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Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey

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Abstract

Sideritis sipylea Boiss. and *Origanum sipyleum* L., an endemic species from Turkey, were extracted with different solvents such as water (WE), ethanol (EE), methanol (ME), acetone (AE). The highest total phenolic contents of *S. sipylea* and *O. sipyleum* were detected in ME and AE as 0.089 ± 0.007 and 0.156 ± 0.014 μg gallic acid/μg extract, respectively. The best IC_{50} values for hydroxyl radical scavenging were determined as 1.1 μg/ml in ME of *S. sipylea* and 3.6 μg/ml in AE of *O. sipyleum*. This value for butylated hydroxyanisole (BHA) was 2.2 μg/ml and 2-times higher than that for *S. sipylea*. Although IC_{50} value for 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical scavenging was 4.5 μg/ml for ascorbic acid, the best values were determined as 0.05 mg/ml for ME of *S. sipylea* and 0.09 mg/ml for EE of *O. sipyleum*. Total antioxidant capacity of *S. sipylea* was similar with BHA and higher than *O. sipyleum*. According to this study, *S. sipylea* in comparison with *O. sipyleum* was generally more active plant as to antioxidant capacity.
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Keywords: *Sideritis sipylea*; *Origanum sipyleum*; Lamiaceae; Total antioxidant capacity; 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; Hydroxyl radical scavenging activity; Total phenolic content

1. Introduction

In all aerobic organisms, including human beings, production of reactive oxygen species (ROS) is balanced by antioxidant defence system. ROS in the forms of superoxide anion, hydroxyl radicals and hydrogen peroxide, which are generated by normal metabolic processes or from exogenous factors and agents, affects DNA, proteins and most biological molecules containing a lipid component of polyunsaturated fatty acids (Liochev & Fridovich, 1994; Halliwell & Gutteridge, 1999). A serious imbalance between the production of ROS and the antioxidant defence system is responsible for oxidative stress. Thus, ROS play an important role in the etiology of many diseases and ageing. Antioxidant defence systems which prevent oxidative damages of ROS consist of flavanoids, carotenoids, phenolic compounds, vitamins and antioxidant enzymes, among item (Fridowich, 1995; Ozturk-Urek, Bozkaya, & Tarhan, 2001). Dietary intake of antioxidant compounds is important for health (Duh, Tu, & Yen, 1999). Although there are some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used in processed foods, it has been reported that these compounds have some side effects (Ito, Fukushima, Hassegawa, Shibata, & Ogiso, 1983). Recently, there has been increasing interest in finding plants with high antioxidant capacities since they can protect the human body from free radicals and retard the progression of many chronic diseases (Miliauskas, Venskutonis, & van Beek, 2004; Moeller, Jacques, & FBlumberg, 2000). Various medicinal properties have been ascribed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products, including medications for ethnoveterinary medicine. Plant products are also known to possess potential for food preservation.

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Sideritis and *Origanum* which have prominent antioxidant capacities are the members of the family of Lamiaceae. Lamiaceae consists of more than 150 species occurring mainly in the Mediterranean area and are used as herbal teas for their (folk) medicinal properties. One of the genus's, *Sideritis* was represented by 38 species in the 7th volume of Flora of Turkey, but this number has increased to 45 (53 taxa) by the year 2000 of which, 45 species of the 39 taxa are endemic in Turkey (Davis, 1982; Aytac & Aksoy, 2000). *Sideritis* species are generally known under the names "adacayi or dagcayi" and widely used as herbal tea and folk medicine in Turkey (Kirimer, Baser, Demirci, & Duman, 2004). In folk medicine, they are used as nervous system stimulants, antispasmodic, carminative, analgesic, sedative, antitussive, stomachic, and anticonvulsant, in the treatment of coughs due to colds and gastrointestinal disorders. Recent studies have shown that extracts of some *Sideritis* species have anti-stress, analgesic, antibacterial, and anti-inflammatory activity (Ezer, Usluer, Gunes, & Erol, 1994). *Sideritis* species contain flavanoids, essential oils, diterpenes, phenylpropanoid glycosides and iridoid glucosides (Ezer, Sakar, Rodriguez, & De la Torre, 1992; Gil, Ferreres, Marrero, Tomas-Lorente, & Tomas-Barberan, 1993). Anti-inflammatory and antibacterial activities of *Sideritis* species have been reported as well (Barberan, Manez, & Villar, 1987; Rios, Manez, Paya, & Alcaraz, 1992).

The other genus, *Origanum* L. is represented in Turkey by 22 species or 32 taxa, 21 being endemic to Turkey. Out of 52 known taxa of *Origanum*, 60% are recorded to grow in Turkey (Akgul & Kivanc, 1988). This high rate suggests that the gene centre of *Origanum* is in Turkey. The volatile oils of *Origanum* species containing 0.05–0.025% concentrations of thymol and carvacrol solutions have inhibitory effect on spore and mycelium development of all the fungi (Baser, 2002). This genus is used as anti-diabetic, carminative, tonic, digestive, stimulant, expectorant, menstrual regulator, diuretic, and for respiratory problems such as asthma.

The purpose of this study was to investigate the total phenolic contents, hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) radicals scavenging activities and total antioxidant capacities in water, ethanol, methanol and acetone extracts of endemic *Sideritis sipylea* and *Origanum sipyleum*.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$), gallic acid, trichloracetic acid (TCA), ascorbic acid, BHA, polyoxyethylenesorbitan monolaurate (Tween 20), and linoleic acid were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate, tiobarbituric acid (TBA), $FeCl_3$ and H_2O_2 were purchased from Merck. All other chemicals used were of analytical grade and obtained from Sigma.

2.2. Plants

S. sipylea is an endemic plant of Mediterranean that is distributed in West and Middle Anatolia. The plant is perennial, 20–60 cm, branched, and densely addressed white or greyish tomentose, glandular. *O. sipyleum* is a polymorphic endemic species growing in western provinces of Turkey. Taxonomical description of these species has been made according to Davis, 1982. The plants were identified by Dr. M. Nakipoglu. Voucher specimens of *S. sipylea* (TIB 92252) and *O. sipyleum* (TIB 050892/502) are deposited in the herbarium of Aegean Agricultural Research Institute.

2.3. Extraction

S. sipylea and *O. sipyleum* were collected from the Spil mountain-Izmir, Turkey. Dried plant samples were crushed in a coffee grinder for 2 min, but at 15 s intervals the process was stopped for 15 s to avoid over heating of the sample. Powdered plant samples (0.15 g) in various solvents such as water, ethanol, methanol and acetone (40 ml) were refluxed for 2 h and then centrifuged at 6640*g* for 10 min, after cooling to room temperature. The obtained extracts in indicated solvents were expressed as water extract (WE), ethanol extract (EE), methanol extract (ME) and acetone extract (AE), respectively.

2.4. Determination of total phenolic contents

Total phenolic contents were measured by using the Prussian Blue Assay, based on oxidation and reduction of iron (Graham, 1992). Gallic acid (0–1.7 μg/ml) was used as the standard and data were expressed as gallic acid equivalents (GAE) in (μg GAE/μg extract) dry material.

The extract (0.10 ml), 50.0 ml distilled water and 3.0 ml 0.10 M $FeNH_4(SO_4)_2$ (in 0.10 M HCI) were mixed. Exactly 20 min after the addition of the ferric ammonium sulphate, 3.0 ml 0.008 M $K_3Fe(CN)_6$ were added and mixed. Twenty minutes after the addition of ferricyanide, absorbance was read at 720 nm against to blank.

2.5. Determination of hydroxyl ($^{\cdot}OH$) radical scavenging capacity

Deoxyribose has often been used to measure the formation of $^{\cdot}OH$ in biochemical systems (Halliwell, Gutteridge, & Aruoma, 1987). Reaction mixture contained in a final volume of 1.0 ml, following reagents at the final concentrations stated: deoxyribose (2.8 mM), $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM), ascorbate (100 μM) and extracts or BHA, as a positive control. If a Fe^{2+}–EDTA chelate is incubated with deoxyribose in phosphate buffer (20 mM) at pH 7.4, $^{\cdot}OH$s are formed. Reaction mixture was incubated at 37 °C for 1 h, and colour developed with tiobarbituric acid (TBA). Then absorbance at 532 nm was

measured as a pink malondialdehyde-TBA chromagen (TBARS).

2.6. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The hydrogen atom or electron donation abilities of the *S. sipylea* and *O. sipyleum* extracts were measured from the bleaching of the purple-coloured methanol solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Half of millilitre of various dilutions of the extracts or ascorbic acid as a positive control was mixed with 1.5 ml of DPPH solution. The samples were incubated for 30 min at room temperature and the decreases in the absorbance values were measured at 517 nm.

2.7. Determination of total antioxidant capacity

Total antioxidant capacities of the extracts and BHA as a positive control were determined according to the thiocyanate method (Yen & Chen, 1995). Linoleic acid emulsion (0.02 M) in phosphate buffer (0.02 M, pH 7.0) was prepared by mixing linoleic acid with an equal amount of Tween 20 (0.02 M). Each extract was mixed with linoleic acid emulsion and incubated in the dark at 37 °C. During linoleic acid oxidation, the formed peroxides oxidize Fe^{2+} to Fe^{3+} and it subsequently forms complex with SCN^{-}, which had maximum absorbance at 500 nm.

2.8. Statistical analysis

Tukey test, one of the multiple comparisons, was used for statistical significance analyses. The values were the mean of three separate experiments ($n = 3$). Comparisons between antioxidant capacities in the extracts were also made with Pearson correlation.

3. Results and discussion

The *in vitro* antioxidant activities such as total phenolic contents, DPPH, $^{\bullet}$OH radicals scavenging and total antioxidant capacities of *S. sipylea* and *O. sipyleum* in the WE, EE, ME and AE's were investigated and compared with those of various controls including ascorbic acid or BHA.

3.1. Total phenolic content

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups (Hatano, Edamatsu, Mori, Fujita, & Yasuhara, 1989). Therefore, phenolic contents of plants may contribute directly to their antioxidant action. Plants belonging to the Lamiceae family are very rich in polyphenolic compounds. The main phenolic compounds identified in this family are rosmarinic acid, carnosic acid, carnosol, methyl carnosate, rosmanol, epirosmanol and rosmadial (Ivanova, Gerova, Chervenkov, & Yankova, 2005). Total phenolic contents in all extracts of *S. sipylea* and *O. sipyleum* are presented in Table 1. Generally, total phenolic contents of *O. sipyleum* were higher than *S. sipylea*. The phenolic content of *O. sipyleum* was highest in the AE and WE as 0.156 ± 0.013; 0.147 ± 0.018μg GAE/μg extract, and there were not statistically different ($p > 0.05$).The values of AE and WE were followed by ME and EE. The order of decreasing phenolic contents of *S. sipylea* was ME > EE > AE > WE and the highest value was 0.089 ± 0.007 μg GAE/μg ME. The total phenolic content of *O. sipyleum* in AE was approximately 2-times higher than those of *Polygonum cognatum* Meissn and *Thymus vulgaris* L. belonging to the Lamiceae family (Yildirim, Mavi, & Kara, 2003; Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003) ($p < 0.01$). Total phenolic levels of these plants used as herbal tea in public are important by reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins (Shahidi & Wanasundara, 1992).

3.2. Hydroxyl radical scavenging

Hydroxyl radical is biologically relevant and extremely reactive oxygen species, which can rapidly react and degrade with susceptible food and biologically relevant substrates, such as polyunsaturated fatty acids, proteins, carbohydrates and DNA (Halliwell, Gutteridge, & Cross, 1992). The $^{\bullet}$OH scavenging activities of the obtained extracts of *S. sipylea* and *O. sipyleum* are depicted in Fig. 1a and b.

IC_{50} is the amount of extract providing 50% inhibition of hydroxyl radical. Lower IC_{50} value reflects better protective action of the extracts. The best IC_{50} values of *S. sipylea* and *O. sipyleum* were determined in ME and AE, as 1.1 and 3.6 μg/ml, respectively. IC_{50} value for BHA was

Table 1
Total phenolic contents of the obtained *S. sipylea* and *O. sipyleum* extracts

Extracts	Total phenolic contents [(μg GAE/μg extract) ± SD]			
	WE	EE	ME	AE
S. sipylea[a]	0.042 ± 0.004	0.075 ± 0.006	0.089 ± 0.007	0.052 ± 0.004
O. sipyleum[a]	0.147 ± 0.018	0.117 ± 0.011	0.138 ± 0.012	0.156 ± 0.013

[a] Data represented the means ±SD, standard deviation ($n = 3$) of one representative experiment.

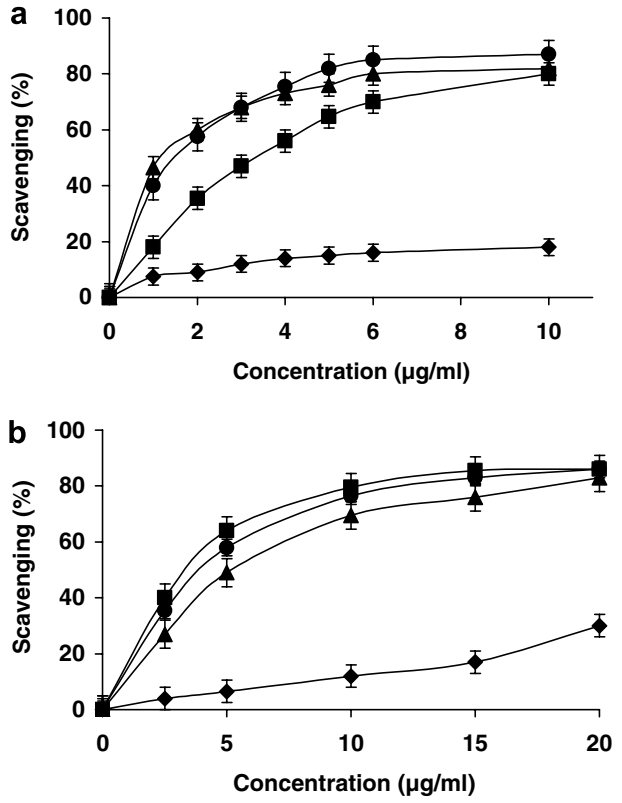

Fig. 1. Hydroxyl radical scavenging activities (%) of *S. sipylea* (a) and *O. sipyleum* (b): ((♦), WE; (■), AE; (▲), ME; (●), EE).

2.2 μg/ml and 2-fold higher than that of IC_{50} value of *S. sipylea*. Supplementation of this ME for food preservation could be more effective and economical than consuming an individual antioxidant such as BHA in protecting them against various type of oxidative stress. The inverse relationship between the amounts in ME of *S. sipylea* and AE of *O. sipyleum* providing IC_{50} values and their total phenolic contents may be derived from component profiles and their effectiveness responsible for antioxidant activities or differences from synergistic effects of both plants extracts ($r = -0.765$, $p < 0.01$). It is known that the total antioxidant activity of the each extract is the sum of the individual activities of each phenolic compound present, and also that these compounds might have synergistic effects. Similarly, Dorman et al. (2003) found that the ·OH scavenging activities did not seem to depend on the total phenolic content. Although rosemary and thyme showed similar scavenging activity, total phenolic content of rosemary was 2-fold higher than thyme.

As compared with the other data, ·OH scavenging activities of *S. sipylea* and *O. sipyleum* were significantly higher than those of *Origanum vulgaris, Rosmarinus officinalis, Salvia officinalis* and *T. vulgaris* ($p < 0.01$) (Dorman et al., 2003). IC_{50} values of *O. vulgaris, R. officinalis, S. officinalis* and *T. vulgaris* for ·OH scavenging were 3375.1, 3764.5, 2158.8 and 3747.3 μg/ml, whereas total phenolic contents were 0.149, 0.185, 0.166 and 0.0956 μg GAE/μg, respectively. It was shown that there was an inverse relationship between these data.

3.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging

DPPH· is a stable free radical and accepts a hydrogen radical to become a stable diamagnetic molecule, yellow coloured diphenylpicrylhydrazine (Soares, Dins, Cunha, & Ameida, 1997). The synthetic nitrogen-centred DPPH· is not biologically relevant, but it is often used as an indicator compound in testing of hydrogen-donation capacity and thus antioxidant activity. The DPPH· scavenging capacity of these extracts may be mostly related to their phenolic hydroxyl groups. However, these properties of putative antioxidants have been attributed to various mechanisms, among which are prevention of radical chain initiation, binding of transition metal ions catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Diplock, 1997).

All *S. sipylea* and *O. sipyleum* extracts were screened for DPPH· scavenging activity (Fig. 2a and b). For DPPH· scavenging, IC_{50} values of ME, EE, AE and WE's of *S. sipylea* were determined as 0.05, 0.07, 0.36 and 0.8 mg/ml, respectively. These values of *O. sipyleum* were also estimated as 0.09, 0.115, 0.135 and 1.05 mg/ml in EE, ME, AE and WE's, respectively. In addition, ascorbic acid concentration providing IC_{50} value was found to be 4.5 μg/ml.

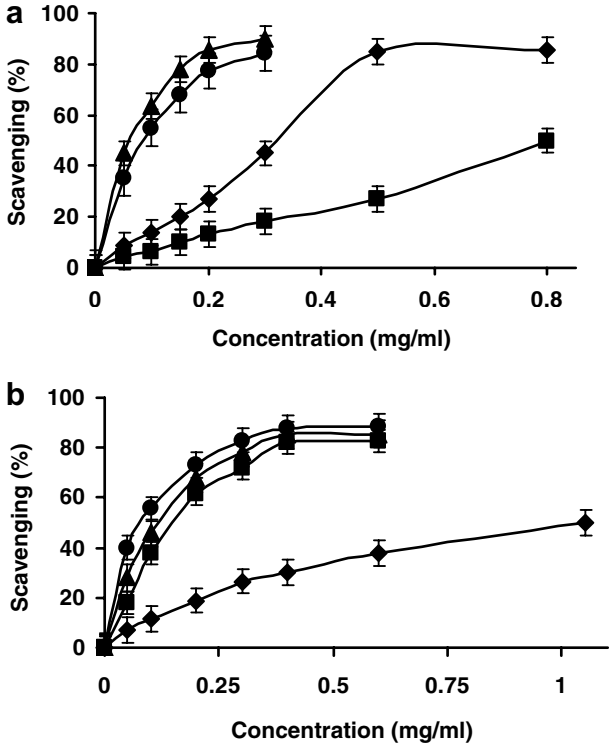

Fig. 2. DPPH radical scavenging activities (%) of *S. sipylea* (a) and *O. sipyleum* (b): ((♦), WE; (■), AE; (▲), ME; (●), EE).

Generally, however, the better ability of ethanolic extract might be due to more hydrogen-donating components extracted by ethanol.

When compared to the other researchers' data; DPPH· scavenging activity of *S. sipylea* was significantly higher than those of potato peel (25-times), *O. vulgaris* (6.7-times), *R. officinalis* (4.7-times), *S. officinalis* (5.3-times), *T. vulgaris* (7.6-times) and *Hypericum hyssopifolium* (1.6-times) (Nandita & Rajini, 2004; Dorman et al., 2003; Cakir et al., 2003) ($p < 0.01$).

3.4. Total antioxidant capacity

Total antioxidant capacities of *S. sipylea* and *O. sipyleum* were determined by the thiocyanite method. The presence of the obtained extracts in the linoleic acid emulsion was able to reduce the formation of peroxides. High absorbance is the indication of high concentration of formed peroxides. Fig. 3a and b showed that there were statistical differences among the control, in which there was no extract, and both plant extracts and also BHA, which is known as a standard antioxidant compound ($p < 0.01$). It was determined that total antioxidant capacities of ME and EE's of *S. sipylea,* and BHA, that were similar values, were significantly higher than the other extracts ($p < 0.01$).

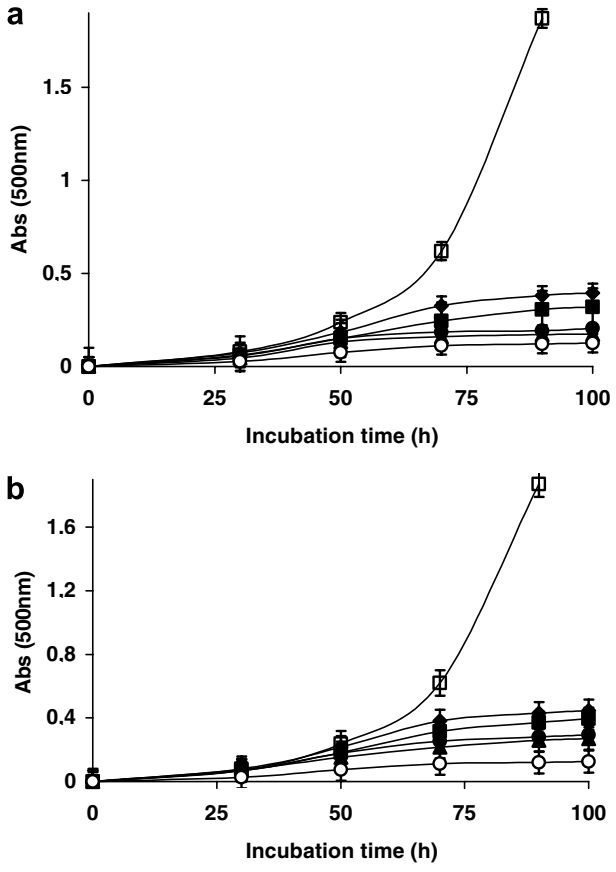

Fig. 3. Total antioxidant capacities of control (■), *S. sipylea* (a) and *O. sipyleum* (b): ((♦), WE; (■), AE; (▲), ME; (●), EE) and BHA (○) in presence of 300 μg/ml concentration.

The antioxidant capacity assayed was the ability to inhibit the peroxidation of linoleic acid. The antioxidant activity of various extracts might be due to the reducing of hydroperoxide, inactivating of free radicals, or complexing with metal ions, or a combination thereof. Total antioxidant capacities of the endemic plant extracts, which minimize the oxidation of lipid components in cell membranes, were higher than that of *Salvia triloba* from Lamiaceae family (Yildirim et al., 2000).

4. Conclusions

S. sipylea and *O. sipyleum*, which are endemic to Turkey, showed radical scavenging antioxidant activities. The role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may be the cause of many diseases including cancer. The total phenolic contents, hydroxyl, DPPH radicals scavenging and total antioxidant capacities of *S. sipylea* were significantly higher than *O. sipyleum*. In addition, the levels determined in the extracts were also considerably higher than some of the results determined in other researches. The results showed that the antioxidant activities of the extracts do not necessarily correlate with high amounts of phenolics.

Although the extracts were found to be effective natural antioxidants, their potential exploitable beneficial effects and safety in humans need to be proven in clinical trials. In a further work, the antioxidant components in endemic *S. sipylea* and *O. sipyleum* will be isolated and identified.

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