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Research Article

COMPARISON OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF FIVE SALVIA SPECIES BY FRAP AND DPPH ASSAY

MEHRSA BEJELI [1], VAHID ROWSHAN [2*], ABDOLRASOL ZAKERIN [1]

[1]Department of Horticulture, Faculty of Agriculture, Islamic Azad University Jahrom, Iran. [2]Department of Natural Resource. Fars Research Center for Agriculture and Natural Resources PO Box 71555-617, Shiraz, Iran. Email: vahid.rowshan@gmail.com

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ABSTRACT

The methanolic extracts of five salvia species growing in Iran were analyzed for total phenolic contents and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and the Ferric Reducing (FRAP) assay. The IC_{50} for antioxidant activity ranged from 386.9 to 2743.05 µg/ml (by DPPH) and inhibition percent ranged from 22.84 to 76.87 µg/ml (by FRAP) and arranged in the following order: *Salvia multicaulis* > *S.verticillata* > *S.lachnocalyx* > *S.mirzayanii* > *S.macrosiphon*. Total phenolic content ranged from 21.74 to 93.65 mg gallic acid equivalent/g of extracts and decreased in the following order: *S.verticillata* > *S.multicaulis* > *S.mirzayanii* > *S.lachnocalyx* > *S.macrosiphon*.

Keywords: Antioxidant activity, DPPH, FRAP, Salvia, Total phenolic.

INTRODUCTION

The genus Salvia with over 900 species is one of the largest members of the family Lamiaceae and is found in both subtropical and temperate parts of the world. The two largest centers of the genus are in America and in South-West Asia[1]. Fifty eight annual or perennial species of the genus are found in Iran of which 17 are endemic[2,3]. Numerous the *Salvia* species have been used since ancient times in folk medicine and have been subjected to extensive pharmacognostic research intended to identify biologically active compounds[4]. These species have been found to possess significant biological activities, including antibacterial, antiviral, antitumor, spasmolytic, antioxidant, anti-inflammatory, antihydrotic activity and have been also used in the treatment of mental, nervous, gastrointestinal conditions and traditionally in foods and cosmetics preparation as well[5].

Antioxidants are a group of substances when present at low concentrations compared to oxidized substrates significantly inhibit or delay oxidative processes, while being oxidized themselves[6]. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others[7]. There are several reports in the literature on the phytochemical analysis of species belonging to *Salvia*. These scientific studies on Salvia species show the presence of many compounds belonging mainly to the groups of phenolic acids, phenolic glycosides, flavonoids, anthocyanins, coumarins, polysaccharides, sterols, terpenoids and essential oils[4,8,9]. Phenolic compounds found in plants have been well known for their ability of scavenging free radicals, which is referred to as antioxidant activity[10]. Several assays have been frequently used to estimate antioxidant capacities for clinical studies including 2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS)[11,12], 2,2- diphenyl-1-picrylhydrazyl (DPPH)[13], ferric reducing antioxidant power (FRAP)[14,15], and the oxygen radical absorption capacity (ORAC)[16]. The DPPH method can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants. Both radicals show similar bi-phase kinetic reactions with many antioxidants. However, the ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferroin analogue, the Fe^{3+} complex of tripyridyltriazine Fe $(TPTZ)^{3+}$ to the intensely blue-coloured Fe^{2+} complex Fe $(TPTZ)^{2+}$ by antioxidants in acidic medium. However, the reducing capacity does not necessarily reflect antioxidant activity, as has been suggested by[17,18]. There are various studies emphasizing that free radicals contribute to the development of many diseases, including hemorrhagic shock, arthritis, ageing, atherosclerosis, ischemia, Alzheimer and Parkinson's disease, gastrointestinal disorders, tumor promotion and carcinogenesis[19]. The aim of this research was to compare the total phenolics contents and efficiency of DPPH and FRAP assays to estimate antioxidant activities in five salvia species.

MATERIALS AND METHODS

Samples extraction

Methanolic extracts of the plants were prepared as follows: 20 g dry plant was macerated in 200 ml methanol/water (90/10) for 2 days with one change of solvent after 1 day. The extract was filtered and then concentrated in a rotary evaporator in less than 10 min. Powders were weighed to calculate the yield, and kept at -20 °C until used. Shortly before each experiment, the powder was dissolved in methanol at the desired concentration and was tested for antioxidant activity and total phenolic content.

Determination of antioxidant using DPPH

The antioxidant activity of plant extract and the standard antioxidants were assessed on the basis of radical scavenging effect of the stable DPPH free radical. Gallic acid was used to prepare a standard solution. In a modified assay[20], 200 µl of a 100 mM solution of DPPH radical in methanol was mixed with 20 µl of 12.5-3200 µg/ml extracts, gallic acid respectively and solutions were left at room temperature for 30 minutes. The DPPH radical inhibition was measured at 515 nm by using a micro-plate reader model Biotek ELx808. The IC_{50} of each sample (concentration in µg/ml required to inhibit DPPH radical formation by 50%) was calculated by Matlab software. The extract methanolic solution without DPPH was used as a blank to be subtracted from all measurements. The antioxidant activity (AOA) is given by:

100- [(A) sample-(A) blank) × 100/(A) control]

The IC_{50} value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract (µg/ml) against the mean percentage of the radical scavenging activity.

Ferric ion reducing activity (FRAP)

The FRAP assay was performed as described previously[21]. Brifly, 180µl of solution of FRAP(10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3.6H_2O$ solution) was mixed with 20 µl of 12.5-3200 µg/ml methanolic extracts microplate in oven at ~37°C. Absorbance was determined at 595 nm after 6 min of incubation at room temperature by micro-plate reader (Biotek ELx808).

Inhibition (%) = [(A) blank–(A) sample / (A) blank] x 100

Determination of total phenol content (TPC)

Total phenolic compound contents were determined by the Folin-Ciocalteau method[22,23]. The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by micro-plate reader (Biotek ELx808) at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg ml^{-1} solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass).

RESULTS AND DISCUSSION

All five plants possess antioxidant potential, but discrepancies were noticed between the species**.** All extracts showed considerable amounts of inhibitory effects from 386.9 µg/ml in *Salvia multicaulis* to 2743.05 µg/ml in *S.macrosiphon* by DPPH (Fig. 1) and inhibition percent ranged from 22.84 to 76.87 µg/ml by FRAP and decreased in the following order: *Salvia multicaulis* > *S.verticillata* > *S.lachnocalyx* > *S.mirzayanii* > *S.macrosiphon* (Fig. 2). These results are in agreement with the data reported by Murat and et al. (2009)[24]. They screened methanolic extracts of 8 *Salvia* species from Turkey for their antioxidant activities by DPPH and beta-carotene/linoleic acid that the most potent plant was *S. verticillata* (IC_{50}=18.3 µg/ml)[24]. Our results showed that, the most potent plant was *S.multicaulis* (IC_{50}= 386.9 µg/ml) and *S.macrosiphon* showed the low antioxidant activity. In the literature, the antioxidant activity of 60 plants of Iran was reported by linoleic acid peroxidation which *S.macrosiphon* (IC_{50}= 2.96 µg) and *S. hypoleuca* (IC_{50}= 5.27 µg) didn't show considerable antioxidant activity (compared to α-tocopherol, IC_{50}= 0.60 µg)[25]. In another study on the antioxidant activity of some medicinal species, using FRAP assay, the *Salvia macrosiphon* (MeOH extract) with 404.12 mmol of FeSO4/100g was showed a considerable antioxidant activity[26]. In present study (MeOH extract) *S.macrosiphon* was showed IC_{50}=2743.05µg/ml by DPPH assay and 22.84 µg/ml inhibition percentage by FRAP assay (Fig. 1, 2).

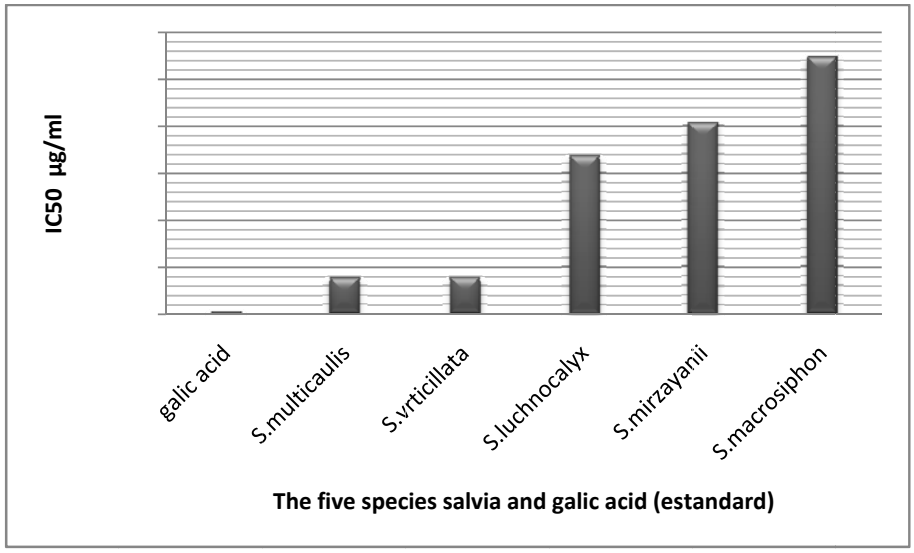

Fig. 1: Comparison of antioxidant activity of the five species *Salvia* and gallic acid by DPPH assay.

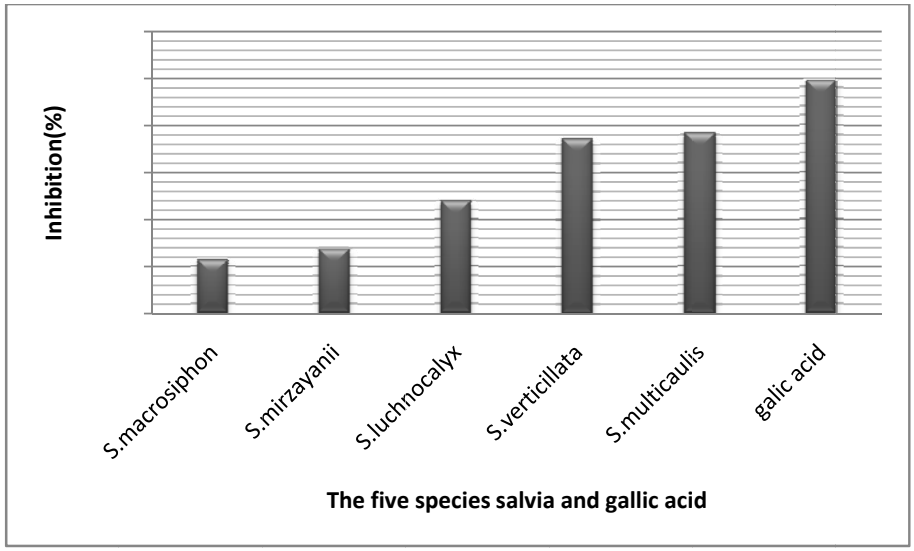

Fig. 2: Comparison of antioxidant activity of the five species *Salvia* and gallic acid by FRAP assay.

The study showed total phenolic content ranged from 21.74 to 93.65 mg gallic acid equivalent/g of extracts (Fig. 3). There were significant differences ($p<0.05$) in total phenolic content between five species, the highest level of phenolics was found in *S.verticillata*, while the lowest was in *S.macrosiphon* (Table 2). Total phenolic contents of the five spices decreased in the following order: *S.verticillata* > *S.multicaulis* > *S.mirzayanii* > *S.lachnocalyx* > *S.macrosiphon*.

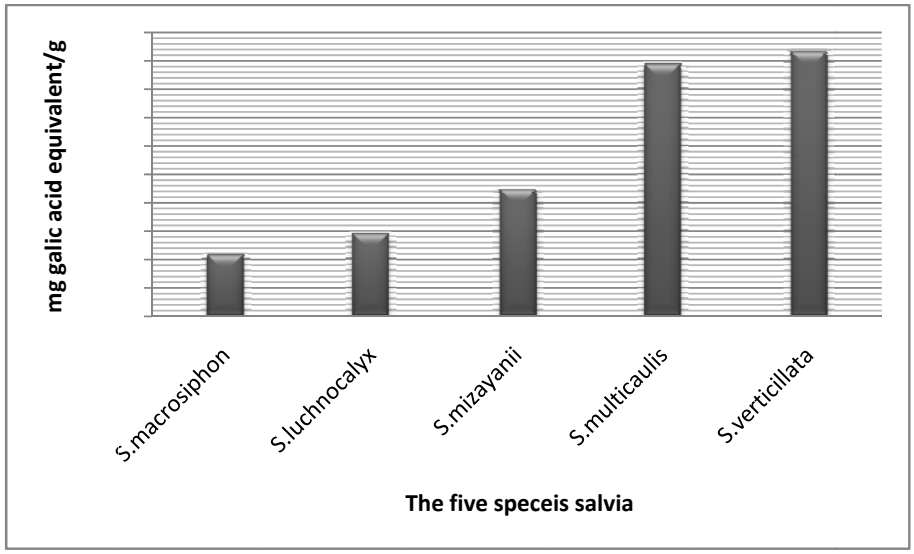

Fig. 3: Comparison of total phenolic content of the five species *Salvia* by Folin-ciocalteu.

Table 1: Comparison of total phenolic content of the five species *Salvia*

Sample	(TPC)[1] Total phenolic content (mg gallic acid equivalent/g)
S.multicaulis	88.88 ± 0.32[b]
S.verticillata	93.65 ± 0.06[a]
S.*luchnocalyx*	29.04 ± 0.25[d]
S.*mirzayanii*	44.92 ± 0.13[c]
S.macrosiphon	21.74 ± 0.25[e]

[1]Data are displayed with mean ± SD, and ($P<0.05$).

Extensive studies have been carried out on antioxidant activity of many species of the Lamiaceae family[27,28,29]. They demonstrated that this family species had a very strong antioxidant capacity. Some of them found that rosemary had the strongest antioxidant effect, but others found this with sage or oregano and basil. Similar our study some authors[30,31] have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity, while others[32] show poor linear correlation or report total antioxidant activity and phenolic content with no comment. The results obtained in our study show good correlation within one species.

CONCLUSIONS

Our results showed that Salvia species were rich in phenolic constituents and demonstrated good antioxidant activity measured by different methods. These plants, rich in phenolic acids could be a good source of natural antioxidants. Therefore, qualitative and quantitative analysis of major phenolics in the spices could be helpful for explaining the relationships between total antioxidant capacity and total phenolic contents in the species. The free radical-scavenging property may be one of the mechanisms by which these plants, notably *Salvia verticillata* and *S.multicaulis*, can be used as natural antioxidants.

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