

## ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT FROM RUMEX CRISTATUS DC

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**Abstract-** *Plants have been used for many years as a source of traditional medicine to treat various diseases and conditions. R. cristatus DC (Polygonaceae) is widely spread in Turkey and used as both herbal medicine and food. This study examined the antioxidant activities of ethanolic extract of R. cristatus DC using different tests. The antioxidant activity of ethanolic extract of R. cristatus leaves was analyzed for total phenolic, flavonoid, ascorbic acid and  $\beta$ -carotene contents, reducing power and DPPH radical scavenging activity. The results were compared with natural and synthetic antioxidants. The results suggest that consumption of R. cristatus DC can be beneficial effects due to its antioxidant properties*

**Keywords:** *Antioxidant activity, Rumex cristatus DC, free radicals, scavenging activity*

### 1. INTRODUCTION

Free radicals play an important role in some pathogenesis of serious diseases, such as arteriosclerosis and cancer [1]. About 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals [2].

Consumption of various types of fruits and vegetables provides excellent health benefits because they are a rich source of phytochemicals that are good for disease risk reduction. High intake of fruits and vegetables has been reported to be associated with a lower incidence of chronic diseases such as cardiovascular disease [3, 4] and cancer [4, 5]. These health benefits are attributed to the antioxidant capacity derived from the phenolic compounds present in edible plants [6].

Phenolic compounds are constituents of both edible and nonedible parts of plants. Many have antioxidant activity, which delays the oxidation of various "important for life" compounds by inhibiting the initiation or propagation of oxidising chain reactions. Ascorbic acid and  $\beta$  carotene levels of the

extract as indicative of antioxidant capacity was aimed.

Natural antioxidants endogenous to food of plant origin can scavenge reactive oxygen and nitrogen species (RONS); evidence suggests that these may be of great importance in preventing the onset of oxidative diseases in the human body [7, 8].

The genus *Rumex* L., (Polygonaceae) is contains roughly 200 species, is widespread throughout the world and 23 species and 5 hibrids in Turkey [9]. The *Rumex* genus comprises several species, of which leaves and roots have been used in traditional medicine for inflammation, blood purification, constipation, purgative and tonic in Turkish traditional medicine [10]. Phytochemical screenings of *Rumex* species have revealed the presence of anthraquinone derivatives, flavonoids, terpenes, organic acids and naphthalene derivatives [11].

In this study, we have investigated the antioxidant activity of the ethanolic extract from *Rumex cristatus* DC. The total phenolic and flavonoid contents were also determined to find out the relationship between free radical scavenging assays. Additional determination of water instead of plant extract

and same procedure applied respectively. Absorbances of the solutions measured by using a UV-vis.

## MATERIAL AND METHODS

### 2.1. Chemicals

2-Deoxy-D-ribose, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 3-(2-pyridyl) - 5,6-bis (4-phenyl-sulfonicacid) - 1,2,4-triazine (ferrozine), 2,2'-azino-bis (3-ethyl benzothiazoline - 6 - sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), (+) catechin hydrate were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2,2-diphenyl-1-picryl-hydrazyl (DPPH),  $\alpha$ -tocopherol and pyrocatechol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), ferric chloride were obtained from Merck. All other reagents used in this study were of analytical grade.

### Plant materials

*R. cristatus* DC leaves were collected in May from Avcilar campus area of Istanbul University and identified by Prof. Dr. Kerim Alpınar (Faculty of Pharmacy, Istanbul University). Leaves were washed with distilled water and dried at room temperature. Dried leaves were stored in a deep freeze at -20 °C.

### 2.3. Preparation of extract

Dried rumex leaves (25 g) were extracted by soxhlet extraction system with ethanol for 4 hours. The extract was then filtered and evaporated to dryness under reduced pressure and controlled temperature (40-50 °C) in a rotary evaporator. The extracts were kept in -20 °C until use.

### 2.4. Determination of total phenolic content

Total phenolic content of *Rumex cristatus* DC extract was determined according to Slinkard and Singleton method [12] with some slight modifications. 0.1 mL aliquots of diluted plant extract were transferred to test tubes. Then diluted with 4.5 mL of distilled water and 0.1 mL Folin-Ciocalteu reagent added. After 3

min 0.3 mL of 2 %  $\text{Na}_2\text{CO}_3$  solution added to these mixtures. All the solutions were vortexed and allowed to stand in a dark place for 2 h. Blank solution is also prepared with 0.1 mL distilled spectrophotometer at 760 nm against blank. Total phenolic content of plant extract was calculated with the standard curve of pyrocatechol and expressed as  $\mu\text{g}$  pyrocatechol equivalent. The samples were analyzed in triplicate.

### Determination of Total Flavonoid Content

Total flavonoid content was measured using the previously developed method by Zhishen [13]. A 0.25 mL aliquots of *Rumex cristatus* extract were mixed with 1.25 mL distilled water and 75  $\mu\text{L}$  of 5 %  $\text{NaNO}_2$  solution. Then incubated at room temperature for 6 min. After incubation 150  $\mu\text{L}$  of 10 %  $\text{AlCl}_3$  solution added to each test tube. All the test tubes incubated again for 5 min, 0.5 mL of 1 M NaOH solution and 275  $\mu\text{L}$  distilled water added to the mixtures respectively. All the solutions vortexed and absorbance of the resulting solution was read at 510 nm against blank. Blank was prepared using distilled water instead of plant extract. Samples were analyzed in triplicate and flavonoid content expressed as  $\mu\text{g}$  (+)-catechin equivalent.

### 2.6. Determination Of Ascorbic Acid Content

Ascorbic acid was determined according to the method of Omaye [14]. 5 mg of plant extract was extracted with 5 mL 1 % metaphosphoric acid solution for 45 min at room temperature and then filtered. The filtrate (2 mL) was mixed with 1 mL of 2,6-dichlorophenol indophenol solution (0.1 mg/mL) and absorbance was measured within 15 seconds at 520 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of L-ascorbic acid. Ascorbic acid content of extract was calculated by using this standart curve. Result was expressed as  $\mu\text{g}$  ascorbic acid per gram of plant extract.

### Determination of $\beta$ -carotene content

25 mg extract of ethanol is solved with 10 mL acetone-hexane mixture (4:6), shaken for 1 min and then filtered. Absorbance of filtrate

was measured at 435, 505 and 663 nm [15]. The result of  $\beta$ -carotene content was calculated according to following formula;  
 $\beta$ -carotene (mg/100 mL plant extract) =  $(0.216 \times A_{663}) - (0.304 \times A_{505}) + (0.452 \times A_{435})$

## 2.8. Reducing power

The reducing power of extract was determined according to the method described of Oyaizu [16]. 1 mL of various concentrations of *R. cristatus* water extracts were mixed with 2.5 mL of 200 mmol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 30 min. After the incubation 2.5 mL of trichloroacetic acid (10 % , w/v) was added. Then vortexed and centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride 0.1 %. The absorbance was measured at 700 nm, higher absorbance indicates higher reducing power.  $\alpha$ -Tocopherol acetate were used as standart antioxidant.

## 2.9. DPPH radical scavenging activity

To determine the hydrogen donating ability of the extract a method based on the reduction of a methanolic solution of the coloured free radical DPPH to the nonradical form was used.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by the method of Brand-Williams [17]. Briefly, 1.5 mL of 20 mg/L DPPH radical in methanol solution was put into test tubes, then 0.75 mL of plant extract at various concentrations added. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 5,10,30,60 min. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm against methanol. The ability to scavenge DPPH radical was calculated by using the formula:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_1 / A_0) \times 100$$

where  $A_0$  is the absorbance of DPPH• in methanol solution without an antioxidant, and  $A_1$  is the absorbance of DPPH• in the presence

of an antioxidant. Synthetic antioxidants; BHA and BHT used as standarts.

## 2.10. Hydroxyl radical scavenging activity

The effect of extract on hydroxyl radicals was assayed by using the deoxyribose method [18].

The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.4), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM FeSO<sub>4</sub>-EDTA, 0.15 ml of 10 mM hydrogen peroxide, 0.525 ml of distilled water and 0.075 mL of extract solution in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37°C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8 % TCA and 0.75 ml 1.0 % of thiobarbituric acid. The mixture was boiled for 10 min, cooled in an ice bath and then measured at 520 nm. Hydroxyl radical scavenging activity was calculated in the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = (A_0 - A_1 / A_0) \times 100$$

$A_0$  is the absorbance of the control reaction,  $A_1$  is the absorbance of sample.

## 3. RESULTS AND DISCUSSION

### 3.1. Total Phenolic, Total Flavonoid, $\beta$ -Carotene And Ascorbic Acid Content

Herbs, fruits, spices and vegetables are important natural antioxidant [19]. Their antioxidant activity has been attributed to the presence of polar phenolic compounds. Many phenolic compounds have been attributed an array of health-promoting benefits, and they are of current interest due to their important biological and pharmacological properties, especially the antioxidant, antiinflammatory, antimicrobial, anti-allergenic, antimutagenic, anticarcinogenic, cardioprotective and vasodilatory effects [20]. The total phenolic content of *R. cristatus* ethanol extract was shown in Table 1. Total phenolic content of the extract obtained from leaves of the *R. cristatus* was determined using Folin-Ciocalteu colorimetric method by using the regression equation of pyrocatechol calibration curve. The amount of phenolics per each extract concentration was expressed as pyrocatechol equivalent. The results obtained

from the assay were expressed as means standard deviation of triplicate analyses and are presented in Table 1. Variations in total phenolic contents among the investigated *R. cristatus* extracts increased with increasing concentration. The highest total phenolic content ( $32.22 \pm 5.34$  mg pyrocatechol/mL) was found at the highest concentration. Phenolic compounds may indicate that the high antioxidant activity of *R. cristatus* extract may be associated with its total phenolic content.

**Table 1.** Total phenolic compounds (TPC) as pyrocatechol equivalents and Total flavonoids as catechin equivalents of ethanol extract from *Rumex cristatus* DC

Extract concentration	TPC as pyrocatechol equivalents ( $\mu\text{g/mL}$ )*	Flavonoids as catechin equivalents ( $\mu\text{g/mL}$ )*
1000	$7.69 \pm 2.58$	$32.94 \pm 31.36$
2000	$22.10 \pm 6.74$	$93.66 \pm 47.43$
3000	$24.25 \pm 4.41$	$128.96 \pm 24.48$
4000	$32.22 \pm 5.34$	$218.24 \pm 92.50$

\*Mean  $\pm$  SD

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants [22]. Flavonoids, including flavanols, flavones and condensed tannins, are plant secondary metabolites. Therefore dietary intake of flavonoid-containing foods was suggested to be of benefit for the preservation from free radical damage. Consumption of the flavonoid-containing fruits and vegetables has been linked to protection against cancer and heart disease [23]. We found flavonoid content of *R. cristatus* ethanol extract as

$218.24 \pm 92.50$   $\mu\text{g/mL}$  as catechin equivalent (Table 1). The results revealed that ethanolic extract of *R. cristatus* contains significant amount of phenols and flavanoids.

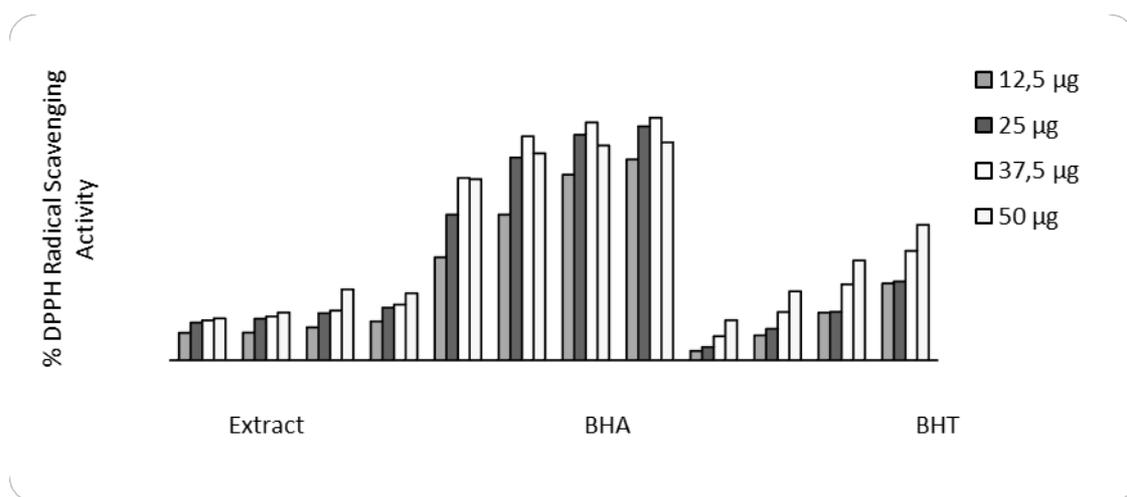
Small molecular weight antioxidants, such as ascorbic acid and carotenoids also play important roles in preventing free radical damage. Ascorbic acid is one of the most important water soluble antioxidants in cells, efficiently scavenging reactive oxygen species such as superoxide, hydroxyl radicals and singlet oxygen, also vitamin C was shown to act as a chain breaking scavenger for peroxy radicals. Carotenoids are very powerful antioxidant agents involved in the scavenging of two of the reactive oxygen species, singlet oxygen and peroxy radicals. A number of epidemiological studies have revealed that an increased consumption of a diet rich in ascorbic acid and carotenoids is correlated with a diminished risk for several degenerative disorders, including various cancer, cardiovascular and various different diseases [24, 25].

In this study, ascorbic acid content of *Rumex* extract was found as  $114.53 \pm 5.58$   $\mu\text{g/g}$ .  $\beta$ -carotene content in our plant extract was found as  $0.66 \pm 0.05$  mg/100 mL.

### 3.2. DPPH radical scavenging activity

The scavenging of the stable DPPH radical was widely used to evaluate antioxidant activity of phenolic compounds extracted from fruit, vegetable, cereal grain, wine, etc. [26]. It is based on the measurement of the reducing ability of antioxidants toward DPPH [27, 28]. The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. Fig 1. shows, the dose response curves of DPPH radical scavenging activity of the extract from *R. cristatus*. The extract was capable of scavenging DPPH radicals in a concentration-dependent manner. BHA, and BHT were used as references for radical scavengers. The scavenging activity of *R. cristatus*, BHA, and BHT on DPPH radicals increased between 12.5-50  $\mu\text{g/mL}$  concentrations. Extract showed the highest radical scavenging activity at 60 min. DPPH radical scavenging activity

of ethanol extract (% 25.75) is lower than the standards used BHA (% 83.75) and BHT (% 52.05) for 60 min. However, we found a good correlation between total phenolic content and DPPH radical scavenging activity. Correlation values of 5,10,30,60 min are ( $r^2= 0.978, 0.988, 0.951$  and  $0.992$ ) respectively. The scavenging effect increased with increasing concentration of the extract. Scavenging abilities on DPPH radicals were in descending order: BHA > BHT > ethanolic extract.



**Figure 1.** DPPH radical scavenging activity of the ethanol extract from *Rumex cristatus* DC. BHA and BHT were used as reference antioxidants.

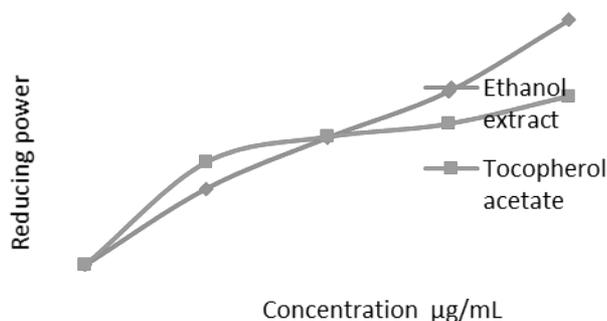
This suggested that scavenging effect of *R.cristatus* extract may depend on hydrogen atom donating by the different polyphenolic and flavonoid compounds and their hydrogen donor capacity, most probably accounts in large part for the antioxidant activity and may provide a basis for the pharmacological activity and therapeutic applications of this extract.

### 3.3 Reducing power

The principle behind reducing power assay is based on its electron donating activity, which is an important mechanism of phenolic antioxidant action [29]. The reducing ability

of *R. Cristatus* extract was measured and was found to increase with increasing concentration of plant extract (Fig 2).

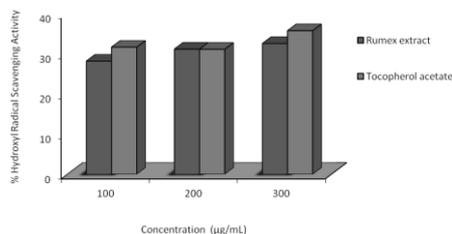
The striking aspect of deoxyribose assay is that it involves the hydroxyl radical which is the most active reactive oxygen species [30]. The effect of extracts and fraction in scavenging OH radicals to prevent oxidative degradation of deoxyribose substrate was determined. The reducing ability of extract, in the range 0–200 µg/mL, was greater than that of  $\alpha$ -tocopherol acetate. The total phenolic content was correlated with reducing power for ethanol extract and correlation value of extracts was found ( $r^2= 0.949$ ).



**Figure 2.** Reducing power of ethanol extract from *R. Cristatus*

### 3.4. Hydroxyl Radical Scavenging Activity

Hydroxyl radical is the most reactive among ROS and possesses the shortest half-life period. Hydroxyl radical causes oxidative damage to DNA, proteins, lipids [31]. The effect of *R. cristatus* on inhibition of free radical-mediated deoxyribose damage was assessed by means of the  $\text{Fe}^{2+}$  dependent DNA damage assay. The Fenton reaction generates hydroxy radical, which degrades DNA deoxyribose, using  $\text{Fe}^{2+}$  salts as a catalyst. Hydroxy radical may attack DNA either at the sugar or the base, giving rise to toxic products. Fig. 3 shows, the dose response curves of radical scavenging activities of the extract and reference antioxidants on the hydroxyl radicals and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron. Rumex extract scavenged hydroxyl radicals by  $22.37 \pm 11.63\%$  at  $300\ \mu\text{g/ml}$ . Tocopherol acetate exhibited higher scavenging activity of  $35.76 \pm 8.94\%$  at a concentration of  $300\ \mu\text{g/ml}$ . These results suggested that *R. cristatus* extracts might be used to provide a hydroxyl radical scavenger for humans.



**Fig 3.** Hydroxyl radical scavenging activity of the ethanol extract from *R. cristatus*.

### 4. CONCLUSION

Several reports have conclusively shown close relationship between total phenolic contents and antioxidative activity of the fruits and vegetables [32]. Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization [33]. These results from various free radical-scavenging systems revealed that the *R. cristatus* DC had significant antioxidant activity and free radical-scavenging activity.

Therefore the leaves of *R. cristatus* could be a good source of antioxidant phenolics. Thus, it can be concluded that ethanol extract can also be used as an accessible source of natural antioxidants with consequent health benefits. Further in vivo studies are needed for a better understanding of their mechanism of action as antioxidant. The findings of this work are useful to further research to identify, isolate and characterize the specific compound which is responsible for higher antioxidant activity.

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