Iranian Food Science and Technology Research Journal
Vol. 13, No. 3, Aug. Sep. 2017, p. 28-38

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 13، شماره 3، مرداد - شهریور 1396، ص. 38-28

Antioxidant Properties of Various Solvent Extracts of Indian Frankincense *(Boswellia serrata)* Oleogum Resin

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Received: 2017.01.01
Accepted: 2017.06.24

Abstract

Methanol, ethanol, acetone and water extracts of Indian Frankincense *(Boswellia serrata)* were evaluated for their total phenolic contents and antioxidant properties using various methods including 2,2-diphenyl-1-picrylhydrazyl, iron (III) reducing power, total antioxidant capacity and oxidative stability index (Rancimat). The four extracts showed varying degrees of antioxidant activity in a dose - dependent manner in each assay. Methanol extract containing the highest amount of phenolic compounds exhibited the strongest antioxidant capacity in all the assays used. Moreover, all the extracts were able to improve the oxidative stability of soybean oil as evaluated by the Rancimat test. On the basis of the results obtained, *B. serrata* oleo-gum resin was found to serve as a potential source of natural antioxidants due to their considerable antioxidant activity.

Keywords: *Boswellia serrata*, Phenolic compounds, Antioxidant properties, Rancimat, Oleogum Resin.

Introduction

Lipid oxidation is one of the most important processes of food deterioration because it can affect food safety, color, flavor and texture (Wasowicz *et al.*, 2004). In addition, oxidation leads to health disorders such as atherosclerosis and carcinogenesis among others. Hence, the presence of antioxidants in foods is recommended for controlling rancidity with its deleterious consequences (Pizzale *et al*., 2002; Koleva *et al*., 2003). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) are widely used to prevent the oxidation of oils and fats and extend the shelf-life of lipid-containing foods (Mohdali, 2010). However, the use of synthetic antioxidants have been recently restricted due to their possible toxic and carcinogenic effects (Padmashree *et al*., 2007; Valentao *et al*., 2002). It has been also suggested that there is an inverse relationship between dietary intake of foods rich in natural antioxidants and human diseases (Yildirim *et al*., 2001). Consequently, there is an increasing interest in finding naturally occurring alternatives from plants for use in food and pharmaceutical industry. The important of plant-based antioxidants in foods is appreciated for preserving foods against oxidative deterioration as well as supplying the essential antioxidants in vivo (Shahidi, 1977). A large number of plants have been screened as viable sources of natural antioxidants including tocopherols, vitamin C, carotenoides and phenolic compounds, which are responsible for maintenance of health and protection from coronary heart disease and cancer (Castenmiller *et al.*, 2002).

The name frankincense is derived from the ancient French term “franc enens” meaning “pure incense”, it is a natural oleogum resin acquired from Boswellia tree that belongs to the family *Burseraceae* comes from three distinct regions India, North Africa and the Middle East. Generally, the frankincense tree

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DOI: 10.22067/ifstrj.v1396i3.61520

is a small, 3 – 6m high and scrubby tree which grows in rough, wild and inhospitable arid mountainous regions. This genus contains 23 species and the major species used for frankincense production are *Boswellia carteri, Boswellia frereana, Boswellia serrata* and *Boswellia papyrifera* (Khan & Farooqi, 1991; Dharmananda, 2003). The resin is harvested by scraping shallow incisions in the bark, this compounds convert into globular, pear or club shaped tears when exposed to the air and sun (Mathe *et al*., 2004). For at least 3000 years frankincense had been a significant trade material for the civilizations located in the North Africa and Arabian Peninsula, In addition to ceremonial and religious purposes, It has also been used in medicine and perfumery (Al-Dubai & Al-khulaidi, 1996; Mothana *et al*., 2011).

The active components of frankincense are various and can be divided in three groups; essential oils (5-9%), alcohol-soluble resins (65-85%) and the remaining water soluble gums (Tucher, 1986). The oleo-gum-resin of frankincense is mainly consisting of volatile oil, monoterpenes, diterpenes and lipophilic pentacyclic triterpene acids of the oleanane (α-boswellic acids), ursane (β- boswellic acids), polysaccharides, and lupine type (Sharma *et al*., 2009).

Frankincense therapeutic effect significantly depends on the amount of oleoresin. These effects include anti-inflammatory, hepatoprotective, anticancerous, anti-HIV, anti-microbial, antifungal, anti ulcerous, gastroprotective, hypoglycemic and antihyperlipidemic properties (Hussein *et al*., 2000; Basar *et al*., 2001; Al-Harrasi & Al-Saidi, 2008; Shen & Lou, 2008; Singh *et al*., 2008; Aman & Balu, 2009; Shah *et al*., 2009).

The objective of this study was to determine the total phenolic contents and the antioxidant properties of various solvent (methanol, ethanol, acetone and water) extracts of *B. serrata* oleogum resin by different methods including DPPH radical scavenging activity, reducing power, total antioxidant capacity and oxidative stability index in vitro.

Materials and Methods

Chemicals and Materials

Tert-Butylhydroquinone (TBHQ) was purchased from Nova international (India), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the Folin–Ciocalteau reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA) while all solvents and reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). *Boswellia serrata* oleogum resin was supplied from a local factory (Gorgan, Iran). The oleo-gum resin was ground to a fine powder, passed through a 40- mesh sieve and kept in an air-tight container at 4°C until further use. Refined soybean oil pure of antioxidant additives was purchased from a local oil refining factory (Alia Golestan Co., Iran).

Extraction of antioxidants from *B.serrata*

The dried powder of *B.serrata* (25g) was extracted overnight in 250 ml each of methanol, ethanol, acetone and water, respectively, in a mechanical shaker (IKA, MTS 2/4) at room temperature and each extract was filtered with Whatman No. 1 filter paper. The filtrates obtained from methanol, ethanol and acetone extractions were evaporated to dryness at 40°C in a rotary evaporator (Buchi,V800, Switzerland) and the water extract was freeze-dried (-40°C) (Operon, FDB-5503, Korea). The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 °C until use.

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin–Ciocalteu method (Slinkard and Singleton, 1977). Briefly, 20 µl of extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) after 8 minutes. Subsequently, the mixture was incubated at oven (Memmert, WB14, Germany) at 40 °C for 30 minutes and its absorbance was measured at 760 nm (Cecil, Aquarius, England). Gallic acid was used as a standard

for the calibration curve. The phenolic content expressed as gallic acid equivalent was calculated using equation obtained by performing linear regression on calibration curve:

$Y=0.0011X-0.0001$ (1) $R^2=0.9949$

Where Y is the absorbance at 720 nm and X is concentration of phenolic compounds as gallic acid equivalents (µg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Blois (1958). Briefly, 1 ml of a 0.1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 100–1000 µg/ml). The mixture was then vortexed and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and antioxidant activity was expressed as percentage DPPH scavenging relative to the control using the following equation:

DPPH scavenging activity (% =[(Absorbance of control− Absorbance of sample)/ Absorbance of control] ×100 (2)

Also IC_{50} was determined by linear correlation analysis of different concentrations of the samples. The methanol is used as experimental control. The control sample was prepared by mixing methanol and DPPH radical solution.

Reducing power assay

The ability of extracts to reduce iron (III) was assessed according to Yildirim *et al*, (2001). The extracts (100– 1000 µg/ml) in 1 ml of the corresponding solvent were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1%) and then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of tri-chloro-acetic acid (100 g/ l) were added and the mixture was centrifuged at 1650g for 10 min. Finally, 2.5 ml of the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of Ferric Chloride ($FeCl_3$, 1g/l) and the absorbance was measured at 700 nm. Higher absorbance indicates better reducing power under the reaction conditions.

Total antioxidant capacity

Total antioxidant activity of the extracts was determined according to Prieto *et al*, (1999). Briefly, a 0.1 ml aliquot of sample solution (containing 100– 1000 µg/ml of different extracts in corresponding solvent) was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of the reagent solution and the appropriate volume of the same solvent used for the sample and was incubated under the same conditions as the rest of the samples.

Oxidative stability index (Rancimat test)

The effectiveness of the *B.serrata* extracts against oxidation of soybean oil was determined by the Rancimat (Metrohm, 743, Switzerland) (AOCS, 2007). That is an accelerated technique most commonly used for the assessment of the oxidative stability of edible fats, oils and fat-containing foods (Farhoosh, 2007). The concentration of the antioxidant agents varied from 100-1000ppm, the air flow rate was set at 20 l/h and the temperature at 110°C The oxidative stability was expressed as induction time while antioxidant index (AI) was calculated from the measured induction times according to Forster *et al*, (2001) using the following formula:

AI= Induction time of soybean oil oxidation with antioxidant/ Induction time of soybean oil oxidation without antioxidant. (3)

Statistical analysis

All the experiments were carried out in triplicate and the collected data were analyzed by ANOVA; the means were compared by the

Duncan's multiple range tests at the 5% level using SPSS version 21 (IBM, USA).

Results and discussion

Extract yield and total phenolics

Solvent extraction has become one of the most popular methods for the preparation of extracts from plant materials (Dai and Mumper, 2010). In general, solvent extraction of polyphenols depends on two events; dissolution of each compound in the plant material matrix followed by their diffusion in the external solvent medium (Shi *et al.*, 2005). Consequently, the extraction efficiency depends on a great number of factors such as polarity of solvent, extraction conditions like time and temperature, chemical and physical properties of the samples as well the solvent/ sample ratio (Dai and Mumper, 2010). The effect of different solvents on the extraction yield and the total phenolic content of *B.serrata* oleo-gum resin are presented in Table 1. The amount of extractable components expressed as percentage by weight of dried material ranged from 11.25% (acetone extraction) to 20.58% (water extraction). According to the results, the water solvent showed the highest yield of extraction because of the highest polarity index. The solvent polarity is an important parameter for extraction of natural antioxidants and the higher polarity leads to better solubility of phenolic compounds (Tomsone *et al.*, 2012).

Table 1. Effect of solvent type on the extraction yield and total phenolics content of *Boswellia Serrata.*

Sample	Yield[1]	Total phenolic content[2]
Methanol extract	11.53 ± 0.19^{c}	254.27 ± 1.22^{a}
Ethanol extract	12.42 ± 0.14^{b}	172.47 ± 0.98^{b}
Acetone extract	11.25 ± 0.09^{c}	158.11 ± 1.31^{c}
Water extract	20.58 ± 0.23^{a}	112.13 ± 1.05^{d}

[1]Grams of extract per 100 g of dried powder.
[2]mg of gallic acid per 100 g dry weight of extract.
Different letters within the same column indicate significant differences ($p<0.05$)

The amount of total phenolics (gallic acid equivalents) expressed as percentage by weight of dried extract ranged from 112.13 in the water extract to 254.27 in the methanol extract. Methanol was found to be the most effective solvent in extraction of phenolic compounds from *B.serrata* oleo gum resin. This is in agreement with previous studies which reported that methanol can be an effective solvent for the extraction of antioxidants from different plants (Siddhuraju *et al.*, 2003; Arabshahi-Delouee & Urooj, 2007; Chirinos *et al.*, 2007). It has been shown that methanol is more efficient in extracting lower molecular weight phenols while aqueous acetone is more suitable for the extraction of higher molecular weight compounds. Consequently, there is no universal extraction procedure that can be applied for the extraction of all phenolics (Dai & Mumper, 2010).

DPPH radical scavenging activity

The DPPH essay has been widely used in order to evaluate the ability of various compounds to scavenge free radicals (Musa *et al.*, 2013). This method is simple and requires mild experimental conditions, which is advantageous in comparison with other methods that require preliminary sample treatment (Musa *et al.*, 2013). However, it can be affected by factors such as the type and amount of the solvent used, the water content, pH and the presence of metal ions (Dawidowicz *et al.*, 2012). The scavenging ability of the extracts in comparison with the synthetic antioxidant TBHQ are presented in Figure 1. As it can be seen, the scavenging activity of the four extracts against DPPH was concentration-dependent.

The methanolic extract was found to be the most active radical scavenger followed by ethanol, acetone and water extracts and this can be attributed to its higher content of total

phenolic compounds. However, it was not as effective as the reference control, TBHQ, since the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture (IC_{50}) was significantly ($p<0.05$) higher than that of TBHQ (Table 2). IC_{50} values of the extracts ranged from 515.46± 0.76 in methanol extract to 982.12± 0.87µg/ml in water extract. A high correlation between total phenolic content and free radical scavenging activity of different plant extracts has been reported by many researchers (Peterson *et al*., 2001; Jimenez-Escrig *et al*., 2001; Arabshahi-Delouee & Urooj, 2007). In general, increasing the concentration of phenolic compounds increases the ability of the extracts to inhibit free radicals but the type of phenolic compounds is crucial as wel.

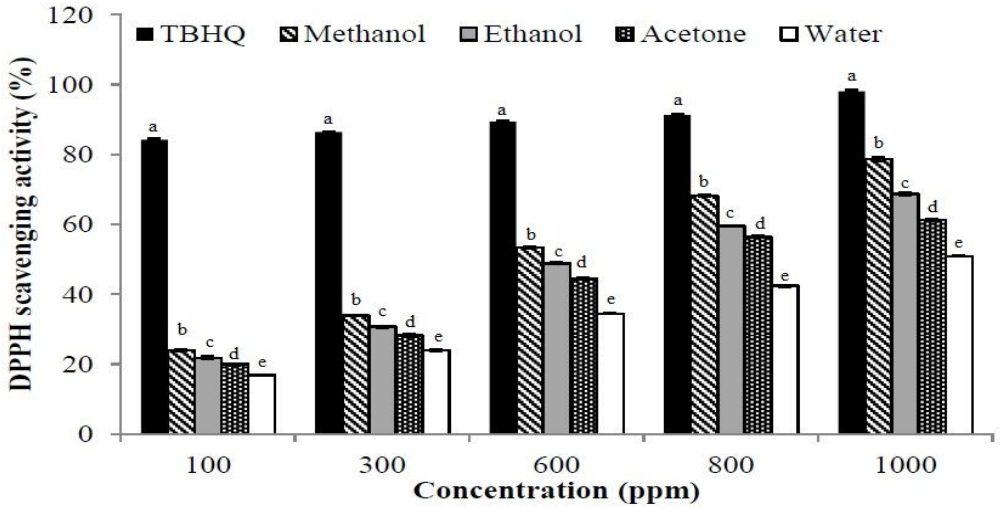

Fig. 1. DPPH radical scavenging activities of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Table 2. Ic_{50}[1] (µg /ml) of different solvent extracts from *Boswellia serrata* oleogum resin and TBHQ.

Sample	DPPH	Reducing power	Total antioxidant capacity
TBHQ	59.32±0.87[a]	40.8±0.79[a]	159.36±0.38[a]
Methanol extract	515.46±0.76[b]	96.15±0.43[b]	359.71±0.61[b]
Ethanol extract	636.76±0.54[c]	185.18±0.62[c]	692.38±0.59[d]
Acetone extract	694.44±0.92[d]	200±0.19[d]	437.6±0.72[c]
Water extract	982.12±0.87[e]	232.55±0.66[e]	ND[2]

[1] The effective concentration in which the absorbance was 0.5 for reducing power and total antioxidant capacity; DPPH radicals were scavenged by 50%.

[2] Not detectable.

Values denoted by different letters within each column are significantly different ($p< 0.05$).

Reducing power

It has been previously reported that the reducing power was associated with the antioxidant activity (Siddhuraju *et al*., 2002). In this assay, the ability of the extracts to reduce iron (III) to iron (II) was determined. All the extracts showed some degrees of electron donation capacity in a concentration-dependent manner, but their capacities were inferior to that of TBHQ (Figure 2). Methanolic extract containing the highest amount of total phenolics, was the most potent reducing agent, whereas the water extract with the lowest phenolic content, was the weakest one.

Regarding the calculated IC50 values for different extracts, they followed the order of water> acetone> ethanol> methanol, which corrected well with their total phenolic contents (Table 2). Similar relations between

iron reducing activity and total phenolics content of plant extracts have been reported previously (Gao et al., 2000; Tsao et al., 2005; Arabshahi-Delouee & Urooj, 2007).

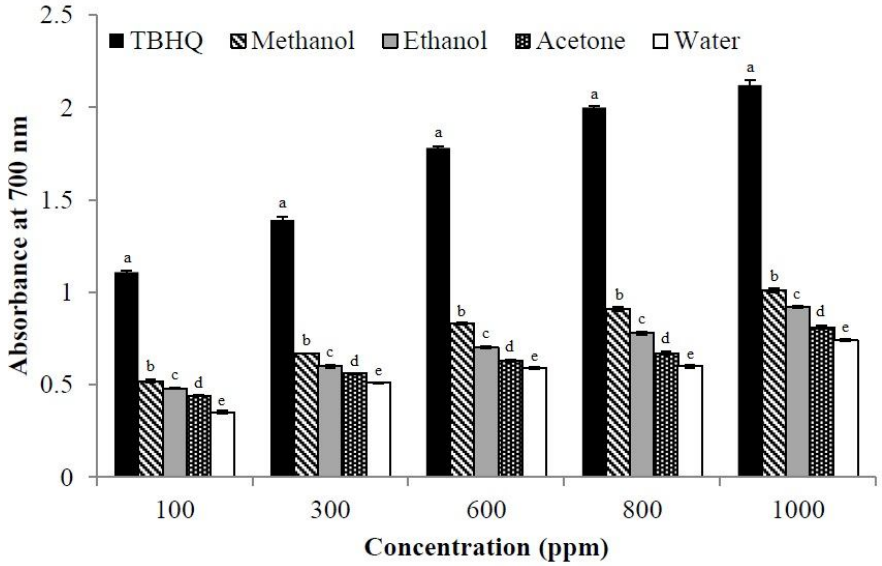

Fig.2. Reducing powers of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Total antioxidant capacity

The phosphomolybdenum assay which is a quantitative method for evaluation of water-soluble and fat soluble antioxidants (Prieto *et al*.,1999) has been widely used to determine the total antioxidant capacity of many plant extracts (Kumaran & Karunakaran, 2007; Arabshahi-Delouee and Urooj, 2007; Jiang *et al*., 2014). As shown in Figure 3, the antioxidant capacity of the extracts was concentration-dependent; however, their capacities were inferior to that of TBHQ. In this assay, methanol extract showed the highest activity followed by acetone, ethanol, and water extracts. The IC_{50} values calculated for various extracts and TBHQ are presented in Table 2. Among the organic solvent extracts, the lowest (359.71 ± 0.61 µg/ml) and the highest (692.38± 0.59 µg/mL) IC_{50} values belonged to the methanol and ethanol extracts, respectively, while the IC_{50} of the water extract could not be determined. Results of this assay demonstrated electron-donating capacity of different extracts of *B. serrata* oleogum resin.

In this study, the DPPH radical scavenging and the reducing power activities assays of the extracts correlated to the amount of their phenolic contents, but in the total antioxidant capacity assay the order was different. Unlike previous assays (DPPH radica scavenging and reducing power), the total antioxidant capacity of the extracts did not correlate well with their total phenolic contents. Singh *et al*, (2008) has reported that apart from the phenols present in the *B. serrata resin*, boswellic acid which is a mixture consisting of four major pentacyclic triterpene acids can be found. The presence of such antioxidant compounds may explain the differences observed in the results of different assays.

Oxidative stability index

The rancimat method is based on the conductivity changes incurred by deionised water after collecting the volatile organic acids produced in the final steps of the accelerated oil oxidation process (García-Moreno *et al*., 2012). The method can provide a fast assessment of the efficiency and the thermal stability of different antioxidants under more challenging conditions than using the Scall oven test (Aladedunye *et al*., 2014). A high

correlation between sensory properties or analytical data and the results of rancimat analysis has been reported (Coppin & Pike, 2001; Anwar *et al*., 2003). Table 3 shows the oxidative stability of soybean oil containing different concentrations of various extracts of *B.serrata* compared to synthetic antioxidant, TBHQ. All the extracts of *B.serrata* were able to improve the oxidative stability of soybean oil, as indicated by longer induction periods compared to that of control without any antioxidant, however the activities were inferior to that of TBHQ. Methanolic extract containing the highest amount of total phenolics, exhibited the greater protective effect against the oxidation of soybean oil, whereas the water extract was the least effective one.

The amount and type of antioxidant components, the solubility in soybean oil, and the stability of the components during thermal processing, are the factors that affect the effectiveness of the antioxidant agents when evaluated with the rancimat test.

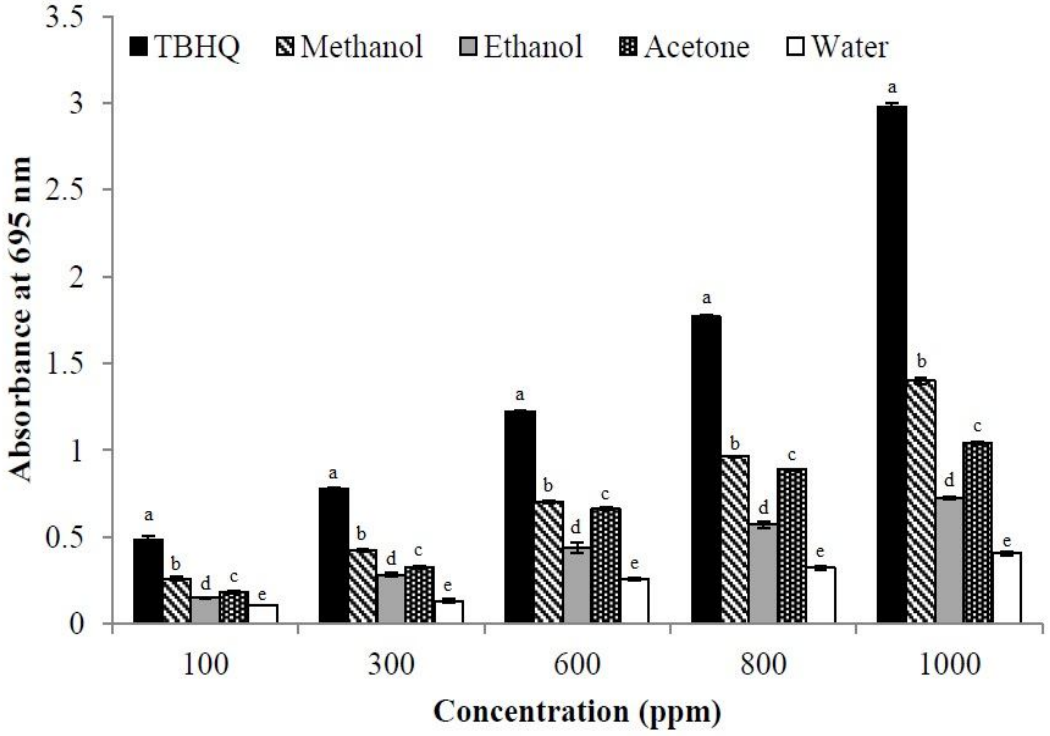

Fig. 3. Total antioxidant activities of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Conclusions

Various solvent extracts of *B.serrata* oleogum resin showed varying degrees of antioxidant activity in different test systems in a concentration dependent manner. The type of extraction solvent and the concentration of the extract were assigned as important parameters in the overall activity of the extracts. Methanol proved to be the most efficient solvent for extraction of antioxidants from *B.serrata* as the related extract contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. Overall, *B.serrata* was found to serve as a potential source of natural antioxidants for utilization in food and biological systems. In addition, potential exploitable beneficial effects and safety in humans need to be proved in clinical trials and the effect of the applied extracts on the sensory attributes of fats and oils should be further studied.

Table 3. Rancimat analysis of soybean oil samples containing different concentrations of *Boswellia serrata* extracts, or TBHQ at 110 °C. Values denoted by different letters within concentrations of the same extract are significantly different (p< 0.05).

Samples	Concentration(ppm)	Induction time (h)	AI[1]
Blank	-	6.14 ± 0.09	-
TBHQ	100	12.25 ± 0.11	1.99
Methanol extract	200	6.98 ± 0.09[d]	1.13
	500	7.30 ± 0.20[c]	1.18
	800	7.67 ± 0.05[b]	1.24
	1000	8.07 ± 0.09[a]	1.31
Ethanol extract	200	6.93 ± 0.10[d]	1.12
	500	7.16 ± 0.06[c]	1.16
	800	7.42 ± 0.04[b]	1.20
	1000	7.64 ± 0.05[a]	1.24
Acetone extract	200	6.71 ± 0.07[d]	1.09
	500	6.92 ± 0.05[c]	1.12
	800	7.28 ± 0.12[b]	1.18
	1000	7.41 ± 0.07[a]	1.20
Water extract	200	6.60 ± 0.06[d]	1.07
	500	6.79 ± 0.01[c]	1.10
	800	7.02 ± 0.05[b]	1.14
	1000	7.25 ± 0.08[a]	1.18

[1]Antioxidant activity index for Rancimat method

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نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 13، شماره 3، مرداد - شهریور 1396، ص. 38-28

Iranian Food Science and Technology Research Journal
Vol. 13, No. 3, Aug. Sep. 2017, p. 28-38

ویژگی‌های آنتی اکسیدانی عصاره‌های مختلف اولئوگم رزین کندر *(Boswellia serrata)*

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تاریخ دریافت: 1395/10/12
تاریخ پذیرش: 1396/04/03

چکیده

ترکیبات فنولی کل و خصوصیات آنتی‌اکسیدانی عصاره متانولی، اتانولی، استونی و آبی اولئوگم رزین کندر توسط روش‌های مختلف مهار رادیکال‌های آزاد DPPH، قدرت احیاءکنندگی، ظرفیت آنتی‌اکسیدانی کل و آزمون رنسیمت مورد ارزیابی قرار گرفت. عصاره‌های مختلف فعالیت آنتی‌اکسیدانی متفاوتی را بروز دادند. عصاره متانولی حاوی بیشترین ترکیبات فنولی بود و ظرفیت آنتی‌اکسیدانی بالاتری را در تمامی آزمون‌های انجام گرفته نشان داد. علاوه بر این، همه عصاره‌ها توانستند پایداری اکسایشی روغن سویا را در آزمون رنسیمت بهبود بخشند. براساس نتایج به‌دست آمده، می‌توان از اولئوگم رزین کندر به‌عنوان منبعی بالقوه از آنتی‌اکسیدان‌های طبیعی استفاده نمود.

واژه‌های کلیدی: *Boswellia serrata*، ترکیبات فنولی، خصوصیات آنتی‌اکسیدانی، رنسیمت، زرشک، اولئوگم رزین

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