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BIOLOGICAL ACTIVITIES AND PHENOLIC CONTENTS OF *Rumex scutatus* COLLECTED FROM TURKEY

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ABSTRACT

Plants come to the fore with their nutritional properties. In addition to its nutritional properties, it is also known to have medicinal properties. In this study, antioxidant, oxidant and antimicrobial activities of the aerial parts of *Rumex scutatus* L. collected from Antalya province were determined. In addition, the phenolic contents of the plant were determined. The above-ground parts of the plant were extracted with ethanol and water. Then, antioxidant and oxidant status were determined using Rel Assay kits. Antimicrobial activities were determined against standard bacterial and fungal strains by agar dilution method. Phenolic contents were measured in HPLC device. As a result of the studies, it was determined that the plant has high antioxidant activity. It also showed high activity especially against gram negative bacteria. In addition, Catechin, Epicatechin, Chlorogenic acid, Sinapic acid, Qumaric acid and Benzoic acid were detected in the plant. As a result, *R. scutatus* was determined to have significant biological activity. It is also thought to be an important source in terms of the compounds detected in its body.

KEYWORDS:
Antimicrobial, Antioxidant, Complementary medicine, Medicinal Plants, Oxidant, Phenolic content

INTRODUCTION

Since ancient times, people have sought different treatments for the treatment of diseases [1]. During these searches, he used many natural materials by observing nature and by trial and error methods [2]. The use of natural materials, which are equivalent to the history of humanity, in the treatment of diseases is at a very high level today [3]. Plants from these natural materials are highly preferred due to the wide variety and biologically active compounds they contain. Many studies have shown that plants have different effects such as antioxidant, antimicrobial, anticancer, DNA protective, antidiabetic, hepatoprotective, antiinflammatory [4-8]. In this context, new studies are important in order to emphasize the importance of different plant species as natural materials. For this reason, biological activities and phenolic contents of *R. scutatus* plant were determined in this study.

The *Rumex* genus is a genus of biennial and perennial plant species belonging to the Polygonaceae family. Species belonging to the *Rumex* genus are widely distributed in many parts of the world. Species belonging to the *Rumex* genus are mostly edible plants. Most species contain oxalic acid and tannin [9]. Because of these properties, it shows mild laxative properties. It is also very common, especially in salads. For this reason, antioxidant, oxidant and antimicrobial activities and phenolic contents of *R. scutatus* were determined. In this context, it is aimed to determine its use in complementary medicine as well as its use as a nutrient.

MATERIALS AND METHODS

Laboratory studies. *R. scutatus* specimens were collected from Antalya/Turkey. The muddy and soiled parts of the above-ground parts of the plant were cleaned using distilled water. Then it was dried in the open air and under suitable conditions in the laboratory environment. After drying, 30 g of the plant samples were weighed and extracted with water and ethanol in a soxhlet device (approximately 6 hours at 50 ^{0}C). After the extraction process, the crude extraction process was made using the Rotary Evaporator device.

Antioxidant and Oxidant Tests. The antioxidant and oxidant values of the plant sample were measured using Rel Assay brand TAS and TOS kits. During the experiment, the procedures were carried out according to the protocol included in the kit. The calibrator Trolox was used in TAS kits and values are expressed in mmol Trolox equiv./L. The calibrator hydrogen peroxide was used in TOS kits and values were expressed in μmol H_2O_2 equiv./L [10, 11]. In

addition, the following formula was used when calculating the oxidative stress index OSI (AU: Arbitrary unit) [12].

$$OSI\ (AU) = \frac{TOS,\ \mu mol\ H_2O_2\ equiv./L}{TAS,\ mmol\ Trolox\ equiv./L\ X\ 10}$$

Determination of Antimicrobial Activities. Agar dilution test was used to test the efficacy of ethanol and water extracts of the plant against bacterial and fungal strains and MIC (minimal inhibitory concentrations) were determined. The MIC value is the lowest concentration value of the extracts that prevents the growth of bacteria and fungi. In this context, bacterial strains (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606). Fungal strains (*Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030) were cultured in RPMI 1640 Broth medium. All extract concentrations were obtained with distilled water [13-17].

Detection of Phenolic Compounds. Phenolic compounds in the plant were detected using HPLC device. The DAD detector was used and the injection volume was set to 20 µL. A: 3% acetic acid and B: methanol were used as the mobile phase and the flow rate was adjusted to 0.8 mL per minute. Chromatographic separation was performed at 30 oC with an Agilent Eclipse XDB-C18 column (250x4.6 mm; id 5 µm) [18]. (Caponio et al., 1999).

RESULTS AND DISCUSSION

Antioxidant Activity Results. Reactive oxygen species are harmful compounds produced as a result of environmental effects and metabolic activities [19]. Endogenous antioxidants come into play in suppressing ROS [20, 21]. When these compounds reach high levels and antioxidants are insufficient, oxidative stress occurs [22]. It is known that oxidative stress has negative effects on people such as cardiological disorders, Alzheimer's and Parkinson's, and cancer [23]. Supplementary antioxidants can be used in cases where the antioxidant defense system is insufficient to reduce the effects of oxidative stress [24, 25].In this context, the antioxidant potential of *Rumex scutatus* was determined in this study. The obtained results are shown in Table 1.

In previous studies, it has been reported that *Rumex scutatus* has antioxidant potential [26]. In our study, it was determined that the TAS value of Rumex scutatus was high. The antioxidant potential of *Rumex scutatus* has not been determined before using Rel Assay kits. It was determined for the first time in our study. In TAS, TOS and OSI studies conducted on different plant species, the TAS value of *Rumex crispus* has been reported as 7.313, TOS value was 4.798, and OSI value was 0.066 [27]. The TAS value of *Mentha longifolia* subsp. *longifolia* has been reported as 3.628, TOS value was 4.046 and OSI value was 0.112 [28]. The TAS value of *Rhus coriaria* var. *zebaria* has been reported as 7.342, TOS value was 5.170, and OSI value was 0.071 [29]. The TAS value of *Ferulago platycarpa* has been reported as 5.688, TOS value was 15.552, and OSI value was 0.273 [30]. The TAS value of *Scorzonera papposa* has been reported as 6.238, TOS value was 11.525, and an OSI value was 0.182 [31]. The TAS value of *Allium calocephalum* has been reported as 5.853, the TOS value was 16.288, and the OSI value was 0.278 [32]. Compared to these studies, the TAS value of *R. scutatus* was found to be higher than *R. crispus*, *M. longifolia* subsp. *longifolia*, *R. coriaria* var. *zebaria*, *F. platycarpa, S. papposa and A. calocephalum*. TAS value shows all endogenous antioxidants produced in living organisms. The increase in TAS value indicates that compounds with antioxidant properties are high. In this context, high TAS value is an indicator of high antioxidant potential [33]. The high TAS value of *R. scutatus* in our study shows that the plant has an important antioxidant potential. As a result, it was determined that *R. scutatus* can be used as a natural antioxidant source.

Oxidant compounds are produced as a result of environmental effects and metabolic activities. TOS value is an indicator of the totality of oxidant compounds in living organisms. A low TOS value indicates that the region where the living organism lives triggers less oxidative stress [34]. In this context, the TOS value of a healthy natural material is expected to be lower. In our study, it was determined that the TOS value of *R. scutatus* was higher than *R. crispus* and *M. longifolia*, and lower than *R. zebaria, F. platycarpa, S. papposa* and *A. calocephalum*. In this context, it is seen that the TOS values of *R. scutatus* are at normal levels.

OSI value shows how much endogenous oxidants are suppressed by endogenous antioxidants in living organisms. The high OSI value indicates that the antioxidant defense system is insufficient against oxidant compounds [35]. In our study, it was determined that the OSI value of *R. scutatus* was lower than *R. crispus, M. longifolia*, *R. zebaria, F. platycarpa, S. papposa* and *A. calocephalum*. This result shows that *R. scutatus* is quite good in terms of antioxidant compounds in suppressing oxidant compounds. As a result, *R. scutatus* is thought to be an important antioxidant source.

Antimicrobial Activity Results. In recent years, there has been an increase in the number of diseases caused by microorganisms [36]. Antibiotics are generally used in the treatment of diseases originating from microorganisms. However, in recent

years, many resistant microorganisms have emerged. The unconscious use of antibiotics draws attention in the resistance of microorganisms to antibiotics [37]. In addition, the side effects of synthetic drugs can be a factor in the formation of different diseases [38]. As a result, the discovery of new antimicrobial drugs has been inevitable. For this reason, researchers have turned to natural products for new antimicrobial drugs. In our study, the effects of *R. scutatus* against standard bacterial and fungal strains were investigated. The obtained results are shown in Table 2.

In previous studies, *R. scutatus* has been reported to be effective at different levels against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, Enterococcus faecalis, Bacillus cereus, Micrococcus flavus, Staphylococcus aureus, Aspergillus fumigatus, Aspergillus niger, A. versicolor, A. ochraceus, Penicillium funiculosum, Penicillium ochrochloron, Penicillium veruccosum* and *Trichoderma viride* [26]. In a different study, it was reported that *R. scutatus* has different levels of effect against *Staphylococcus epidermidis*, *Bacillus subtilis, Moraxella catarrhalis, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae, Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* [39]. In our study, ethanol and water extracts of *R. scutatus* were used. As a result of the findings, it was determined that water extracts showed higher activity than ethanol extracts. In addition, water extract of plant showed the highest activity against *E. coli* and *P. aeruginosa* at 25 µg/mL extract concentration. The ethanol extract of plant showed the highest activity against *P. aeruginosa* at 25 µg/mL concentration. The water extract of the plant was effective against gram-positive bacteria at 100-200 µg/mL concentrations. Ethanol extract of the plant was effective against gram-positive bacteria at 200 µg/mL concentration. In addition, the extracts of plant were effective against fungal strains at 100-200 µg/mL concentrations. As a result, it was determined that the plant extracts were effective against test microorganisms at 25-200 µg/mL concentrations. In this context, it has been determined that *R. scutatus* has antimicrobial potential and can therefore be used as a natural antimicrobial agent.

Phenolic Contents. Plants produce many biologically active compounds within their body. Although these compounds do not have nutritional properties, they have important medicinal properties [40]. In this context, the determination of phenolic compounds in plants is very important in terms of their use as natural materials [41]. In this study, phenolic compounds in *R. scutatus* were determined. The obtained results are shown in Table 3.

Phenolic compounds form the basis of many drugs used in the treatment of diseases. Catechin has been reported to have anti-metastatic, antioxidant, antibacterial, antifungal, and anticancer activities [42-47]. It has been reported that epicatechin has anticancer, antioxidant, antimutagenic and antimicrobial activities [43, 46, 48, 49]. Chlorogenic acid has been reported to have antimicrobial, anti-inflammatory, analgesic, antipyretic and antioxidant activities [50-52]. It has been reported that sinapic acid has activities such as antioxidant and anti-thrombosis [53,54]. It has been reported that P-Coumaric acid has antioxidant, antimicrobial and anticancer activities [55,56]. It has been reported that benzoic acid has antimicrobial and antihepatotoxic activities [57-59]. In our study, the presence of Catechin, Epicatechin, Chlorogenic acid, Sinapic acid, P-Coumaric acid and Benzoic acid was found in *R. scutatus*. The highest P-Coumaric acid was determined in the plant. As a result, it was determined that *R. scutatus* could be a natural source for the detected compounds.

TABLE 1
Antioxidant and Oxidant Status of *Rumex scutatus*

	TAS	TOS	OSI
Rumex scutatus	8.656±0.199	4.951±0.409	0.057±0.006

Values are presented as mean±SD

TABLE 2
MIC values of *Rumex scutatus*

Extracts	A	B	C	D	E	F	G	H	J
Water	100	200	200	25	25	50	100	200	100
EtOH	200	200	200	50	25	50	200	200	100

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*
*200, 100, 50 ve 25 µg/mL extract concentrations

TABLE 3
Phenolic content of *Rumex scutatus*

	Rumex scutatus (ppm)
Catechin	17.54
Epicatechin	20.22
Chlorogenic acid	5.49
Sinapic acid	14.63
P-Coumaric acid	33.58
Benzoic acid	7.20

CONCLUSIONS

In this study, the antioxidant and antimicrobial activities of the edible wild plant *R. scutatus* were investigated. According to the results obtained, it has been determined that the plant can be an important antioxidant and antimicrobial agent. In addition, phenolic compounds in *R. scutatus* were determined. As a result, it has been seen that the plant can be a source for the detected compounds. In this context, it is thought that the plant may be a natural agent in pharmacological designs.

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