complex with H_2O_2 (Fenton reaction) and spectrophotometrically determined – *via* hydroxylation of a salicylate probe – by the HRS-CUPRAC method [17]. The ability of the synthesized complexes to scavenge H_2O_2 (% inhibition) was determined without interference by directly measuring the concentration of H_2O_2 using the modified CUPRAC method [18] at 450 nm in the presence of a Cu(II) salt. Results were expressed as the mean ± standard deviation (SD) of five replicates.

2.4. LC-MS/MS Analysis

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed using a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Türkiye) mass spectrometry. The analyses were performed using equip a Synergy Max C18 column (250 x 2 mm i.d., 5mm particle size) [19]. To analyze polyphenolics, the mobile phase consisted of two solvents, *i.e.* 0.1 % of formic acid in bidistilled water (A) and methanol (B). The polyphenolics were analyzed using gradient program: (Flow rate= 0.25 mL/min; column temperature: 30 ^{o}C): 0-1.00 min 55% A- 45% B; 1.01-20.00 min 100% B; 20.01-23.00 min 55% A – 45% B. Using the above working mode, these polyphenolics were identified by matching retention times and mass spectral data with those of calibration standards.

2.6. Statistical Analysis

The experimental findings were performed in triplicate. The data were recorded as mean ± ($t_{.05}$ $s/n^{1/2}$), where $t_{.05}$ is the Student's table t-value for 4 degrees of freedom and s is the standard deviation of *N*=5 measurements and analyzed by Excel software (Microsoft Office 2007). In related figures, these values were depicted within error bars.

3. Results and Discussion

3.1. Optimization of Microwave-Assisted Extraction (MAE) Conditions

Prior to assessment of antioxidant characteristics of medicinal herb extracts, the conditions of microwave-assisted extraction process were optimized by means of TAC measurements. *Achillea vermicularis* Trin. was selected as representative for the optimization of MAE process. Particularly, MeOH is widely used in extraction of polyphenolics from medicinal herbs, and it has been shown that MeOH is very effective solvent on extraction of polyphenolics with lower weights [20].

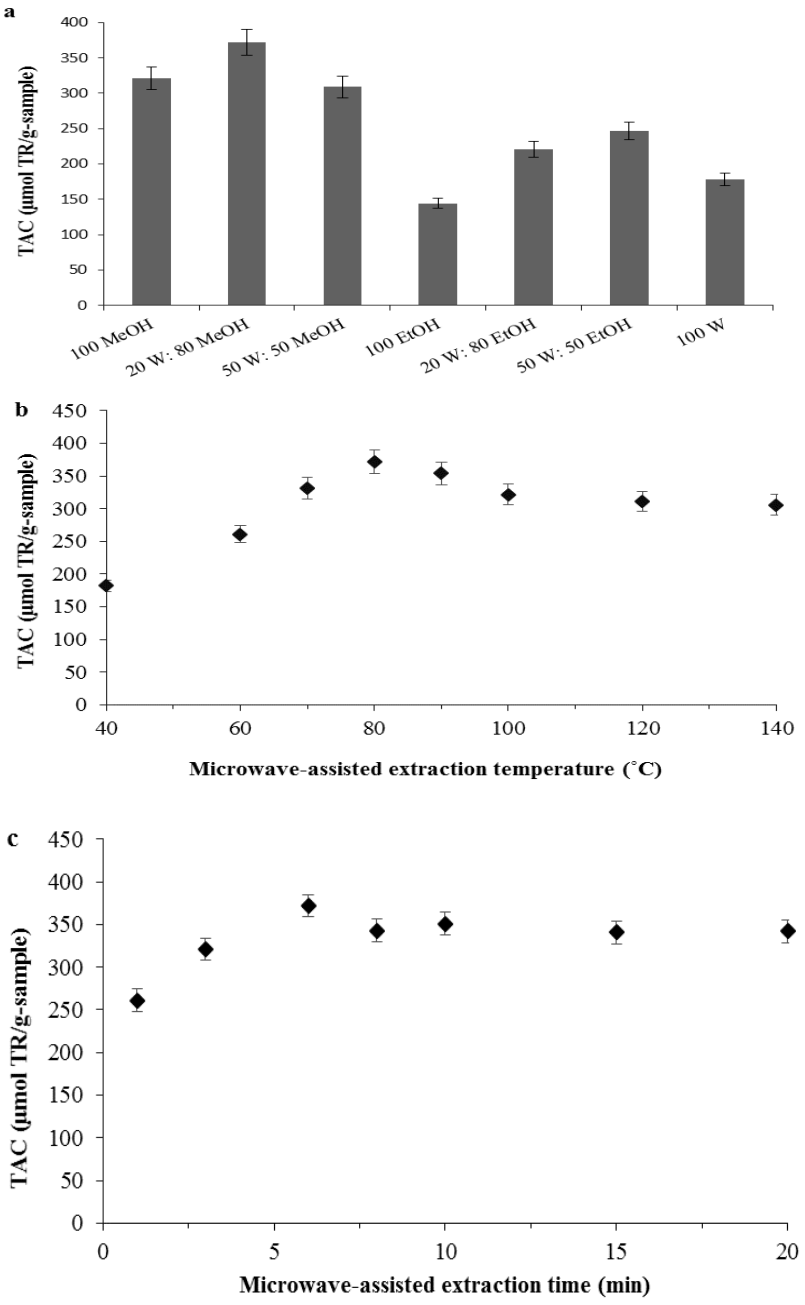

Figure 1. Effect of MAE conditions on the TAC of *Achillea vermicularis* Trin. methanolic extract: **(a)** solvent type (W:H_2O) and concentration, **(b)** temperature, and **(c)** time.

It was also reported that MeOH is the best supporting of ionizing among the solvents by simplifying electron transfer in anion solvation [21]. The influences of the solvent type and concentration were examined. Figure 1a depicts that the TAC of extract was greatly influenced by the solvent type. While 80% (v/v) MeOH concentration in water, maximum TAC value of extract was obtained. This concentration was selected as optimum solvent concentration for microwave assisted extraction.

The examination of the MAE temperature effect on the TAC extract (Figure 1b) indicated that TAC was increased consistently with the increasing temperature up to 80 °C, when the temperature was above 80 °C, a little TAC decrease was observed, presumably owing to fractional degradation of some antioxidant components. This temperature of was above the boiling point of 80% (v/v) methanol (*i.e.*, 64.5 °C), probably it causes internal pressure increase in the methanolic extract *via* microwave radiation, and therefore 80 °C was used in the following experiments.

The results obtained from the study consists of MAE time effect on the TAC values of extract (Figure 1c) show that the TAC of extract changed with extraction time and its maximum value was reached at sixth minute of extraction, and therefore MAE time of 6 min was employed in following experiments.

3.2. LC-MS/MS Characterization

Table 1. Phenolic characterization of methanolic herb extracts (μg/g dw herb) by LC-MS/MS.

Polyphenolics	***Hypericum scabrum* L.**	***Papaver fugax* Poiret var. *platydiscus* Cullen**	***Achillea vermicularis* Trin.**
Quercetin-3-*O*-arabinoside	1615.89±107.4	334.89±22.2	-
Quercetin	687.92±91.4	20.19±2.6	163.74±21.7
Gallic acid	18.89±1.3	2.82±0.2	2.57±0.2
Ellagic acid	220.33±14.7	-	26.72±1.7
Kaempferol	415.55±29.3	422.66±29.8	551.12±38.9
Quercitrin	434.55±27.7	-	126.78±8.1
p-Coumaric acid	636.73±44.1	1088.87±75.5	1096.98±76.1
Pyrogallol	98.63±6.5	38.33±2.5	-
p-Hydroxybenzoic acid	-	-	25.78±2.1
Ferulic acid	-	-	15.88±0.5
Syringic acid	-	-	-
Apigenin	2.43±0.2	7.48±0.6	91.07±7.3
Luteolin	153±39.3	15.44±3.9	716.15±183.9
Isorhamnetin	-	-	413.75±36.5
Chlorogenic acid	-	24.2±3.3	6270±868.2
Rosmarinic acid	5.1±0.3	9.28±0.7	6.91±0.5
Luteolin-7-*O*-glucoside	155.59±15.8	29.14±2.9	479.25±48.7
Luteolin-5-*O*-glucoside	97.19±6.2	10.4±0.6	313.31±20.1
Isoquercetin	1745.41±500.8	287.82±82.5	-
Kaempferol-3-*O*-rutinoside	456.71±41.2	18.67±1.6	47.67±4.3
Rutin	4964.44±325.1	532.79±34.9	80.07±5.2

Phenolic characterization (flavonoids and derivatives; hydroxycinnamic acids, phenolic acids, and others) of the samples was performed by using LC-MS/MS technique (Table 1) [19]. LC and MS-MS library containing 21 polyphenolics was established by ESI-LC/MS under negative ion mode. Methanolic extract of *Achillea vermicularis* Trin. contains the highest amount of chlorogenic acid

(6270±868.2 µg/g dw herb) and rutin (1096.98±76.0 µg/g dw herb) whereas microwave assisted extract of *Hypericum scabrum* L. showed highest amount of rutin (4964.44±325.1 µg/g dw herb) and quercetin-3-*O*-arabinoside (1615.89±107.4 µg/g dw herb). On the other hand, methanolic extract of *Papaver fugax* Poiret var. *platydiscus* Cullen exhibited highest fumaric acid (1088.87±75.5 µg/g dw herb). Flavonoids and their derivatives commonly exist in *Achillea vermicularis* Trin. and *Papaver fugax* Poiret var. *platydiscus* Cullen extract. Syringic acid was not found in the extracts. Ferulic acid, *p*-hydroxybenzoic acid, and isorhamnetin were only found in *Achillea vermicularis* Trin. extract. According to the Table 1, this herb was also very rich in hydroxycinnamic acids. The LC-MS/MS data and the results obtained by FC assay showed a positive correlation (R^2 = 0.957).

3.3. Total Phenolic Content (TPC)

Polyphenolics are found as the major contributor of antioxidative capacities of herbs [22]. Since phenols and all antioxidant compounds donate electrons to the reagent which has high redox potential, molybdotungstophosphate heteropolyanion ($3H_2O–P_2O_5–13WO_3–5MoO_3–10H_2O$), generating the FC chromophore [12]. Table 2 depicts the comparatively TAC and TPC of the herb extracts with each other in terms of µmol of TR equivalents per g of extract. TPC values of the extracts were higher than the corresponding TAC values (Table 2) because of the high redox potential of the FC reagent. *Hypericum scabrum* L. had the highest TPC (1238.2 ±14.3 µmol TR/g) among the medicinal herbs. The TPC values of *Achillea vermicularis* Trin. and *Papaver fugax* Poiret var. *platydiscus* Cullen were found as (756.1± 8.0 µmol TR/g), and (412.9± 5.1 µmol TR/g), respectively. The highest TPC values of *Hypericum scabrum* L. extract might be probably based on it's the highest total phenolic content.

Table 2. TPC, TAC (Voltammetric-CUPRAC, Spectrophotometric-CUPRAC, and ABTS methods), and EC_{50} values of methanolic herb extracts.

Samples	TPC (µmol TR/g)	Voltammetric CUPRAC-DPV (µmol TR/g)	Spectrophotometric -CUPRAC (µmol TR/g)	ABTS (µmol TR/g)	FRS activity (EC_{50}[a])	HPS activity (EC_{50}[a])	HRS activity (EC_{50}[a])
Hypericum scabrum L.	1238.2±14.3	436.5±5.1	469.8±6.1	251.1±3.2	2.53±0.1	6.75±0.3	6.27±0.4
Papaver fugax Poiret var. *platydiscus* Cullen	412.9±5.1	289.4±2.8	287.5±3.6	111.1±1.4	5.49±0.3	9.20±0.5	4.47±0.2
Achillea vermicularis Trin.	756.1±8.0	372.1±3.1	369.7±4.0	185.9±2.2	3.87±0.2	8.36±0.4	5.09±0.3

Each value is expressed within confidence interval as {mean±($t_{.05}$ $s/n^{½}$)} where $t_{.05}$ is the Student's table t-value for 4 degrees of freedom and s is the standard deviation of *N*=5 measurements.
[a]EC_{50} (mg/mL): effective concentration at which 50% of radicals are scavenged.

3.4. Total Antioxidant Capacity (TAC)

TAC represents the result of many parameters (*i.e.*, antioxidants localization, redox potentials of the compounds present in the matrix, their cumulative and synergistic interaction, and the nature of the oxidizing substrate. TAC considers the cumulative action of all the antioxidants present in the plant [23]. ABTS-TEAC assay used ABTS radical cation ($ABTS^{\bullet+}$) as probe, and based on the degree of color change which is correlated to the concentration of polyphenolics in the plant. The developed DPV−CUPRAC electrochemical method is an alternative method to the spectrophotometric methods for determining the TAC of herb extracts. Table 1 shows the TAC of the medicinal herb extracts expressed as µmol of TR equivalents per g of extract. The TAC values with respect to the voltammetric method were found as 436.5±5.1, 372.1±3.1 and 289.4±2.8 µmol/g for *Hypericum scabrum* L., *Achillea vermicularis* Trin., and *Papaver fugax* Poiret var. *platydiscus* Cullen, respectively. The LC-MS/MS data and the results obtained by FC assay showed a positive correlation

(R^2 = 0.938). The corresponding spectrophotometric findings of *Hypericum scabrum* L., *Achillea vermicularis* Trin., and *Papaver fugax* Poiret var. *platydiscus* Cullen with the conventional CUPRAC method were found as 469.8±6.1, 369.7±4.0 and 287.5±3.6 μmol/g, respectively. TAC values of microwave-assisted extracts were also determined by ABTS-TEAC method. The order of TACs of herb extracts were the same with three methods: *Hypericum scabrum* L., *Achillea vermicularis* Trin., and *Papaver fugax* Poiret var. *platydiscus* Cullen (Table 1). TAC values of herb extracts were also much lower than their corresponding TPC values.

It has been demonstrated that *Achillea vermicularis* Trin. shows very high TPC and strong scavenging activity toward DPPH radical [24]. Monoterpenes and diterpenes are the main components of this plant, and their antioxidant activity was established in many studies *in vitro*. In addition, sesquiterpenes have been found to exhibit antioxidant activity. Presumably their antioxidant properties are owing to reaction mechanism that acts by chain carrying perhydroxyl *radicals* ($HOO^{\bullet}$), which react quickly with linoleylperoxyl radicals and thus terminate the chain reaction. Synergistic interaction between terpenoids and other antioxidants such as α-tocopherol or flavonoids, like rutin, has been demonstrated [25]. Probably the highest TPC and TAC values for *Achillea vermicularis* Trin. found in this study are due to its high content of polyphenolics (especially hydroxycinnamic acid: chlorogenic acid) (Table 1) and terpenoids. Structural properties of hydroxycinnamic acids should normally dictate that chlorogenic acids (two –OH bearing) should exhibit higher trolox equivalent antioxidant capacity (TEAC) coefficient ($TEAC_{\text{chlorogenic acid}}$=2.5) than ferulic ($TEAC_{\text{ferulic acid}}$=1.2) and *p*-coumaric acids (one –OH bearing) ($TEAC_{p\text{-coumaric acid}}$=0.6).

Quercetin and its derivatives were abundant flavonoid in *Hypericum scabrum* L. and this plant contained more flavonoid compounds with highest TAC compared to other studied medicinal herbs. This result revealed that quercetin, its derivative and other flavonoids may be responsible for the antioxidant potential of this medicinal herb and suggested that there seemed to be a good correlation among these compounds and TAC. Quercetin is a more powerful antioxidant with high $TEAC_{CUPRAC}$ coefficient ($TEAC_{\text{quercetin}}$=4.38) than other flavonols. Quercetin and its derivatives has also been reported to exhibited various pharmacological activities such as antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory and hepatoprotective activities [26,27].

Antioxidant properties of a kind of *Papaver fugax* Poiret var. *platydiscus* Cullen has been demonstrated [28]. Numerous studies have indicated that *Papaver fugax* Poiret var. *platydiscus* Cullen has a wide variety of alkaloids. It has been demonstrated that alkaloids exhibited relatively good antioxidant potentials [29]. *In vitro* studies showed that some of alkaloids can act as effective scavengers of ROS, and also inhibit lipid peroxidation [30].

3.5. Free Radical Scavenging (FRS) Activity

Free radicals are generated in normal cell metabolism. Oxidation is essential to many living organisms for the production of energy. The main characteristic of an antioxidant is its ability to trap free radicals. DPPH-FRS activity assay is very popular to assess antioxidant activity of plant extracts. On receiving hydrogen from a corresponding donor, its solutions deceive the intense deep purple color [31]. The rate of reaction of various polyphenolics with DPPH differs. The FRS activities of tested medicinal herb extracts are expressed as decrease of concentration of DPPH or as EC_{50} (concentration of a compound decreasing the absorbance of a DPPH solution by 50 %) [32,33]. The DPPH-FRS activity of methanolic herb extracts increased with increasing concentration (Figure 2).

Figure 2 depicts DPPH-FRS abilities of the herb methanolic extracts. The concentration of extracts was increased gradually. When the concentration was 1 mg/mL, the values of DPPH radical scavenging activity were 41.2%, 36.4% and 20.6% for *Hypericum scabrum* L., *Achillea vermicularis* Trin., and *Papaver fugax* Poiret var. *platydiscus* Cullen, respectively. The values were dramatically increased to 97.2%, 92.1%, and 70.7% in the case of the concentration of the extract was 15 mg/mL. The order of free radical scavenging activities of herb methanolic extracts as follows: *Hypericum scabrum* L. > *Achillea vermicularis* Trin. > *Papaver fugax* Poiret var. *platydiscus* Cullen. The order of results showed similarity with that of both TAC and TPC values.

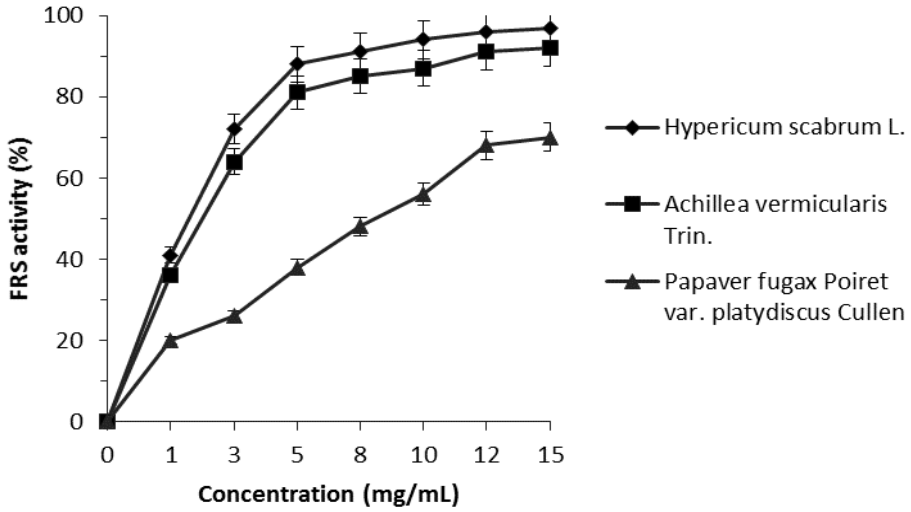

Figure 2. FRS activity (%) of the herb extracts (results showed within error bars).

3.6. Hydroxyl Radical Scavenging (HRS) Activity

The hydroxyl radical ($OH^{\bullet}$) is the most reactive of the ROS species due to its very fast kinetic and high redox potential and it induces oxidative damage in various biological macromolecules. Because of its over reactivity, HRS activity is very significant for assessing the radical scavenging activity of herbs. An HRS method using a salicylate probe for detecting $OH^{\bullet}$ generated by Fenton reaction, was employed to evaluate HRS of the microwave assisted herb extracts [17]. As seen in Figure 3, inhibition of $OH^{\bullet}$ are influenced intensely with the increasing concentrations of herb extracts. The initial concentration of the extracts was 1 mg/mL, and their inhibitions were 24.3%, 28.6%, and 32.7% for *Hypericum scabrum* L., *Achillea vermicularis* Trin., and *Papaver fugax* Poiret var. *platydiscus* Cullen, respectively. When the concentration was increased to 15 mg/mL, the order of the inhibition of the extracts was the same (*Papaver fugax* Poiret var. *platydiscus* Cullen > *Achillea vermicularis* Trin. > *Hypericum scabrum* L.) and were 68.4%, 58.1%, and 48.5%, respectively. The scavenging activities of methanolic extracts on the $OH^{\bullet}$ were decreased in the order of 93.1%, 75.3%, and 71.4% at the concentration of 15 mg/mL, respectively.

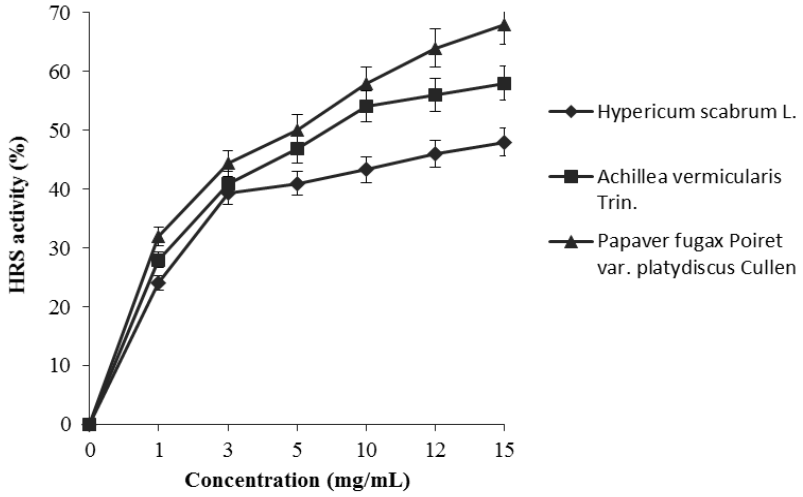

Figure 3. HRS activity (%) of the herb extracts (results showed within error bars).

3.7. Hydrogen Peroxide Scavenging (HPS) Activity

Hydrogen peroxide (H_2O_2) is a non-radical oxidant, and it can diffuse across biological membranes. H_2O_2 might lead to cell death by the so-called enzyme-suicide mechanism in many ways [34]. Therefore, the measurement of HPS activity of plant extracts is crucial. The HPS activities of the herb extracts were evaluated by a concentration-dependent method (HPS–CUPRAC) in the presence of Cu(II) as catalyst [18]. Figure 4 shows that in the existence Cu(II) catalyst the scavenging reaction of the extracts with H_2O_2. The HPS activities increased with the increasing concentration of the extracts, the activities were followed in that order; *Hypericum scabrum* L. > *Achillea vermicularis* Trin. > *Papaver fugax* Poiret var. *platydiscus* Cullen. When the concentration of extracts was 15 mg/mL, the HPS activities of the extracts was increased up to 59.6%, 44.7%, and 39.3%, respectively. This order of HPS activities was same with the test results of TPC and TAC.

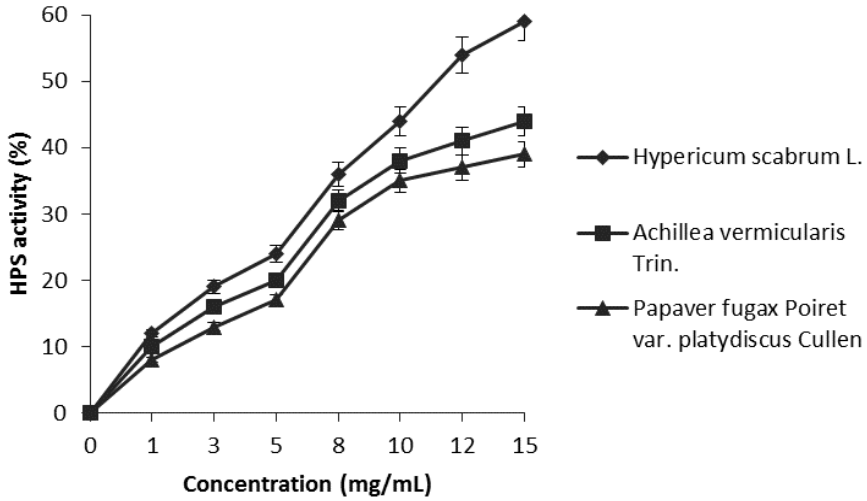

Figure 4. HPS activity (%) of the herb methanolic extracts (values depicted within error bars).

4. Conclusions

Three medicinal herbs (*Achillea vermicularis* Trin., *Hypericum scabrum* L., and *Papaver fugax* Poiret var. *platydiscus* Cullen), which grown particularly in Eastern part of Turkey, were evaluated for their antioxidant properties by different antioxidant assays and the obtained results from their methanolic microwave assisted extracts have shown that their antioxidant properties are substantial. Antioxidant activities of the herb methanolic extracts were depicted in Table 2 in terms of EC_{50} values (mg extract per mL). EC_{50} values inversely proportional with their antioxidant capacities. The three of medicinal herbs were analogous in antioxidant effectiveness of their methanolic extracts. The scavenging activity of DPPH radicals and H_2O_2 was in the same order: *Hypericum scabrum* L. > *Achillea vermicularis* Trin. > *Papaver fugax* Poiret var. *platydiscus* Cullen. There was a correlation found between these activities and both of TAC and TPC values. Scavenging activity order of $OH^{\bullet}$ were exactly the opposite to the others. *Hypericum scabrum* L. had the highest antioxidant properties in all of the tested methods than the other herbs. Finally, these medicinal herbs provide meaningful profits to the human health with their high antioxidant properties.

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