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E-mail: bjsir07@gmail.com

Utilization of *Salicornia fruticosa* herb for producing antioxidants

***E. M. Elsebaie, S. Y. A. Elsanat, M. S. Gouda and K. M. Elnemr**

Food Technology Department, Faculty of Agric. Kafrelsheikh University, Egypt

Abstract

The present work was aimed to study the effect of extracted phenolic compounds from *Salicornia* air part by several solvents as natural antioxidants on preservation of corn oil comparing with synthetic antioxidant (TBA) on the oil stability against oxidative rancidity during storage at 70 °C for 5 days. The results indicate that the best solvent for extracting polyphenolic compounds was methanol followed by ethanol, chloroform and water. HPLC analysis for the total polyphenols extracted from the air part of *salicornia fruticosa* indicated to presence high percentages of Pyrogallol, Ellagic, B-OH Benzoic and Catechin. The extracted phenolic acids were tested against corn oil keeping quality. Results show that peroxide value and TBA values of corn oil that treated by different types of extracts at different levels were lower than control. Keywords: Salicornia fruticosa; DPPH; Corn oil; Phenolic extract.

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Introduction

There is a growing body of evidence suggesting that oxidative stress through an increased production of reactive oxygen and nitrogen species (ROS and RNS) plays an important role in the development of tissue damage and pathological events in living organisms (Peuchant *et al.,* 2004).

In order to limit the harmful effects of ROS, a high performance antioxidant system, consisting of enzymes, proteins, vitamins, carotenoids, polyphenols, trace elements and small molecules, such as glutathione, may interact with ROS and regulate their production within a physiological range.

Antioxidants may therefore be of major importance in preventing the onset and/or the progression of oxidative pathologies and may provide protection to foods (Spignoli, 2000). The physiological benefits of the plant phenolics have been attributed to their potential role in inhibiting lipid peroxidation, modulating cell signal transduction pathways and inducing apoptosis (Hou *et al.,* 2004). The development and utilisation of more effective antioxidants of natural origin could, therefore, afford potential benefits for the optimization of human health (Moure *et al.,* 2001; Panico *et al.,* 2005). Increased concern over the safety of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Tsuda *et al.,* 1998) has lead to an increased interest in exploration of effective and economical natural antioxidants. There is increasing evidence that changing one's diet to an increased intake of food in selected natural antioxidants, such as plant polyphenols, vitamin C or flavonoids, can reduce the incidence of chronic and degenerative diseases (Laandrault *et al.,* 2001). Several sources of natural antioxidants have been investigated, including plants and microorganisms (Arai *et al.,* 2002 and Bandoniene *et al.,* 2000). The extraction and purification of phytochemicals from natural sources is desired, since these bioactive substances are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceuticals and cosmetic products (Radwan e*t al.,* 2007). Different solvent systems have been used for extraction of polyphenols from plant materials (Chavan *et al.,* 2001). Extraction yield is dependent on the solvent and method of extraction (Goli *et al.,* 2005). The extraction method must enable complete extraction of the compounds of interest and must avoid their chemical modification (Zuo *et al.*, 2002).

Salicornia fruticosa L. (also known as glasswort) is annual succulent herb of Chenopodiaceae family and one of the most salt tolerant plants. It is growing on salt marshes and muddy seashores (Kim *et al.,* 2009) and this family is represented in Egypt by 25 genera and about 300 species. Several species possess antibacterial and antihypertensive properties,

*Corresponding author. e-mail: essamelsbay@yahoo.com

also mentioned in folk medicine for relief of toothache and chronic rheumatic. Investigation of certain species of this family revealed that they contain large amounts of minerals, essential amino acid, essential fatty acids, coumarins, phenolic compounds and alkaloids (Radwan *et al.,* 2007).

The aim of this study is to isolate Phenolic compounds extracted from Salicornia fruticosa, then identify and using it as natural antioxidants for preservation corn oil during storage.

Materials and methods

Materials

Salicornia fruticosa (L.) was collected from international coastal road near El- Boroles city, Kafr El- Sheikh Governorate, Egypt during January 2012. The plant was identified by Dr. M. El-Gebaly and Dr. S. El Kawasshty, taxonomists, National Research Center, Cairo, Egypt (Radwan *et al.,* 2007). Corn oil was obtained from Tanta company for oils and soaps, Tanta, Egypt. Commercial antioxidant; butylated hydroxyanisole (BHA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma chemical company (St. Louis, Mo., U.S.A.). All chemicals and solvents used in this investigation (HPLC spectral grade) were purchased from El- Gomhorea company, Tanta, Egypt.

Methods

Sample preparation for antioxidant extracts:

After roots separation, the air part of the plant were cut into small pieces (1cm) then dried at 40ºC ± 2°C in an air hot oven until the moisture content reached less than 10 %. The dried product was ground into powder to pass through a 60 mesh sieve. These fractions were used in analyses for the chemical composition.

Gross chemical composition

Moisture; crude protein; ether extract; ash and crude fiber content were determined according to the methods described in the A. O.A. C. (2000). Total carbohydrates were calculated by difference.

Total phenolic compounds determination

Extraction of total phenolic compounds

The prepared ground sample (5 g) were macerated in 50 mL of each solvent (absolute methanol, ethanol, chloroform and water) for 24 hours at room temperature. The crude solvent extracts were filtrated through filter paper (Whatman No. 1), filtrates were evaporated under vacuum in rotary evaporator at 45°C and weighted to determine the extracted yield of each sample (McGrath *et al.,* 1982).

Quantitative determination of total Phenolic compounds

The concentrations of total phenols in each extract were estimated with Folin-Ciocalteau reagent (Gutfinger, 1981).

HPLC determination and identification of Phenolic compounds

Phenolic compounds of samples were extracted according to the method outlined by Evangelisti, *et al.*(1995). A known weight of dried powder sample was soaked in 25ml sterilized water and agitated on a rotary shaker for 24hrs at 200 rpm. Sullary was filtered through WhatmanNo3 filter paper under vacuum, followed by centrifugation at 12.500g for 30min at 80°C. The aqueous extract was acidified to pH(2.5) using diluted phosphoric acid . Each sample was partitioned three times with an equal volume of diethyl -ether. The combined diethyl -ether layers were evaporated to dryness under reduced pressure at 30°C.The resulting residue was redissolved in 3ml of spectral grade methanol and filtered through a 0.2 µm filter sterilized membrane prior HPLC analysis.

Identification of individual Phenolic compounds of the plant samples were performed on a HEWLLET packared HPLC (Model 1100), using a hypersil C18 reversed-phase column (250 ×4.6mm) with 5 µm particle size. Injection by means of a Rheodyne injection valve (Model 7125) with 5 µl fixed loop was used . A constant flow rate of one ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A)and ending with (B)over 35 min, using an UV detector set at wavelength 254 nm. Phenolic compounds of each sample were identified by comparing their relative retention time with those of the standards mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg Phenolic /g dry weight. Sixteen standard Phenolic compounds were purchased from Sigma (St. Louis, USA) and from Merck-Schuchardt (Munich, Germany) Chemical Companies.

Scavenging effect assay of Salicornia fruticosa air part extracts

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method of Sousa *et al.* (2008). . Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (100 & 200 ppm).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys10S UV: Thermo electron corporation). Butylated hydroxyanisole (BHA) was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

% scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Antioxidative efficiency of Salicornia fruticosa extracts

The antioxidative activities of polyphenol extracts from Salicornia fruticosa were assayed by addition of each extracts 0, 100, 200, 300 ppm to corn oil (control), then Pyrex bottles filled up with treated oils. The treated oils were stored in an air oven at 70° C for five days and the degree of oxidation was determined at different times as follows:

Peroxide values (PV)

The peroxide values (PV) of stored oils were determined as described by Leonard *et al.,* (1987). Expressed as mill-equivalents of peroxide per 1000g of sample.

Thiobarbituric acid number (TBA)

Thiobarbituric acid values were carried out according to the method described by Sidwell *et al.* (1990). Expressed as mg malondialdhyde/ kg oil.

Statistical analysis

Data were treated by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system, 1988). Duncan's multiple range test (Duncan 1955) was used to compare differences among individual means. Treatment effects were considered significant at $P \leq 0.05$ levels.

Results and discussion

Gross chemical composition of Salicornia fruticosa air part:

Table (I) shows gross chemical composition of *salicornia fruticosa* air part. Data indicate that moisture, crude protein, ether extract, ash, crude fiber and available carbohydrate of *salicornia fruticosa* air part were 77.63, 18.04, 14.69, 13.39, 30.65 and 23.23 % respectively. These results are in agreement with those reported by Min *et al.* (2002) and Lee *et al.* (2004).

Table I. Gross chemical composition of *Salicornia fruticosa* air part (%on dry weight basis)

Component(%)	Moisture	Crude protein	Ether extract	Ash	Crude fiber	available carbohydrate*
salicornia fruticosa air part	77.63	18.04	14.69	13.39	30.65	23.23

*Total carbohydrates were calculated by difference

Effect of using different solvents on extraction yield and total polyphenol content extracted from salicornia fruticosa air part

Extraction yield and total polyphenol content extracted from *salicornia fruticosa* air part are given in Table II. The data indicate that water used as extraction solvent was the highest

Table II. Effect of different solvents on extraction yield and total polyphenol content extracted from *salicornia fruticosa* air part

Extraction solvent	Extraction yield (%)	Total poly phenols(ppm)
Methanol	18.46[b]	1520[a]
Ethanol	8.76[c]	1240[b]
Chloroform	4.60[d]	940[c]
Water	48.6[a]	872[d]

Values followed by the same letter in the same columns are not significantly different P≤0.05

amount of extracted yield reaching 48.6%, followed by methanol 18.46%. These results are agreement with the results obtained by Kang *et al.* (2011). On the other hand, the data indicate that methanol was the best solvent for extracting polyphenols from *salicornia fruticosa* air part. High amount of extracted polyphenolic compounds by methanol was 1520 (ppm), comparing with other solvents. These results are in agreement with the results obtained by Kahkonen *et al.* (1999) and Min *et al* (2002).

Phenolic compounds extracted from salicornia fruticosa air part

High performance liquid chromatography method (HPLC) was used to fractionate and identificate the polyphenolic compounds extracted from *salicornia fruticosa* air part. The obtained results, were listed in Table (II).

Data in table (III) indicated that, *salicornia fruticosa* air part methanolic extract contains 12 phenolic compounds. Pyrogallol, Ellagic, B-OH Benzoic and Catechin were the major phenolic compounds presented and identified in the methanolic extract.

Table III. Phenolic compounds in methanolic extract of *salicornia fruticosa* air part

Phenolic compound	Concentration (%)	Phenolic compound	Concentration (%)
Pyrogallol	28.14	Caffeine	4.38
Protocatechuic	5.16	B- coumaric	0.42
Catechin	10.26	B-OH Benzoic	10.92
Chlorogenic	8.09	Ellagic	25.41
caffeic	1.81	Colchecien	2.49
Vanillic	2.88	Chrysin	0.04

Table IV. Antioxidant activities of various solvent extracts from *salicornia fruticosa* air part in DPPH assay

Extract type	DPPH reduction %	
	100 ppm	200 ppm
BHA	83.760[a]	94.550[a]
Methanol extract	57.510[b]	76.060[b]
Ethanol extract	43.427[c]	60.330[c]
Chloroform extract	34.510[d]	43.896[d]
Water extract	7.280[e]	13.380[e]

Values followed by the same letter in the same columns are not significantly different $P \leq 0.05$.

Antioxidant activity

Antioxidant activity of *salicornia fruticosa* air part extracts were analyzed measuring scavenging activities of DPPH. DPPH radical is a stable dark purple colored radical, which are changed to yellow by accepting an electron from antioxidants (Sasidharan *et al.,* 2007). Data in table IV showed that methanolic extract has the most potent activity followed by ethanol extract, chloroform extract and water extract at the low levels of concentrations 100 and 200 ppm. These results are in agreement with the results obtained by Min *et al.* (2002).

Utilization of salicornia fruticosa air part phenolic extracts as natural antioxidants

Antioxidant activity of phenolic compounds extracted from *salicornia fruticosa* air part was assayed against corn oil (control). Measurement of peroxide value (PV) and Thiobarbituric acid (TBA) are suitable potent parameters to characterize oxidative changes in the tested oils. In this relation, different levels (100, 200 and 300 ppm) of phenolic s*alicornia fruticosa* air part extracts were added to corn oil as natural antioxidants then PV and TBA were determined during storage for 5 days at 70 °C and compared with oil treated by 200 ppm of TBA as a synthetic antioxidant.

Effect of adding different levels of natural extracts and synthetic antioxidants on peroxide value (PV) of corn oil stored at 70° C

Table (V) showed the change in PV of corn oil free from antioxidants and those treated with synthetics and natural antioxidants. In general, addition of such compounds caused to decrease the increment of PV compared with oil free from antioxidants. However, the results showed that PV was gradually increased storage and the level of the increase for untreated oils were greater than all samples of treated oils. These results are consistent with findings of Yanping *et al.* (1999) who reported that lipid peroxides were significantly reduced by the addition of antioxidants in processed foods and oil.

Effect of adding different levels of natural extracts and synthetic antioxidants on Thiobarbituric acid (TBA) of corn oil stored at70°C.

Thiobarbituric acid (TBA) of fresh and heated corn oil were determined and the results are presented in Table (VI). The levels of TBA values were rapidly increased with increasing the storage period of control. Comparing to heated control (3.11 mg malonaldhyde/kg oil) for five days storage, all antioxidant extracts and BHA decreased TBA value to 1.823, 1.859, 1.983, 2.155 and 2.475 mg malonaldhyde/kg oil. Similar results were obtained by Hassan (2002).

Table V. peroxide value per milli-equivalent O_2/ 1000 g oil (PV) of corn oil treated by *Salicornia fruticosa* air part phenolic extracts during storage at 70 °C

Extract	Control	BHA	Methanol			Ethanol			Chloroform			Water		
Time (hr)		200 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm
0	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	00.39	0.39	0.39	0.39
24	4.62^{a}	3.19^{j}	3.53^{f}	3.38^{h}	3.32^{i}	3.96^{c}	3.45^{g}	3.41^{g}	4.1^{b}	3.67^{e}	3.49^{f}	4.28^{b}	3.99^{c}	3.88^{d}
48	5.62^{a}	3.86^{i}	4.41d^{e}	4.28^{f}	3.91^{h}	4.53^{c}	4.39^{e}	4.00^{g}	4.67^{b}	4.52^{c}	4.33^{f}	4.71^{b}	4.55^{c}	4.46^{d}
72	6.30^{a}	4.14^{j}	4.64^{f}	4.47^{h}	4.30i	4.81^{c}	4.63^{f}	4.48^{h}	4.98^{b}	4.69^{e}	4.51^{g}	5.09^{b}	4.76^{d}	4.69^{e}
96	8.91^{a}	5.878^{k}	6.65^{f}	6.42^{h}	6.06^{j}	6.88^{d}	6.63^{f}	6.29^{i}	7.12^{c}	6.76^{e}	6.48^{g}	7.25^{b}	6.84^{d}	6.73^{e}
120	10.783^{a}	7.071^{k}	8.014^{f}	7.74^{h}	7.29^{j}	8.26^{d}	7.99^{f}	7.57^{i}	8.56^{c}	8.13^{e}	7.8^{g}	8.7^{b}	8.21^{d}	8.08^{e}

Values followed by the same letter in the same row are not significantly different P≤ 0.05.

Table VI. Thiobarbituric acid number mg malondialdhyde/ kg oil (TBA)of corn oil treated by *Salicornia fruticosa* air part phenolic extracts during storage at 70 °C

Extract	Control	BHA	Methanol			Ethanol			Chloroform			Water		
Time (hr)		200 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 ppm
0	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234
24	0.647^{a}	0.437^{k}	0.538^{h}	0.53^{h}	0.476^{j}	0.577^{e}	0.554^{g}	0.515^{i}	0.619^{c}	0.585^{d}	0.569^{f}	0.632^{b}	0.608^{c}	0.593^{cd}
48	1.131^{a}	0.749^{k}	0.803^{h}	0.772^{i}	0.764^{j}	0.889^{e}	0.835^{g}	0.803^{h}	0.998^{c}	0.905^{e}	0.858^{f}	1.061^{b}	0.983^{d}	0.920^{d}
72	2.067^{a}	1.232^{k}	1.482^{h}	1.271^{i}	1.256^{j}	1.537^{e}	1.404^{g}	1.334^{h}	1.724^{c}	1.568^{e}	1.451^{f}	1.895^{b}	1.732^{c}	1.669^{d}
96	2.513^{a}	1.492^{k}	1.766^{h}	1.543^{j}	1.523^{jk}	1.866^{f}	1.705^{h}	1.623^{i}	2.106^{c}	1.9027^{e}	1.761^{g}	2.313^{b}	2.108^{c}	2.012^{d}
120	3.11^{a}	1.823^{k}	2.167^{h}	1.879^{j}	1.859^{jk}	2.288^{f}	2.084^{h}	1.983^{i}	2.59^{c}	2.335^{e}	2.155^{g}	2.856^{b}	2.595^{c}	2.475^{d}

Values followed by the same letter in the same row are not significantly different P≤ 0.05.

Conclusion

In summary, the results from this study have shown that methanol was the most suitable solvent for polyphenolic compounds extraction from *salicornia fruticosa* air part. Additionally, different extracts of air parts of *salicornia fruticosa* showed potent antioxidant activity lower than the standard BHA. The antioxidative activities observed can be attributed to presence of phenolic compounds. HPLC analysis for the total polyphenols extracted indicated to presence high percentages of Pyrogallol, Ellagic, B-OH Benzoic and Catechin. The extracted phenolic acids were tested on preservation of corn oil comparing with synthetic antioxidant (TBA) on the oil stability against oxidative rancidity during storage at 70 °C for 5 days. Results show that peroxide value and TBA values of corn oil that treated by different types of extracts at different levels were lower than control and the recommended concentration for natural antioxidants (methanolic extract) is 300ppm.

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