



## Antioxidant Properties of Ethanol and Water Extracts of Different Parts of *Salvia russellii* Bentham Plant

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In this study, the antioxidant activity of water and ethanol extracts of *Salvia russellii* leaves and flowers was evaluated by various antioxidant assay, ABTS\* (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging capacity, DPPH\* (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, superoxide anion radical scavenging capacity, hydrogen peroxide scavenging capacity, reducing power and metal chelating activities. Various antioxidant activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and  $\alpha$ -tocopherol. The extracts of *Salvia russellii* showed strong antioxidant activity. The extracts of *Salvia russellii* have effective antioxidant assay. This antioxidant property depends on concentration and increasing with increased amount of sample. The results obtained in the present study indicated that *Salvia russellii* leaves and flowers is a potential source of natural antioxidant.

**Key Words:** *Salvia russellii*, Antioxidant activity, *In vitro*, Leaves and Flowers.

### INTRODUCTION

The human body has several antioxidant defense systems to protect healthy cell membranes from active oxygen species and free radicals<sup>1,2</sup>. The innate defense systems may be supported by antioxidative compounds taken as foods, cosmetics and medicine. Therefore, the antioxidative compounds provided by the diet may enrich the antioxidative status of living cells and thus reduce the damage, particularly in old age<sup>3</sup>. The most widely used antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted recently because of serious concerns about their carcinogenic potential<sup>4,5</sup>. Therefore, there is great interest in finding new and safe antioxidants from natural sources<sup>6,7</sup>. Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens<sup>8</sup>. Numerous studies have been carried out on some plants such as rosemary, sage and oregano, which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals<sup>9</sup>.

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids, dietary glutathionine, vitamins and endogenous metabolites and such natural products are rich in antioxidant activities<sup>10,11</sup>. Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals ( $O_2^{\bullet-}$ ), hydroxyl radicals ( $OH^{\bullet}$ ), singlet oxygen ( $^1O_2$ ) and non-free radical species such as hydrogen peroxide ( $H_2O_2$ ) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors<sup>12,13</sup>. Electron acceptors, such as molecular oxygen, react easily with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS)<sup>14</sup>. Reactive oxygen species have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn on the attention of many experimental works<sup>15,16</sup>.

There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans<sup>1,17,18</sup>. Several methods have been developed to measure the free radical scavenging capacity (RSC), regardless of the individual compounds which contribute towards the total capacity of a plant product in scavenging free radicals. The

methods are typically based on the inhibition of the accumulation of oxidized products, since the generation of free radical species is inhibited by the addition of antioxidants and this gives rise to a reduction of the end point by scavenging free radicals. The reliable method to determine RSC involves the measurement of the disappearance of free radicals, such as 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid radical (ABTS<sup>•+</sup>), the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•+</sup>) or other coloured radicals, with a spectrophotometer<sup>19,20</sup>. Owing to the increasing demand for information about the total RSC of all types of plant extracts, an easy, rapid and reliable method for the determination of RSC of various samples might be useful. The method should not be time-consuming, but sensitive enough to screen differences between plants parts used for herbal medicine, which include the flower, top, aerial and roots<sup>21</sup>.

*Salvia*, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world<sup>22</sup>. *Salvia* genus (Lamiaceae) is represented by 95 species in Turkey<sup>23,24</sup>. Some members of this genus are of economic importance since they have been used as flavouring agents in perfumery and cosmetics. Sage (*S. officinalis*) has been credited with a long list of medicinal uses, e.g. spasmolytic, antiseptic, astringent<sup>25</sup>. Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial activity, as well as scavenging activity of active oxygen, as in superoxide anion radicals, hydroxyl radicals and singlet oxygen<sup>26</sup>, inhibiting lipid peroxidation<sup>27</sup>. Consequently, the corresponding extracts have been widely used to stabilize fat and fat-containing foods<sup>28</sup>. Despite the medicinal potential of plants in Turkey being considerable, knowledge of this area and studies on these plants are scarce<sup>29</sup>.

It was reported that aimed to screen another 55 taxa of the *Salvia* genus growing in Turkey for their anti-AChE activity by the spectrophotometric method of Ellman on ELISA microplate reader (Ellman, Courtney andres, & Featherstone, 1961) as well as antioxidant activity by two methods as 2,2-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radical scavenging activity and iron-chelating capacity. For this study the dichloromethane, ethyl acetate and methanol extracts (165 extracts in total) were prepared from *Salvia* species. Antioxidant activity of dichloromethane extracts screened here in the best scavenging activity against DPPH<sup>•</sup> radical was caused by *S. russellii* (86.36%) at 100 µg/mL. The ethyl acetate extracts of *S. russellii* showed DPPH<sup>•</sup> radical scavenging effect over 90 %, while the methanol extracts exerted very high scavenging effect at all concentrations tested<sup>30</sup>.

## EXPERIMENTAL

Ferrous chloride, α-tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

**Plant material and extraction:** The herbal parts of *S. russellii* was collected from Kusakçi Mountain, Elazig, Turkey when flowering (June 2009). The voucher specimens were deposited at the Herbarium of the Department of Biology, Firat University, Elazig, Turkey.

The aerial parts of the plant material were air dried to dryness at room temperature and under shade and then powdered to a fine grade by using a laboratory scale mill. For water extraction, 10 g sample was put into a fine powder in a mill and was mixed with 200 mL boiling water by magnetic stirrer for 15 min. For ethanol extraction 10 g sample was put into a fine powder in a mill and was mixed with 200 mL ethanol. The residue was re-extracted until extraction solvents became colourless. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected and then ethanol and water was removed by a rotary evaporator (IKA RV 05 basic) at 50 °C to obtain dry extract. Both extracts were placed in a plastic bottle and then stored at -20 °C until used.

**ABTS<sup>•+</sup> radical scavenging capacity:** ABTS also forms a relatively stable free radical, which decolourizes in its non-radical form<sup>31</sup>. The spectrophotometric analysis of ABTS<sup>•+</sup> radical scavenging capacity was determined according to the method of Re *et al.*<sup>32</sup>. In this method, an antioxidant is added to a pre-formed ABTS<sup>•+</sup> radical solution and after a fixed time period the remaining ABTS<sup>•+</sup> is quantified spectrophotometrically at 734 nm<sup>33</sup>. ABTS<sup>•+</sup> was produced by reacting 2 mM ABTS in H<sub>2</sub>O with 2,45 mM potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), stored in the dark at room temperature for 12 h. The ABTS<sup>•+</sup> solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer (pH 7.4). Then, 1 mL of ABTS<sup>•+</sup> solution was added to 3 mL of *S. russellii* extracts in ethanol at 100 µg/mL concentrations. The absorbance was recorded after 0.5 h of mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolourization is calculated as percentage reduction of absorbance.

The scavenging capability of test compounds was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance in the presence of the sample of *S. russellii* extracts or standards.

**DPPH<sup>•</sup> radical scavenging capacity:** The free radical scavenging capacity of *S. russellii* extracts was measured by 2,2-diphenyl-1-picrylhydrazil (DPPH<sup>•</sup>) using the method of Shimada *et al.*<sup>34</sup>. In brief, 0.1 mM solution of DPPH<sup>•</sup> in ethanol was prepared and 1 mL of this solution was added 3 mL of *S. russellii* extracts solution in water at different concentrations (50, 100 and 250 µg/mL). After 0.5 h, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH<sup>•</sup> radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ Scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where  $A_0$  is the absorbance of the control reaction and  $A_1$  is the absorbance in the presence of the sample of *S. russellii* extracts.

**Superoxide anion scavenging capacity:** Measurement of superoxide anion scavenging capacity of *S. russellii* extracts was based on the method of Liu *et al.*<sup>35</sup> with slight modification. 1 mL of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT in 100 mmol/L phosphate buffer, pH 7.4), 1 mL NADH solution (468 mmol/L in 100 mmol/L phosphate buffer (pH 7.4) and 100  $\mu$ L of sample solution of *S. russellii* extracts in water were mixed. The reaction started by adding 100  $\mu$ L of phenazine methosulphate (PMS) solution (60 mmol/L PMS in 100 mmol/L phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging capacity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of *S. russellii* extracts or standards<sup>36</sup>.

**Hydrogen peroxide scavenging capacity:** The ability of the *S. russellii* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*<sup>37</sup>. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically measuring absorption with extinction coefficient for  $H_2O_2$  of 81  $M^{-1} cm^{-1}$ . Extracts (50, 100 and 250  $\mu$ g/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *S. russellii* extracts and standard compounds was calculated:

$$\% \text{ Scavenged } [H_2O_2] = \frac{(A_0 - A_1)}{A_0} \times 100$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance in the presence of the sample of *S. russellii* extracts or standards.

**Reducing power:** The reducing power of *S. russellii* extracts was determined by the method of Oyaizu<sup>38</sup>. Different concentrations of *S. russellii* extracts (50, 100 and 250  $\mu$ g/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide  $K_3[Fe(CN)_6]$  (2.5 mL, 1 %). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) were added to the mixture, which was then centrifugated for 10 min at 3000 rpm (Universal 320R 2005, UK). The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and  $FeCl_3$  (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

**Metal chelating activity:** The chelating of ferrous ions by the *S. russellii* extracts and standards was estimated by the method of Dinis *et al.*<sup>39</sup>. Briefly, extracts (50, 100 and 250  $\mu$ g/mL) were added to a solution of 2 mM  $FeCl_2$  (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine- $Fe^{2+}$  complex formation was calculated using the formula given below:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance in the presence of the sample of *S. russellii* extracts or standards. The control does not contain  $FeCl_2$  and ferrozine, complex formation molecules.

## RESULTS AND DISCUSSION

**ABTS radical-scavenging capacity:** All the tested compounds exhibited effective radical cation scavenging activity (Fig. 1). The scavenging effect of *S. russellii* and standards on  $ABTS^{+}$  decreased in the order: BHA > BHT >  $\alpha$ -tocopherol > water extract of *S. russellii* flowers > water extract of *S. russellii* leaves > ethanol extract of *S. russellii* flowers > ethanol extract of *S. russellii* leaves (100, 97.3, 96.9, 90.5, 90.3, 89.6 and 89.5, respectively) at the concentration of 100  $\mu$ g/mL (Table-1). No significant differences in  $ABTS^{+}$  scavenging potential were found among *S. russellii* extracts, BHA, BHT and  $\alpha$ -tocopherol.

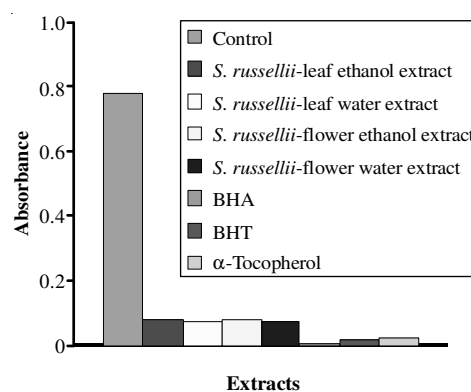


Fig. 1.  $ABTS^{+}$  radical-scavenging capacity of water and ethanol extracts of *S. russellii* leaves and flowers, BHA, BHT and  $\alpha$ -tocopherol

TABLE-1  
(%)  $ABTS^{+}$  RADICAL-SCAVENGING CAPACITY OF WATER AND ETHANOL EXTRACTS OF *S. russellii* LEAVES AND FLOWERS, BHA, BHT AND  $\alpha$ -TOCOPHEROL

Extracts (100 $\mu$ g/mL)	ABTS assay (%)
<i>Salvia russellii</i> -leaf ethanol extract	89.5
<i>Salvia russellii</i> -leaf water extract	90.3
<i>Salvia russellii</i> -flower ethanol extract	89.6
<i>Salvia russellii</i> -flower water extract	90.5
BHA	99.9
BHT	97.3
$\alpha$ -Tocopherol	96.9

**DPPH<sup>•</sup> radical scavenging capacity:** DPPH<sup>•</sup> is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule<sup>40</sup>. The reduction capability of DPPH<sup>•</sup> radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants (Fig. 2). Hence, DPPH<sup>•</sup> is often used as a substrate to evaluate antioxidative activity of antioxidants<sup>41</sup>. We used  $\alpha$ -tocopherol as standards (Fig. 3). The scavenging effect of water and ethanol extracts of *S. russellii* leaves and flowers and standards on the DPPH<sup>•</sup> radical decreased in order:  $\alpha$ -tocopherol > ethanol extract of *S. russellii* flowers > ethanol extract of *S. russellii* leaves > water extract of *S. russellii* leaves > water extract of *S. russellii* flowers (Fig. 3). 100  $\mu$ g of water and ethanol extracts of *S. russellii* leaves and flowers exhibited 77, 85, 75 and 88 % DPPH<sup>•</sup> scavenging capacity, respectively. On the other hand, at the same dose,  $\alpha$ -tocopherol exhibited 95 % DPPH<sup>•</sup> scavenging capacity. These results indicates that both *S. russellii* leaves and flowers extracts have a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

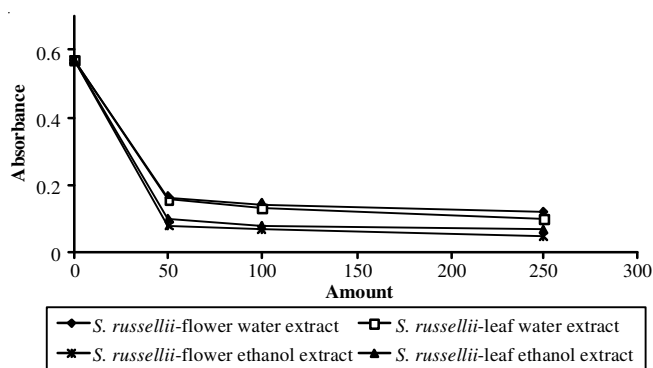


Fig. 2. DPPH<sup>•</sup> radical scavenging capacity of water and ethanol extracts of *S. russellii* leaves and flowers.

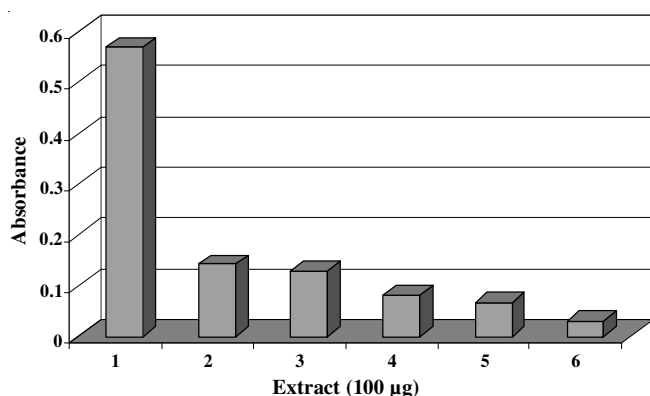


Fig. 3. DPPH<sup>•</sup> radical scavenging capacity of water and ethanol extracts of *S. russellii* leaves and flowers (100  $\mu$ g),  $\alpha$ -tocopherol. (1. Control 2. Water extract of *S. russellii* flowers 3. Water extract of *S. russellii* leaves 4. Ethanol extract of *S. russellii* leaves 5. Ethanol extract of *S. russellii* flowers 6.  $\alpha$ -tocopherol)

**Superoxide anion scavenging capacity:** In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction

mixture (Fig. 4). Table-2 shows the % inhibition of superoxide radical generation of 100  $\mu$ g/mL of water and ethanol extracts of *S. russellii* leaves and flowers and comparison with same doses of BHA, BHT and  $\alpha$ -tocopherol. The extracts of *S. russellii* leaves and flowers have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and  $\alpha$ -tocopherol. The percentage inhibition of superoxide generation by 100 mg doses of BHA, water and ethanol extracts of *S. russellii* leaves and flowers was found as 99, 95, 94, 91 and 88.5 % and greater than that same doses of BHT and  $\alpha$ -tocopherol (88 and 81 %), respectively. Superoxide radical scavenging activity of these samples followed the order: BHA > water extract of *S. russellii* flowers > water extract of *S. russellii* leaves > ethanol extract of *S. russellii* flowers > ethanol extract of *S. russellii* leaves > BHT >  $\alpha$ -tocopherol.

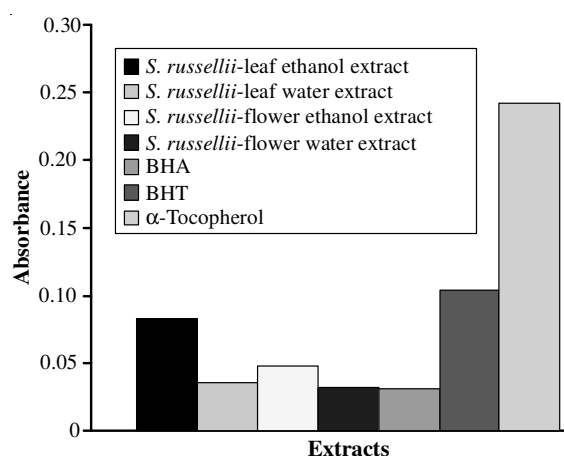


Fig. 4. Superoxide anion radical scavenging activity of water and ethanol extracts of *S. russellii* leaves and flowers, BHA, BHT and  $\alpha$ -tocopherol by the PMS-NADH-NBT method

TABLE-2 % SUPEROXIDE ANION SCAVENGING ACTIVITY OF WATER AND ETHANOL EXTRACTS OF <i>S. russellii</i> LEAVES AND FLOWERS, BHA, BHT AND $\alpha$ -TOCOPHEROL	
Extracts (100 $\mu$ g/mL)	% Superoxide anion scavenging activity (100 $\mu$ g)
<i>Salvia russellii</i> -leaf ethanol extract	88.5
<i>Salvia russellii</i> -leaf water extract	94.0
<i>Salvia russellii</i> -flower ethanol extract	91.0
<i>Salvia russellii</i> -flower water extract	95.0
BHA	99.0
BHT	88.0
$\alpha$ -Tocopherol	81.0

**Hydrogen peroxide scavenging activity:** The ability of the both extracts of *S. russellii* leaves and flowers to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*<sup>37</sup>. The scavenging ability of water and ethanol extracts of *S. russellii* leaves and flowers on hydrogen peroxide is shown Fig. 5 and compared with BHA, BHT and  $\alpha$ -tocopherol as standards. Both *S. russellii* leaves and flowers extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. 250  $\mu$ g of water and ethanol extracts of *S. russellii* leaves and flowers exhibited 88.75, 95.5, 83.0 and 69.8 % scavenging activity on hydrogen peroxide,

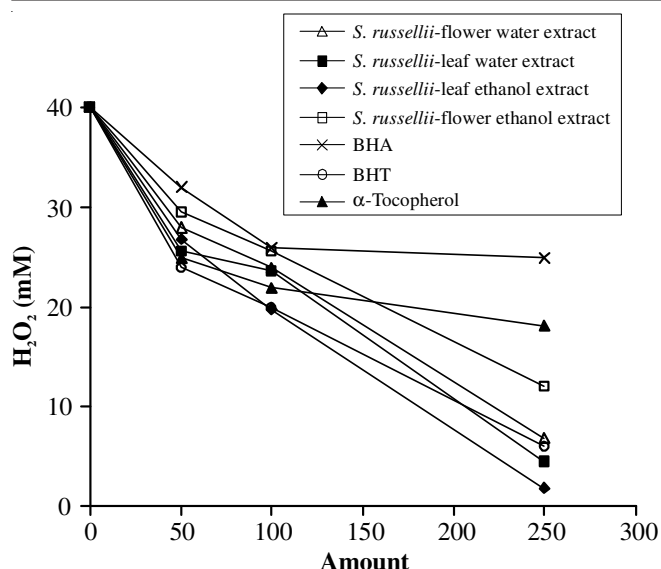


Fig. 5. Hydrogen peroxide scavenging activities of water and ethanol extracts of *S. russellii*, BHA, BHT and  $\alpha$ -tocopherol

respectively. On the other hand, BHA, BHT and  $\alpha$ -tocopherol exhibited 37.5, 84.7 and 54.5 % hydrogen peroxide scavenging activity. These results showed that *S. russellii* leaves and flowers extracts had stronger hydrogen peroxide scavenging activity. Those values close to BHA, but lower than that BHT and  $\alpha$ -tocopherol. The hydrogen peroxide scavenging effect of 250  $\mu$ g of extracts of *S. russellii* leaves and flowers and standards decreased in the order of ethanol extract of *S. russellii* leaves > water extract of *S. russellii* leaves > BHT > water extract of *S. russellii* flowers > ethanol extract of *S. russellii* flowers >  $\alpha$ -tocopherol > BHA. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells<sup>42</sup>. Thus, the removing of  $H_2O_2$  is very important for antioxidant defence in cell or food systems.

**Reducing power:** Fig. 6 shows the reductive capabilities of samples *S. russellii* leaves and flowers extracts compared to BHA, BHT and  $\alpha$ -tocopherol. For the measurements of the reductive ability, we investigated the  $Fe^{3+}$ - $Fe^{2+}$  transformation in presence of *S. russellii* leaves and flowers extracts samples using the method of Oyaizu<sup>38</sup>. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity<sup>43</sup>. However the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging<sup>44,45</sup>. Like the antioxidant activity, the reducing power of both *S. russellii* leaves and flowers extracts increased with increasing amount of sample. Reducing power of water and ethanol extracts of *S. russellii* leaves and flowers and Standard compounds followed the order: BHA > BHT > water extract of *S. russellii* flowers >  $\alpha$ -tocopherol > water extract of *S. russellii* leaves > ethanol extract of *S. russellii* leaves > ethanol extract of *S. russellii* flowers.

**Metal chelating activity:** The chelation of ferrous ions by the extracts of *S. russellii* leaves and flowers was estimated

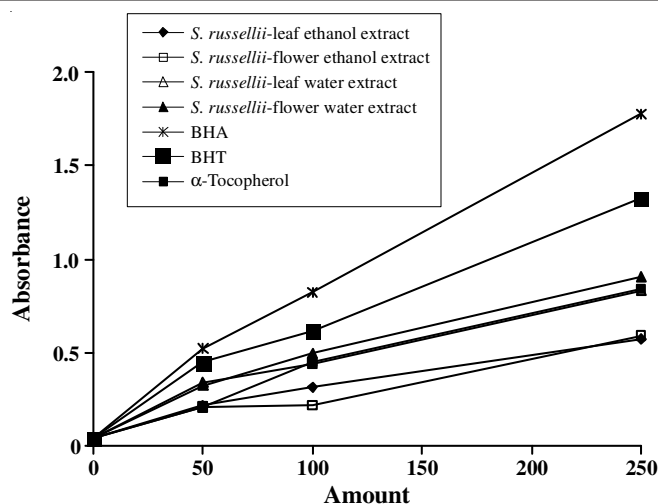


Fig. 6. Reducing power of water and ethanol extracts of *S. russellii* leaves and flowers, BHA, BHT and  $\alpha$ -tocopherol. (Spectrophotometric detection of the  $Fe^{3+}$ - $Fe^{2+}$  transformation, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene)

by the method of Dinis *et al.*<sup>39</sup>. Ferrozine can quantitatively form complexes with  $Fe^{2+}$ . In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimation of the metal chelating activity of the coexisting chelator<sup>46</sup>. In this assay both extracts of *S. russellii* leaves and flowers and standard compounds are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

As shown in Fig. 7, the formation of the  $Fe^{2+}$ -ferrozine complex is not complete in presence of water and ethanol extracts of *S. russellii* leaves and flowers, indicating that both extracts of *S. russellii* leaves and flowers chelate with the iron.

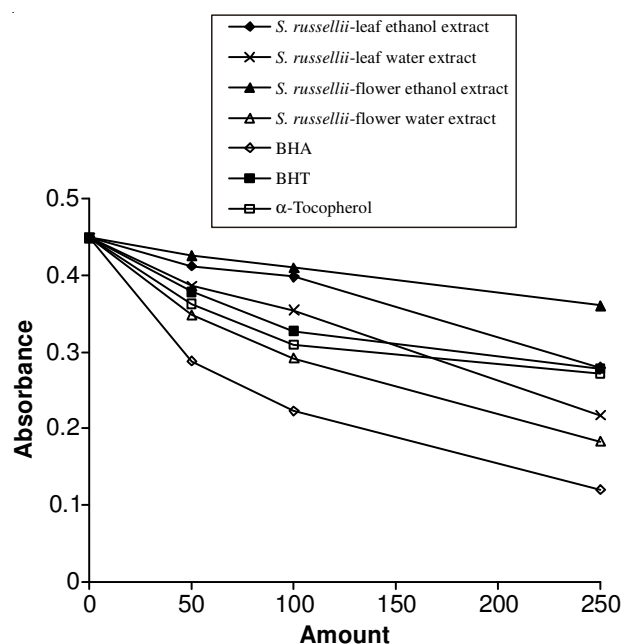


Fig. 7. Metal chelating effect of different amount of water and ethanol extracts of *S. russellii*, BHA, BHT and  $\alpha$ -tocopherol. (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene)

The absorbance of Fe<sup>2+</sup>-ferrozine complex was linearly decreased dose dependently (from 50 to 250 µg/mL). The percentages of metal scavenging capacity of 250 µg concentration of water and ethanol extracts of *S. russellii* leaves and flowers, α-tocopherol, BHA and BHT were found as, 51.7, 37.5, 59.0, 20.0, 39.2, 72.9 and 38 %, respectively. The metal scavenging effect of both extracts of *S. russellii* leaves and flowers and standards decreased in the order of BHA > water extract of *S. russellii* flowers > water extract of *S. russellii* leaves > α-tocopherol > BHT > ethanol extract of *S. russellii* leaves > ethanol extract of *S. russellii* flowers.

Metal chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation<sup>41</sup>. It was reported that chelating agents, which form s-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion<sup>47</sup>. The data obtained from Fig. 7 reveal that both extracts of *S. russellii* leaves and flowers demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

As a conclusion, the water and ethanol extracts of *S. russellii* leaves and flowers showed strong antioxidant activity, reducing power, DPPH• radical, superoxide anion scavenging, hydrogen peroxide scavenging and metal chelating activities when compared to standards such as BHA, BHT and α-tocopherol. The results show that the water and ethanol extract of *S. russellii* leaves and flowers can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. It can be used in stabilizing food against oxidative deterioration. However, the polyphenolic compounds or other components responsible for the antioxidant activity of water and ethanol extracts of *S. russellii* leaves and flowers are already unknown. Therefore, it is suggested that further work must be performed on the isolation and identification of the antioxidant components in *S. russellii* leaves and flowers.

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