

Chemical Composition and Antioxidant Activity of the Essential Oils of *Salvia palaestina* (Bentham) and *S. ceratophylla* (L.)

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Abstract: This study is designed to examine the chemical composition and *in-vitro* antioxidant activity of the essential oils of *Salvia palaestina* (Bentham) and *S. ceratophylla* (L.). GC-EIMS analyses of the essential oils were resulted in the determination of 70 and 53 different compounds, representing 92.50% and 95.80% of the total oils, respectively. In *S. palaestina* oil, the major compounds were caryophyllene oxide (16.1%) and (*E*)-caryophyllene (4.5%). In *S. ceratophylla* oil, γ -muurolene (11.4%) and α -pinene (7.6%) were the major compounds. Antioxidant activities of the samples were determined by four different test systems namely β -carotene/linoleic acid, DPPH, reducing power and chelating effect. In all systems, *S. palaestina* oil showed greater activity profile than that of *S. ceratophylla*. None of the essential oils showed metal chelating effect.

Keywords: *Salvia palaestina*; *Salvia ceratophylla*; essential oil; antioxidant activity.

1. Introduction

Plant volatile and nonvolatile secondary metabolites have wide applications in dietary regimens, food flavoring and preservation, folk medicine and fragrance industry [1,2]. Application of plant materials as dietary regimens and preservatives is mainly due to their antioxidant, antimicrobial and other biological potentials. Antioxidants can inhibit or delay the oxidation of oxidizable substrates and this appears to be very important in the prevention of oxidative stress which is suggested as the leading cause of many oxidation related diseases [3]. Recently, and mainly due to undesirable side effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for finding naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine [4,5]. In this regard, a growing rate of research was conducted on many plant species in order to find new natural bioactive compounds in them.

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Salvia (sage) is the largest genus of the Lamiaceae family and comprises about 900 species. From its Latin name “*Salvia*”, meaning to cure; it is clear that sage has a historical reputation for promotion of health and treatment of ailments [6]. Until the discovery of antibiotics, *Salvia* was a frequent component of herbal tea mixtures, recommended to patients with tuberculosis to prevent sedation and was found to be an active ingredient in combined plant preparations for the treatment of chronic bronchitis. Several species are used in folk medicine all around the world to treat microbial infections, cancer, malaria, inflammation and to disinfect homes after sickness [7]. Plants belonging to this genus are known for their many biological activities, such as antibacterial, antioxidant, antitumor, antidiabetic, antimicrobial, anxiolytic, sedative and anti-inflammatory activities [8-12], which could partly explain the reasons why this plant is so beneficial in the treatment of many human diseases. The essential oils produced by the foliage are acknowledged worldwide because of their beneficial uses [13]. There are a number of literature reports on analyses of essential oils from plants of this genus and morphological and genetic variations are also observed according to their geographical origin [14-18].

To the best of our knowledge, essential oil composition of *S. palaestina* (Benthams) has previously been reported elsewhere [19-23], while no literature data is available for the oil composition of *S. ceratophylla* (L.). From this point of view, data presented in Table 1 could be assumed as the first report on *S. ceratophylla*.

Antioxidant activities of the various extracts of these species have previously been reported, but essential oils of both species have not been reported elsewhere. The aim of this study is determining the chemical composition and antioxidant activities of the essential oils of *S. palaestina* and *S. ceratophylla*.

Table 1. Chemical composition of the essential oils of *S. palaestina* and *S. ceratophylla*

Compounds	Identification ^a	LRI ^b	Relative Concentration (%)	
			<i>S. palaestina</i>	<i>S. ceratophylla</i>
Tricyclene	Ri, MS	0932	-	0.4
α -Thujene	I, MS	0935	0.9	-
α -Pinene	Ri, MS, Co-GC	0944	1.6	7.6
Camphene	Ri, MS, Co-GC	0957	-	0.5
Sabinene	Ri, MS	0982	1.1	1.0
β -Pinene	I, Co-GC, MS	0986	2.4	-
Myrcene	I, MS	0998	0.5	-
α -Terpinene	I, MS	1019	0.6	-
<i>p</i> -cymene	I, MS	1026	1.2	3.2
1,8-Cineole	Ri, MS, Co-GC	1032	-	1.5
Limonene	Ri, MS, Co-GC	1033	-	0.4
Δ -3-Carene	I, MS	1034	0.4	-
Phenylacetaldehyde	I, Co-GC, MS	1049	1.8	-
γ -Terpinene	I, Co-GC, MS	1057	2.2	-
<i>cis</i> -Sabinene hydrate	I, MS	1074	1.0	-
<i>trans</i> -Sabinene hydrate	I, MS	1101	1.5	-
Phenylethyl alcohol	I, MS	1106	0.8	-
α -Thujone	Ri, MS	1114	-	4.6
β -Thujone	Ri, MS	1116	-	1.0
<i>cis-p</i> -Menth-2-en-1-ol	I, MS	1123	0.9	-
<i>trans</i> -Pinocarveol	Ri, MS	1136	1.4	1.0
Camphor	Ri, MS, Co-GC	1149	2.0	1.7
Menthone	I, MS	1157	1.0	-
Isomenthone	I, MS	1161	0.5	-
<i>neo</i> -Menthol	I, MS	1165	0.9	-
Pinocarvone	Ri, MS	1169	-	0.3
Borneol	I, Co-GC, MS	1170	1.0	-
<i>p</i> -Cymene-8-ol	I, MS	1180	0.1	-
Naphthalene	I, Co-GC, MS	1184	0.7	-
Terpineol-4	Ri, MS, Co-GC	1186	4.0	2.3
α -Terpineol	I, Co-GC, MS	1191	1.6	-

1-Dodecene	<i>I</i> , Co-GC, MS	1194	0.3	-
Myrtenol	<i>Ri</i> , MS	1199	1.3	0.9
<i>cis</i> -Piperitol	<i>I</i> , MS	1201	0.4	-
Decanal	<i>I</i> , MS	1206	0.4	-
Citronellol	<i>I</i> , MS	1222	0.2	-
Pulegone	<i>I</i> , MS	1245	4.1	-
Piperitone	<i>I</i> , MS	1249	0.4	-
Indole	<i>I</i> , MS	1297	<i>t</i> ^c	-
<i>trans</i> -Pinocarvyl acetate	<i>Ri</i> , MS	1299	-	5.3
Thymol	<i>I</i> , Co-GC, MS	1301	0.7	-
Carvacrol	<i>Ri</i> , MS, Co-GC	1303	3.0	1.5
α -Cubebene	<i>Ri</i> , MS	1360	2.5	<i>t</i>
Eugenol	<i>Ri</i> , MS, Co-GC	1364	1.2	1.4
Ylangene	<i>Ri</i> , MS	1372	-	<i>t</i>
α -Copaene	<i>Ri</i> , MS	1379	3.0	4.7
β -Bourbonene	<i>Ri</i> , MS	1391	-	0.9
β -Cubebene	<i>I</i> , MS	1394	0.7	2.8
(<i>E</i>)-Caryophyllene	<i>Ri</i> , MS, Co-GC	1425	4.5	1.0
α - <i>trans</i> -Bergamotene	<i>I</i> , MS	1442	<i>t</i>	-
Pentadecan-2-one	<i>I</i> , MS	1447	0.4	-
Aromadendrene	<i>I</i> , MS	1449	0.4	-
(<i>E</i>)-Geranylacetone	<i>I</i> , MS	1453	0.6	-
α -Humulene	<i>Ri</i> , MS	1459	0.2	2.4
<i>allo</i> -Aromadendrene	<i>Ri</i> , MS	1466	0.5	1.0
Tridecan-2-one	<i>I</i> , MS	1480	0.3	-
γ -Muurolole	<i>Ri</i> , MS	1481	0.8	11.4
γ -Gurjunene	<i>Ri</i> , MS	1482	-	0.3
<i>cis</i> - β -Guaiene	<i>Ri</i> , MS	1496	-	3.4
α -Muurolole	<i>Ri</i> , MS	1508	-	3.0
γ -Cadinene	<i>Ri</i> , MS	1510	0.6	5.7
δ -Cadinene	<i>I</i> , MS	1529	2.0	-
<i>trans</i> -Calamenene	<i>Ri</i> , MS	1533	-	1.7
1- <i>S-cis</i> -Calamenene	<i>I</i> , MS	1540	0.8	1.0
α -Cadinene	<i>Ri</i> , MS	1541	-	0.4
α -Calacorene	<i>Ri</i> , MS	1547	0.5	1.3
(<i>E</i>)-Nerolidol	<i>I</i> , MS	1565	0.7	-
β -Calacorene	<i>Ri</i> , MS	1569	-	0.4
Palustrol	<i>I</i> , MS	1570	<i>t</i>	-
Caryophyllene oxide	<i>I</i> , MS	1580	16.1	-
Spathulenol	<i>Ri</i> , MS	1584	<i>t</i>	0.7
Globulol	<i>I</i> , MS	1589	1.2	-
Humulene epoxide II	<i>Ri</i> , MS	1614	-	1.6
1- <i>epi</i> -Cubenol	<i>I</i> , MS	1629	0.4	3.9
T-Cadinol	<i>Ri</i> , MS	1641	-	0.4
Cubenol	<i>Ri</i> , MS	1643	0.9	1.5
T-Muurolol	<i>I</i> , MS	1647	0.7	-
α -Cadinol	<i>Ri</i> , MS	1651	<i>t</i>	0.5
β -Eudesmol	<i>I</i> , MS	1656	0.2	-
Cadalene	<i>Ri</i> , MS	1680	-	2.6
Heptadecane	<i>I</i> , Co-GC, MS	1705	1.1	-
Octadecane	<i>I</i> , Co-GC, MS	1810	0.2	-
Hexahydrofarnesyl acetone	<i>I</i> , MS	1852	2.4	0.6
Farnesyl acetone	<i>I</i> , MS	1940	3.1	-
Hexadecanoic acid	<i>Ri</i> , MS	1948	1.8	0.5
(<i>Z</i>)-Phytol	<i>Ri</i> , MS	1954	1.4	1.4
Eicosane	<i>I</i> , Co-GC, MS	2020	0.1	-
Heneicosane	<i>Ri</i> , MS, Co-GC	2106	-	0.9
Tricosane	<i>Ri</i> , MS, Co-GC	2314	-	1.0
Tetracosane	<i>Ri</i> , MS, Co-GC	2418	0.7	<i>t</i>

Pentacosane	R _i , MS, Co-GC	2516	-	1.0
Heptacosane	R _i , MS, Co-GC	2723	0.9	1.3
Nonacosane	R _i , MS	2941	0.7	1.3
Hentriacontane	R _i , MS	3122	-	0.2
Dotriacontane	R _i , MS	3219	-	0.4
TOTAL			92.50	95.80

^a R_i: linear retention index; MS: mass spectrum; CoGC: coinjection with authentic compound

^b LRI: linear retention indices (HP-5 column).

^c tr: trace

2. Materials and Methods

2.1. Collection of plant material

Salvia palaestina was collected in 20.08.2009 between C6 Gaziantep-Nizip highways (13-15 km, altitude 350 m) at its flowering season. *Salvia ceratophylla* was collected from B5 Kayseri-Incesu highway (altitude 1000 m) in 18.06.2009. Taxonomic identification of the plant material was confirmed by a senior taxonomist Dr. H. Aşkın AKPULAT, in Cumhuriyet University, Faculty of Education, Department of Secondary School Science and Mathematics Education. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (Voucher No: AKPULAT 4355 and AKPULAT 4317, respectively).

2.2. Isolation of the essential oil

The air-dried and ground aerial parts of the plants were submitted for to water-distillation using a Clevenger-type apparatus for 3 hours. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4 °C until tested and analyzed (yields 1.35% and 0.80% v/w, respectively).

2.3. Gas chromatography (GC) /EIMS

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 5.0 µL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data [24-29]. Moreover, the molecular weights of some substances identified were confirmed by the mass spectral data of their standards.

2.4. Antioxidant activity

2.4.1. 2, 2'-Diphenyl-1-picrylhydrazyl assay

Hydrogen atoms or electrons donation ability of the corresponding oils was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [30,31]. 50 µL of various concentrations of the oils in methanol was added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min

incubation period at 20°C the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used. Tests were carried out in triplicate. Values are presented as means \pm S.D. of three parallel measurements.

2.4.2. β -Carotene-linoleic acid assay

In this assay antioxidant capacity is determined by indirectly measuring the inhibition of the volatile organic compounds and the conjugated diene hydro peroxides arising from linoleic acid oxidation [32]. A stock solution of β -carotene-linoleic acid mixture was prepared as following: 0.5 mg β -carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 μ L linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL distilled water saturated with oxygen (30 min 100 ml/min.) was added with a vigorous shaking. 2.5 mL of this reaction mixture was dispersed to test tubes and 350 μ L portions of the oils prepared at 2 g L⁻¹ concentrations were added and emulsion system was incubated up to 48 hours at room temperature. After this incubation period absorbance of the mixtures were measured at 490 nm. Antioxidative capacities of the oils were compared with those BHT and blank (contains EtOH instead of essential oil). Values are presented as means \pm S.D. of three parallel measurements.

2.4.3. Reducing power

The reducing power was determined according to the method of Oyaizu [33]. Each of the samples in methanol and water (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. Finally, absorbance was measured at 700 nm against a blank.

2.4.4. Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. [34]. Briefly, 2 mL of various concentrations of the samples in methanol was added to a solution of 2 mM FeCl₂ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorption readings at 562 nm were taken after 10 min against a blank sample consisting of a 2 ml extract solution with 2 mM FeCl₂ (0.05 mL) and water (0.2 mL) without ferrozine. The inhibition percentage of ferrozine-Fe²⁺ complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect (\%)} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$$

Where A_{Control} is the absorbance of control (The control contains FeCl₂ and ferrozine, complex formation molecules) and A_{sample} is the absorbance of the test compound.

3. Results and Discussion

3.1. Chemical composition of the essential oils

The hydro distillation of dried *S. palaestina* and *S. ceratophylla* buds gave yellowish essential oils (yields 1.35% and 0.80% v/w, respectively). The identified compounds are shown in Table 1.

The GC analysis of the essential oil of *S. palaestina* led to the identification of 70 different components, representing 92.5% of total oil constituents (Table 1). The major compounds of the essential oil were found as caryophyllene oxide (16.1%), (*E*)-caryophyllene (4.5%), pulegone (4.1%), terpineol-4 (4.0%), farnesyl acetone (3.1%), carvacrol and α -copaene (3.0%).

In the case of *S. ceratophylla*, 53 compounds were identified, which represented about 95.8% of the total detected constituents. The major constituents of the oil were γ -muurolene (11.4%), α -pinene (7.6%), γ -cadinene (5.7%), *trans*-pinocarvyl acetate (5.3%), α -copaene (4.7%), α -thujone (4.6%), 1-*epi*-cubenol (3.9%), *cis*- β -guaiene (3.4%), *p*-cymene (3.2%) and α -muurolene (3.0%).

As far as our literature survey could ascertain, no report is available for the essential oil composition of *S. ceratophylla*. Therefore, this study could be assumed as the first report on this topic. On the other hand, several reports are available in the literature for the essential oil composition of *S. palaestina* [19-23].

According to a report Salehi *et al.* [19], GC and GC-MS analysis of *S. palaestina* essential oil has been resulted in the identification of 60 components (91.7% of total oil). The main components were determined as germacrene D (14.0%), β -bisabolene (11.9%), 1-*epi*-cubenol (9.8%), decanal (7.0%), β -caryophyllene (6.1%) and isobornyl butanoate (5.8%).

Rustaiyan *et al.* [20] has also published a report on the chemical composition of *S. palaestina* oil. In this report, *S. palaestina* oil was found rich in sesquiterpenes. According to these researchers, β -caryophyllene (36.4%) was found as the major compound. One another report has been reported by Senatore *et al.* (2005) on the chemical composition of this plant species. According to this report, sclareol (20.2%), β -caryophyllene (16.6%) and linalool were found as the major compounds.

As can be seen from the reports given above, oil compositions of the different *S. palaestina* samples showed great diversity, and our results are not agreed with the literature data. However, this is the first report on the oil composition of *S. ceratophylla*.

3.2. Antioxidant activity

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts [35]. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. The methods chosen are the most commonly used for the determination of antioxidant activities of plant extracts and/or essential oils.

The reduction ability of DPPH radicals' formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [36].

The scavenging ability of the essential oils showed a concentration-dependent activity profile (Table 2). The strongest free radical scavenging activity was exhibited by *S. palaestina* essential oil ($80.57\% \pm 1.51$ at 2.00 mg mL^{-1}). Free radical scavenging capacity of *S. ceratophylla* was determined as $62.84\% \pm 0.65$ at the same concentration. Free radical scavenging potentials of the synthetic antioxidant BHT, α -tocopherol and quercetin were determined as $41.53\% \pm 0.26$, $72.25\% \pm 1.16$ and $98.73\% \pm 1.31$, respectively at 0.20 mg mL^{-1} concentration.

As clearly indicated by Lucarini *et al.* [37] radical-scavenging capacity is directly related to the hydrogen atom donating ability of a compound and not correlated to the redox potentials alone, as observed by this researcher when studying the antioxidant capacity of phenothiazine and other related compounds.

In β -carotene-linoleic acid system, β -carotene undergoes a rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β -carotene destruction by "neutralizing" the linoleate free radical and any other free radicals formed within the

system [38]. Table 2 depicts the inhibition of β -carotene bleaching by the essential oils of *S. palaestina* and *S. ceratophylla*.

As can be seen from Table 2, the most active sample was the essential oil of *S. palaestina*. At 2.0 mg mL⁻¹ concentration, antioxidant activity of the oil was measured as 68.15 \pm 1.25%. At the same concentration, the essential oil of *S. ceratophylla* also exhibited moderate activity value (65.37% \pm 1.21). In this test system, oxidation of linoleic acid by the synthetic antioxidant BHT, α -tocopherol and quercetin were determined as 96.04% \pm 0.58, 96.43% \pm 0.32 and 98.39% \pm 0.58, respectively at 0.40 mg mL⁻¹ concentration.

The reductive potential measures the ability of a sample to act as electron donor and, therefore, reacts with free radicals converting them to more stable products and thereby terminates radical chain reactions.

Reducing power of the samples is also presented in Table 2. As can be seen from the table, reducing power of the essential oils of the both plant species found as 0.362 nm \pm 0.002 and 0.248 nm \pm 0.003 at 1.0 mg mL⁻¹ concentration, respectively. It is extremely important to point out that, reductive potentials of the extracts and/or essential oils are strictly related with the polarities of their phytochemicals. The essential oil, which contains the non-polar secondary metabolites (terpenoids), remains almost inactive. Reducing powers of BHT, α -tocopherol and ascorbic acid were measured as 0.800 nm \pm 0.004, 0.512 nm \pm 0.007 and 1.184 nm \pm 0.083, respectively at 0.2 mg mL⁻¹ concentration.

The reductive potential, measured by the absorbance at 700 nm, may be due to the di- and monohydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities as described by Shimada et al. [39]. Nevertheless our results seem to reveal the existence of some minor components responsible for the significant reductive ability of the oils.

Transition metal ions can stimulate lipid peroxidation by two mechanisms, namely by participating in the generation of initiating species and by accelerating peroxidation decomposing lipid hydro peroxides into other components which are able to abstract hydrogen, perpetuating the chain of reaction of lipid peroxidation [40].

Data revealed from the chelating effect experiments are presented in Table 2. In this system; essential oils of the both plant species did not show metal chelating effect. Metal chelating potential of EDTA was measured as 99.42% \pm 0.07 % at 0.25 mg mL⁻¹ concentration.

The differences found with the different methodologies can be to a certain extent explained by the diverse relative amounts of minor compounds in the oils but that can have a major impact in the final antioxidant effect of the oil. Further work is needed to fully understand the variables that can affect the evaluation of the antioxidant capacity by different methodologies.

In general, essential oil of *S. palaestina* exhibited slightly greater antioxidant activity than that of *S. ceratophylla*. As far as our literature survey could ascertain, antioxidant activity of the essential oil of *S. palaestina* and *S. ceratophylla* have not previously been reported. From this point of view, the results presented in Table 2 could be assumed as the first data on these plant species. On the other hand, antioxidant activities of the various extracts of both species, especially obtained by methanol, have been reported [41-44].

Despite all the efforts, too many plant species in the world still has not been investigated. In this manner, the scientists are still working to uncover the hidden biological richness. To the best of our knowledge, antioxidant activities of the plant species presented here have not previously been reported elsewhere. Therefore, data presented here could be assumed as the first report. We believe that this work will contribute to the discovery of new plant species having antioxidant properties.

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Table 2. Antioxidant activity of the essential oils of *S. palaestina* and *S. ceratophylla* ^a

Test systems	Concentrations (mg ml ⁻¹)	Samples						
		<i>S. palaestina</i>	<i>S. ceratophylla</i>	BHT	α -tocopherol	Quercetin	Ascorbic acid	EDTA
DPPH (%)	0.20	-	-	41.53 ± 0.26	72.25 ± 1.16	98.73 ± 1.31	-	-
	0.40	68.70 ± 2.21	49.24 ± 1.33	-	-	-	-	-
	1.00	75.26 ± 1.86	55.43 ± 1.50	-	-	-	-	-
	2.00	80.57 ± 1.51	62.84 ± 0.65	-	-	-	-	-
β -Carotene / Linoleic acid (%)	0.4	52.53 ± 1.68	42.41 ± 1.26	96.04 ± 0.58	96.43 ± 0.32	98.39 ± 0.58	-	-
	1.0	59.24 ± 1.23	51.64 ± 1.61	-	-	-	-	-
	2.0	68.15 ± 1.25	65.37 ± 1.21	-	-	-	-	-
Reducing Power (absorbance at 700 nm)	0.2	0.154 ± 0.005	0.114 ± 0.012	0.800 ± 0.004	0.512 ± 0.007	-	1.184 ± 0.083	-
	0.4	0.273 ± 0.011	0.189 ± 0.005	-	-	-	-	-
	1.0	0.362 ± 0.002	0.248 ± 0.003	-	-	-	-	-
Chelating Effect (%)	0.25	N.A.	N.A.	-	-	-	-	99.42 ± 0.07
	0.50	N.A.	N.A.	-	-	-	-	-
	1.0	N.A.	N.A.	-	-	-	-	-

^a Values expressed are means ± S.D. of three parallel measurements

N.A.: Not Active

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