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Influence of extraction method on antioxidant properties of *Rheum ribes* root extract

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Abstract. *Rheum* species are important medicinal herbs, often used in pharmacological research, due to the presence of anthracene derivatives in the subterranean parts of the plant. In this study, we intended to assess its antioxidant capacity, in correlation with the method of extraction. For this purpose, *Rheum ribes* extraction was realized with four solvents of different polarities (50% methanol, 70% ethanol, 80% acetonitrile, and petroleum ether). We used different extraction techniques, such as orbital shaker, ultrasonic stirrer, microwave, and Soxhlet extraction, and the total phenolic content of the *Rheum ribes* extracts was determined by modified Folin–Ciocalteu method. The reducing power and radical scavenging activity of the extracts were also evaluated. The results shown that the antioxidant activity of the extracts depends on the extraction methods especially through the used solvent and decreases in the order: ethanol > methanol > acetonitrile > petroleum ether.

Keywords: *Rheum ribes*; antioxidant activity; Folin–Ciocalteu assay; DPPH; FRAP.

1. Introduction

In the developing world, the tendency towards natural antioxidants has increased due to the harmful effects of synthetic antioxidant use on the health. Hence, the researches on the antioxidant components in root, leaf and fruit of plant material are increasing day by day. *Rheum ribes* L. *(Polygonaceae*) is eaten as a vegetable, being the only native *Rheum* species growing in Eastern of Turkey. Its young shoots and roots are widely used in folk medicine due to pharmacological properties, such as promote digestion, improve appetite, treat diarrhea, and as stomachic, antiemetic [1, 2]. *Rheum ribes* enhances the memory in old patients. Extract of root and stem of *Rheum ribes* has high antioxidant activity. The findings reveal that foods containing antioxidants delay the progress of Alzheimer's disease probably due to prevention or neutralization of detrimental effects of free radicals [3]. There are many assays in vitro used to measure the antioxidant effect of *Rheum ribes* extract [1, 4-8]. Some authors reported the antioxidant properties of *Rheum ribes* extracts [1, 9-11].

As a follow-up to this, we focused on the influence of extraction method on the antioxidative properties of *Rheum ribes* extracts. as. We used four solvents (methanol, ethanol, acetonitrile and petroleum ether) and four extraction techniques (orbital shaker, ultrasonic stirrer, microwave and Soxhlet extraction) to figure out which method was more efficiently for the antioxidant activity. The antioxidative properties were examined by using spectrophotometric methods, i.e. 2,2-diphenyl-2-picryl-hydrazyl free radical scavenging (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays [9]. In addition, Folin-Ciocalteu modified method was used for determining the total phenolic content [10-12].

2. Experimental

The roots of *Rheum ribes* were purchased from a local market in Urfa, Turkey. Folin-Ciocalteu's reagent, iron (III) chloride hexahydrate, methanol, ethanol, acetonitrile, petroleum ether and sodium carbonate were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid (GA), and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All chemicals and solvents were of analytical grade. Deionized and pure water (Millipore-Q System) was used for the study.

2.1. Preparation of plant extracts. The extraction procedure was as described by Apak *et al.* [13]. The roots of *Rheum ribes* materials were dried at the room temperature and chopped into small parts by using a blender. On 2 g chopped roots were added 25 mL of extraction solvent (i.e. 50% methanol, 70% ethanol, 80% acetonitrile, and petroleum ether).

The extraction temperature and time were: 60 ^{o}C and 30 min for ultrasonic extraction [IU]; 25 ^{o}C and 4 h for Soxhlet extraction [IS]; 40 ^{o}C and 1 h for microwaves extraction (0.25 g root powder) [IM]; 25 ^{o}C and 2 h for orbital shaker extraction [IO]. The extracts were filtered using Whatman blue band filter paper and stored at -20 ^{o}C until analysis.

2.2. Total phenolic content

Total phenolic content of *Rheum ribes* was determined with Folin-Ciocalteu reagent according a modified method, using GA as a standard phenolic compound [12-17]. In brief, 100 µL sample were added to 4.0 mL of distilled water and 100 µL Folin-Ciocalteu reagent, and

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the mixture was incubated for 5 min at 30 ºC. 800 µL of 6% Na_2CO_3 solution were added and the mixture was incubated in the amber straight sided beaker for 30 min at 30 ºC. The absorbance was measured in 685-760 nm domain (i.e. 687 nm for methanol extract, 688 nm for ethanol extract, 685 nm acetonitrile extract and 686 nm fore petroleum ether extract) by using a spectrophotometer (UV 1601, Shimadzu Co., Ltd., Kyoto, Japan) [16].

The results were expressed as gallic acid equivalent equivalents (GAE, mg of GA per 1 mL sample) [8, 9]. The calibration equation for GA is $A = 0.0019x + 0.0095$ ($R^2 = 0.9996$). Each test was repeated three times.

2.3. Determination of radical scavenging ability by using DPPH method

The DPPH radical scavenging assay was applied by Marinova [15] and was later modified by Yilmaz and Seyhan [16] with some minor changes. In brief, 1.5 mL of the extract were mixed with 1.5 mL of 0.1 mM DPPH solution (in methanol). The reaction mixture was incubated at 30 ˚C for 30 min (200 rpm). The absorbance was measured in 515-528 nm domain (i.e. 521 nm for methanol extract, 515 nm for ethanol extract, 525 nm acetonitrile extract, and 528 nm for petroleum ether extract) by using spectrophotometer. The results were expressed as millimolar of Trolox equivalent antioxidant capacity (TEAC) per 1 mL sample. The calibration equation for Trolox is $A = -0.0134x + 0.6375$ ($R^2 = 0.9951$). Each test was repeated three times.

2.4. Ferric reducing/antioxidant power assay

We used the method described by Benzie and Strain [17] with some modifications. The working solutions were used on the day of preparation. FRAP reagent: acetate buffer (300 mM, pH 3.6), tripyridyltriazine (TPTZ) (10 mM in HCl, 40 mM) and $FeCl_3$ (20 mM). The required sample was added to the FRAP reagent (1:30, v:v) and incubated at 37 ºC for 4 min. The absorbance of the reaction mixture was read at 596 nm for methanol extract, 597 nm for ethanol extract, 597 nm acetonitrile extract, and 595 nm for petroleum ether extract. The antioxidant capacity, based on the ability of the sample to reduce ferric ions, was expressed as millimolar Trolox equivalents per sample by using a calibration curve [14]. The calibration equation for Trolox is $A = 0.0011x + 0.1188$ ($R^2 = 0.9893$). Each test was repeated three times.

3. Results and discussion

The antioxidant potential of *Rheum ribes* extracts, obtained by four extraction methods and also in different solvents, was evaluated and the results were compared.

3.1. Total phenolic content

The values of total phenolic content for analyzed samples are presented in Fig. 1. The ethanol extract of *Rheum ribes* showed the highest phenolic concentration, and contrarily the petroleum extract of this plant showed lowest antioxidant activity. As indicated in Fig. 1, the total polyphenols values varied from 0.30 to 1.22 mg GAE /mL.

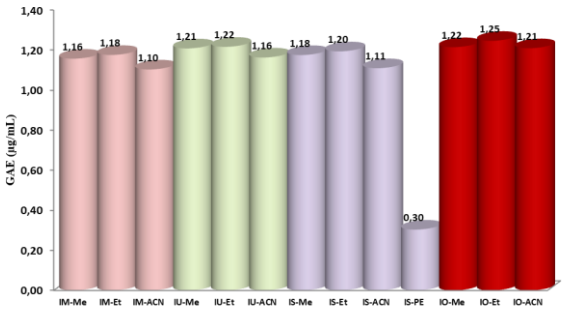

Figure 1. Total phenolic content in the *Rheum ribes* extracts expressed in terms of GAE.

3.2. Antioxidant capacity

The antioxidant capacity of *Rheum ribes* species were widely studied *in vitro* by DPPH and FRAP methods. To the best of our knowledge, some authors have been reported that this plant has antioxidant activity and a potential activity to protect the body from some diseases [7, 18-25].

The FRAP values of studied samples are shown in Fig. 2. The best antioxidant capacity was acquired using ethanol. Contrarily, petroleum ether extracts contain considerably smaller concentration of phenols. As indicated in Fig. 2, the FRAP values varied from 8.88 to 62.70 mM Trolox/mL.

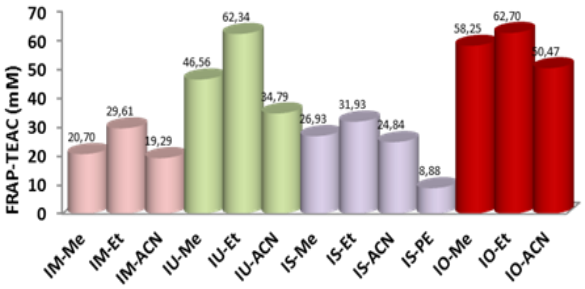

Figure 2. FRAP values for *Rheum ribes* extracts.

The DPPH values of samples are shown in Fig. 3. The higher value was determined for ethanolic extract. By contrast, the lowest antioxidant capacity was obtained by using petroleum ether as solvent. As indicated in Fig 3, the DPPH values varied from 2.84 to 28.98 mM TEAC/mL.

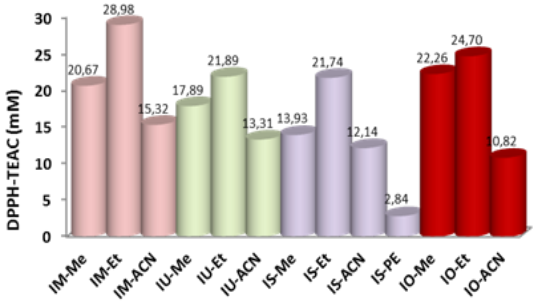

Figure 3. DPPH scavenging activity of investigated *Rheum ribes* extracts.

4. Conclusion

The antioxidant potential of the *Rheum ribes* extracts was evaluated using different antioxidant tests. According to

the results, the ethanol extract showed high antioxidant activity. Therefore, this ethanol fraction will have highest amount of antioxidant compounds which are thought to be phenol in nature. In addition, among the four extraction methods were compared, ultrasound and microwave extract techniques showed the best results.

Particularly, the petroleum ether extract was found to be the less active. The polyphenol extracted in the optimum conditions showed high antioxidant activities *in vitro*. Therefore, polyphenol concentrates with high phenolic contents and powerful antioxidant properties can be obtained from roots of *Rheum ribes* using the optimized conditions. For the medicinal properties of this plant due to its antioxidant potential further *in vivo* studies of these species are required.

Conflict of interest

The authors declare no conflict of interest.

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