BIODIESEL PRODUCTION AND ANTIOXIDANT CAPABILITY FROM SEEDS OF *SALICORNIA BEGELOVII* COLLECTED FROM AL JUBAIL, EASTERN PROVINCE, SAUDI ARABIA

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Abstract

Salicornia begelovii Torr displays excessive biotechnological prospective as a salt-water irrigated crop. Qualitative and quantitative compositions of fatty acids were analyzed in the seeds of *Salicornia begelovii* collected from the eastern region, Al Jubail, Saudi Arabia. Hexane extraction of the seed oil from *Salicornia begelovii* yielded 29% of total lipids. The GC-MS (Gas Chromatography-Mass Spectroscopy) investigation of the hexane extracts revealed five major peaks for the seed oil: 72.5 wt.% linoleic-ω6 acid (18:2), 7.4 wt.% palmic acid (16:0), 13.3 wt.% oleic acid (18:1), 2.14 wt.% stearic acid (18:0) and 2.3 wt.% linolenic-ω3 acid (18:3). The quantity of the both saturated palmitic and stearic acids amounted (9.18%) in *S. begelovii* seed oil. The antioxidant capability of *S. begelovii* seed oil were determined and expressed by hydroxyl radical scavenging assay, nitric oxide (NO) scavenging activity and radical scavenging effects of the extract on DPPH free radical were studied. The composition of the oil was nutritive and medical health value was high, in addition to, it's composition very similar to that of safflower oil. No unwanted fatty acid constituents were established in *S. begelovii* seed oil, and it could be suggested for biofuel fabrication.

Key words: *Salicornia,* Fatty acids, Biodiesel, Antioxidant.

Introduction

Globally, soil salinity establishes a growing difficult that cause land degradation with nearly 7% of the soil's terrestrial surface having "salt-affected soils". The manner of accumulating the concentration of overall liquefied salts (in soil and water) is identified as salinization. It can be formed either by "natural manners" such as mineral weathering and steady seawater interference or by "artificial methods" such as irrigation. On a universal measure, it has been assessed that each minute 3 ha of presently arable land converts infertile owing to salinization. It has been estimated that about 100 million hectare of soil have become salty (due to lowly irrigation running) which counterparts 11% of the universal watered zones. Soil salinity has strictly disturbed the agricultural manufacture in above half of the world's countries. Countries which are characterized by disproportionately high areas of saline land are Australia, Pakistan, Bangladesh, Thailand, and several countries in Central Asia. Farming of these lands might give the rise in foodstuff fabrication to feed a rising world population, which is anticipated to reach 9.1 billion people by 2050 and hereafter worldwide food fabrication will require to rise by up to 70 % by this period to face this evolution.

Biological saline cultivation is an operative procedure of decreasing the influence of salinity in saline soils. Salt sensitive plants are the common plants applied in advanced agronomy and cannot tolerate saline soils even though at little concentrations. Cultivation for saline tolerant plants is the conventional method for evolving salt resistant types. Though, propagation old-style harvests for salinity tolerance is a time-consuming, labor concentrated and dense method at plant and cellular level. Salt loving plants, instead, establish of plants that succeed once grown-up in aggressive saline environments, wherever other normal crops cannot survive. The Central and South West Asia are the main middles of diversification of halophytes, in common and the family Chenopodiaceae, in particular. The great expansion of saline soils and varied climate and topography in Saudi Arabia with widespread saline habitations in hot deserts provide conditions for diversification of this fascinating group. One of the potential uses of halophytes is as feedstock's for biofuels (Abideen *et al.*, 2012; Abideen *et al.*, 2011). However, as the main component of the halophyte biomass is lignocellulose, significant processing (high temperature pretreatment and enzymatic hydrolysis) is needed to release the fermentable sugars needed for biofuel production.

Halophyte metabolites are a combination of components typical for lignocellulosic biomass and components unique for a family or species. The most commonly found components include primary metabolites such as amino acids, protein, carbohydrates and lignin (Bandaranayake, 2002). Secondary metabolites or phytochemicals include compounds of pharmacological and biological importance, such as alkaloids, fatty acids and lipids, flavonoids, phenolics, quinines, tannins, terpenoids, steroids and saponins, coumarins to name a few (Bandaranayake, 2002). Content of the secondary metabolites can vary depending on the particular habitat where the plant grows.

Dwarf glasswort *Salicornia bigelovii*, is a leafless, fast-growing, succulent, small-seeded, annual saltmarsh plant, with potential as a saline water crop. It belongs to the family Chenopodiaceae, a well-known family for its salt-tolerant species. It is also a potential oilseed, forage, biomass crop, and a promising carbon sequestration plant. Halophyte with growing scientific and public recognition as a plant owing to its great salt-tolerance and numerous uses. *S. bigelovii* has an exceptional salt tolerance, adaptation to marginal lands and hot climates, therefore has great potential as a domesticated biomass, oilseed, and forage crop plant (Masters *et al.*, 2007). *S. bigelovii* has been successfully cultivated as an oilseed and vegetable crop in the desert coastlines of Mexico, India, the Middle East,

Africa and in Southeast China (Lu *et al.*, 2010). The leaf tips of the halophyte can be consumed by human either fresh or as pickled vegetable. The fresh (green) biomass can also be used in mixture with other forages for livestock feed. *S. bigelovii* seeds have high concentrations of good quality oil (≈30%) and low salt content (<3%), characteristics that make it promising as an oilseed halophytic crop especially for biofuel purposes. Seedcake can also be used as animal feed due to its high protein contents (≈45%).

Biodiesel is an alternative source of fuel produced from plant and animal oils (Marchetti *et al.*, 2007). During the production of biodiesel, two steps are necessary: i. extraction of triacylglycerols from the raw material; and ii. Trans-esterification to produces fatty acid methyl esters (FAME) (Chisti, 2007). Fatty acids include medium-chain (C10– C14), long-chain (C16 –18) and very-long-chain (C20) species and fatty acid derivatives. Under unfavorable environmental or stress conditions for growth, however, many plants alter their lipid biosynthetic pathways towards the formation of triacylglycerol (TAG). Triacylglycerols can be converted to the fatty acid methyl esters present in biodiesel (Chisti, 2007). The most commonly synthesized fatty acids have chain lengths that range from C16 to C18 similar to those of higher plants (Ohlrogge & Browse, 1995).

Antioxidants are substances that markedly delay or prevent the oxidation of oxidizable substrate when present in foods or body at low concentrations. There are two types of antioxidants, (1) enzymatic antioxidants (e.g., superoxide dismutase, ascorbic peroxidase, polyphenol oxidase and catalase) and (2) non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids) (Krishnaiah *et al.*, 2011). Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases including cardiovascular diseases, cancers (Gerber *et al.*, 2002), neurodegenerative diseases and Alzheimer's disease (Di Matteo and Esposito, 2003). Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years. The phenolic compounds may contribute directly to the antioxidant action due to the presence of hydroxyl functional groups around the nuclear structure that are potent hydrogen donors. These phenolic compounds of plant origin show their antioxidant effect by various mechanisms including their ability to scavenge free radicals, chelate metal ions that serve as the catalysts for production of free radicals or activate various antioxidant enzymes and inhibit oxidases (Kulkarni *et al.*, 2004).

The cultivation of *S. bigelovii* has been successful in the U.S., Mexico, Saudi Arabia, the United Arabic Emirates, Egypt, Eritrea and Pakistan (Clark, 1994, El-Mallah *et al.*, 1994, Anwar *et al.*, 2002, Zerai *et al.*, 2010).

S. bigelovii has a great biotechnological potential as a salt-water irrigated crop. However, insufficient information is available regarding the oil quality and content in this species. The aim of this work was to determine both qualitative and quantitative compositions of fatty acids in the seeds of *S. bigelovii* cultivated in Saudi Arabia and assessment of its potential for biodiesel production. Moreover, to determine the antioxidant capabilities of *S. bigelovii* seeds.

Materials and Methods

Plant material: For the determination of fatty acids and the total lipid content of *S. bigelovii*, seeds were collected from Al Jubail area, which is located in the gulf coast, eastern province, Saudi Arabia during April 2014. The electrical conductivity of the soil surface (0 and 5 cm deep) in the study area were 6.3-8.4 ds m $^{-1}$, respectively. Earlier to extraction, collected seeds were kept in the dark at -5°C for 24 h. The seeds were cleaned and washed to eliminate adhering remains. The seeds were then dried at 55°C for 24 hours and afterward weighed and ground. Average of seed weight ranged between 0.10 ± 0.04 mg (n = 100).

Pure hexane, ethanol, acetone and methanol were purchased from E. Merck Co (Germany). Folin Ciocalteus and antioxidant compounds were purchased from Merck Co (Germany) Fatty acid profiles were analyzed by gas chromatography GC Model, Shimadzu-8A, equipped with a FID detector connected to an Innowax capillary column (60m x 0.25mm x 0.25μm), from Agilent Technologies.

Oil extraction: The total oil from the seeds was extracted using cholorform: methanol mixture (2:1 v/v). About 100 g powdered *S. bigelovii* seed immersed in solvent was agitated in a conical flask for 24 h. The residue was allowed to settle and supernatant was decanted and heated to 40°C until solvent was completely evaporated. The final weight of solid material left after evaporation was noted.

Trans esterification of *S. bigelovii* oil: Potassium methoxide (23 g methanol/ 2g KOH) was added to the extracted oil and vigorously stirring at 400 rpm for 30 min at 30 °C. Use separating funnel to separate the glycerol layer and then add methanol/KOH mixture to the top ester layer using the same previous conditions (Meda *et al.*, 2005). Mixture was allowed to stand for at least 12 h to separate the biodiesel and glycerol. The glycerol was removed by gravity settling (low layer), whereas the ester layer (biodiesel) as transferred to the flask of rotary evaporator to remove the rest of methanol in the biodiesel at 65°C. Further purification was carried out to remove the remaining catalyst and glycerol, then the biodiesel was weighted and used for different analyses.

Physico-chemical properties of produced biodiesel: For physical and physico-chemical properties of oil, specific density and refractive index were determined at room temperature (28∘C) using a specific density bottle and a refractometer respectively. For determination of acid, peroxide, iodine and saponification values, standard Association of Official Analytical Chemists (AOAC), 2000 methods were followed. The fatty acid composition and free fatty acid content were measured in *S. bigelovii* seeds. The cetane number (CN) and higher heat value (HHV) of fatty acid methyl ester composition of oil with the help of equations [Association of Official Analytical Chemists (Anon., 2000).

GC analysis for fatty acid profile in the biodiesel: The derivatization of the lipid fraction of *S. bigelovii* was carried out according to the method described by D'oca *et al.* (2011). A lipid fraction sample (300 mg) was placed in

a test tube, a 3 mL mixture of boron trifluoride methanol was added and then the test tube and its contents were heated in a water bath at 70°C for 20 minutes. To recover the fatty acid methyl esters, the derivative mixture was washed into a separating funnel with 15 mL of hexane and 20 mL of distilled water. The organic and aqueous phases were then separated. The organic phase containing the fatty esters was dried and the solvent was evaporated at 50°C. Fatty acids profiles of glasswort (*S. bigelovii*) seeds oil were then determined using gas chromatography (GC Model, Shimadzu-8A, equipped with a FID detector connected to an Innowax capillary column (60m x 0.25mm x 0.25μm), from Agilent Technologies, under the following: Column 5% Degs on 80/100 Chromo Q, Detector temperature 270°C, H_2 Flow rate 75 mL/ min, sensitivity 16 ×10 2, Column temperature 150-180 °Cat rate 2°C/ min, N_2 flow rate 20 mL/min, air flow rate 0.5 ml/ min and start speed 2.5 mm/ min). The identification of fatty acids was performed through a comparison with the retention time of standards.

$$CN = \frac{46.3 + 5458}{SV - 0.225} \times IV$$

$$HHV = 49.43 - [0.041 (SV) + 0.015 (IV)]$$

Antioxidant activities of *S. bigelovii* seeds oil extraction: Known weight of powdered seeds of *S. bigelovii* was weighed (25 g) and 150 ml of the extracting solvent (80% methanol) was added. The extraction was carried out at 50°C for about 30 min, and the extract was filtered through cotton wool. The residue was extracted again by 100 ml of the same extracting solvent for about 5 min on a boiling water bath and left overnight in the fridge and filtered through cotton wool plug in the neck of filter funnel. The two extracts were combined and evaporated by using rotary evaporator apparatus under vacuum at 40°C until no more water can be distilled. The obtained heavy extract were weighed and stored at-80°C to be used for further studies.

Hydroxyl radical scavenging assay*:* The hydroxyl radicals (˙OH) in aqueous media were generated through the Fenton system (Strlic *et al*., 2002). The ˙OH scavenging activity of *S. bigelovii* extract was determined according to the method described by Li *et al*. (2007). An extract stock solution was prepared with DMF (1 mg/ml). Different concentrations (10, 25, 100, and 250μg mL^{-1}) of the stock solution were transferred into different test tubes. To each test tube 1 ml of safranin solution (1.14 mM) in phosphate buffer solution (PBS) (67 mM, pH 7.4), 0.5 ml of EDTA solution (0.04 M) in PBS, 0.5 ml of Fe^{2+} solution (0.04 M) in PBS, and 2 ml of H_2O_2 solution (3%) were added and adjusted the volume to 5 ml with PBS. The assay mixtures were incubated at 37°C for 30 min in a water-bath. After which, the absorbance was measured at 520 nm. The suppression ratio for ˙OH radical was calculated from the following expression:

Hydroxyl radical
scavenging assay (%) = $[(A_i - A_0)/ (A_c - A_0)] \times 100$,

where A_i is the absorbance in the presence of the tested compound, A_0 is the absorbance in the absence of the tested compound, and A_c is the absorbance in the absence of the tested compound, EDTA-Fe(II) and H_2O_2.

Nitric oxide (NO) scavenging activity: Nitric oxide was generated from spontaneous decomposition of sodium nitroprusside (20 mM) in phosphate buffer (pH 7.4). Once generated NO, it interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The nitric oxide scavenging activity of extract was determined as described by Shirwaikar *et al.* (2006) with a slight modification. Briefly, a stock solution of extract, was prepared to contain 1 mg/ml. Different amounts (25, 50, 100 and 250 μg mL^{-1}).of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. 0.2 ml of sodium nitroprusside (20 mM) in phosphate buffer solution (pH 7.4), and 1.8 ml of PBS solution was added and incubated at 37°C for 3 h. 1 ml of each solution was taken and diluted with 1 ml of Griess reagent [1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-anphthyl) ethylenediamine]. Similarly, a blank was prepared containing the equivalent amount of reagents (only sodium nitroprusside and vehicle), but without the extract. The absorbance of these solutions were measured at 540 nm against the corresponding blank solutions. Ascorbic acid was used as a positive control (100μg/1 ml). The percentage inhibition of nitric oxide was calculated as follows:

The NO scavenging activity (%) of *S. bigelovii* seed extract = $[(A_b - As)/Ab] \times 100$;

where A_b is the absorbance of the blank and A_s is the absorbance in the presence of sample extract or positive control.

DPPH radical scavenging activity: The antioxidant activities of extract were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Brand-Williams *et al*., 1995). A methanolic stock solution of each sample was prepared to contain 1 mg/ml. Different amounts (25, 50, 100 and 250 μg mL^{-1}) of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. Two milliliters of DPPH (0.06 M in methanol) was added to each test. A positive control (Vit. C, 100μg/1ml) was prepared in the same way as samples. Finally, a solution containing only 1 ml methanol and 2 ml of DPPH solution was prepared and used as a blank. All test tubes were incubated in a dark place at room temperature for 1 h. The spectrophotometer was set at 517 nm and the absorbance was adjusted at zero for methanol. The absorbance of blank, positive control, and samples were recorded. The disappearance of DPPH was recorded and the percent inhibition of the DPPH radical by samples and positive control, was calculated as follows:

% Inhibition (or % radical scavenging activity) = $[(A_b - A_s)/A_b] \times 100$;

where A_b is the absorbance of blank (has the highest value) and A_s is the absorbance of sample or positive control (Vit. C).

Statistical analysis: All data are represented as the mean ± SE of at least three independent experiments. Statistical analyses were performed using SAS statistical software (SAS Institute, Cray, NC, USA) using one-way analysis of variance. p<0.05 was considered statistically significant.

Results

Seed oil from *S. bigelovii* yielded 29% of total lipids, which is the highest value in comparison with the other reported seeds of *Salicornia brachiata*, *Salicornia fruticosa* and *Salicornia europaea*, containing 22.4, 26.4 and 27.8 respectively. Table 1 presents physical and physico-chemical properties of *Salicornia bigelovii* seeds oil in comparison with other edible oils. These comparison showed that unsaturation (measure of iodine value) of *S. bigelovii* was significantly very high compared with *S. brachiata* seed oil and slightly higher than the iodine value in *S. fruticosa*. The saponification value of *S. bigelovii*, which is an indication of average molecular weight of fat, was the lowest (189.3) compared with the corresponding values for *Salicornia brachiata*, *Salicornia fruticosa* and *Salicornia europaea* (547.5, 195.6 and 197.3). The low content of saponins in the seeds of *S. bigelovii* makes the oil likely for edible purposes especially the total percentage of oil in seeds is 29% (Table 1).

The experimental results showed that there were five components of fatty acid prominent in the oil of *Salicornia bigelovi* L. seed by GC. They were 72.5% linoleic-ω6 acid (18:2), 13.3 wt.% oleic acid (18:1), 7.40 wt.% palmitic acid (16:0), 2.4wt.% stearic acid (18:0) and 2.3 wt.% linolenic-ω3 acid (18:3) as shown in Fig. (1).

In the present study three methods were used to assess the antioxidant activity of the extracted seed oil of *S.*

bigelovii. Therefore three methods were followed to evaluate their ability to scavenge free radicals. As shown in Fig. 2, the extracted seed oil of *S. bigelovii* exhibited a significant antioxidant capabilities in a concentration-dependent manner. These antioxidant activities were expressed by hydroxyl radical scavenging assay, nitric oxide scavenging activity and radical scavenging capacity on DDPH free radical. A progressive increment of the antioxidant activities of the extracted seed oil of *S. bigelovii* was observed in a concentrations dependent manner. The maximum antioxidant capabilities were observed at a concentration of 250 µg/ml (p<0.05). Regarding the hydroxyl radical scavenging activities of seed oil from *S. bigelovii*, they were noticed to be low to intermediate and increased significantly in a dose-dependent manner (Fig. 2). According to the present results, the seed oil from *S. bigelovii* showed a high ability to scavenge NO at their tested higher concentrations (Fig. 2), and may be of considerable interest in preventing the negative effects of excessive NO generation in the human body.The new findings showed that most tested concentrations of the seed oil had lower ability than Vit. C to scavenge nitric oxide from the reaction media. However, the higher concentrations (100 and 250 µg mL^{-1}) of the extract had significantly greater ability of NO scavenging activity. The present work has shown that the extracted oil from seeds of *S. bigelovii* exhibited a marked DPPH scavenging activity (Fig. 2). As a result, the new findings showed that the increase of oil concentration caused a significant decrease in the concentration of DPPH due to the free radical scavenging effect of seed oil of *S. bigelovii*. Since the hydrogen donating of the tested extract was comparable to Vit. C, it was evident that the extract could serve as hydrogen donors and consequently terminating the radical chain reaction.

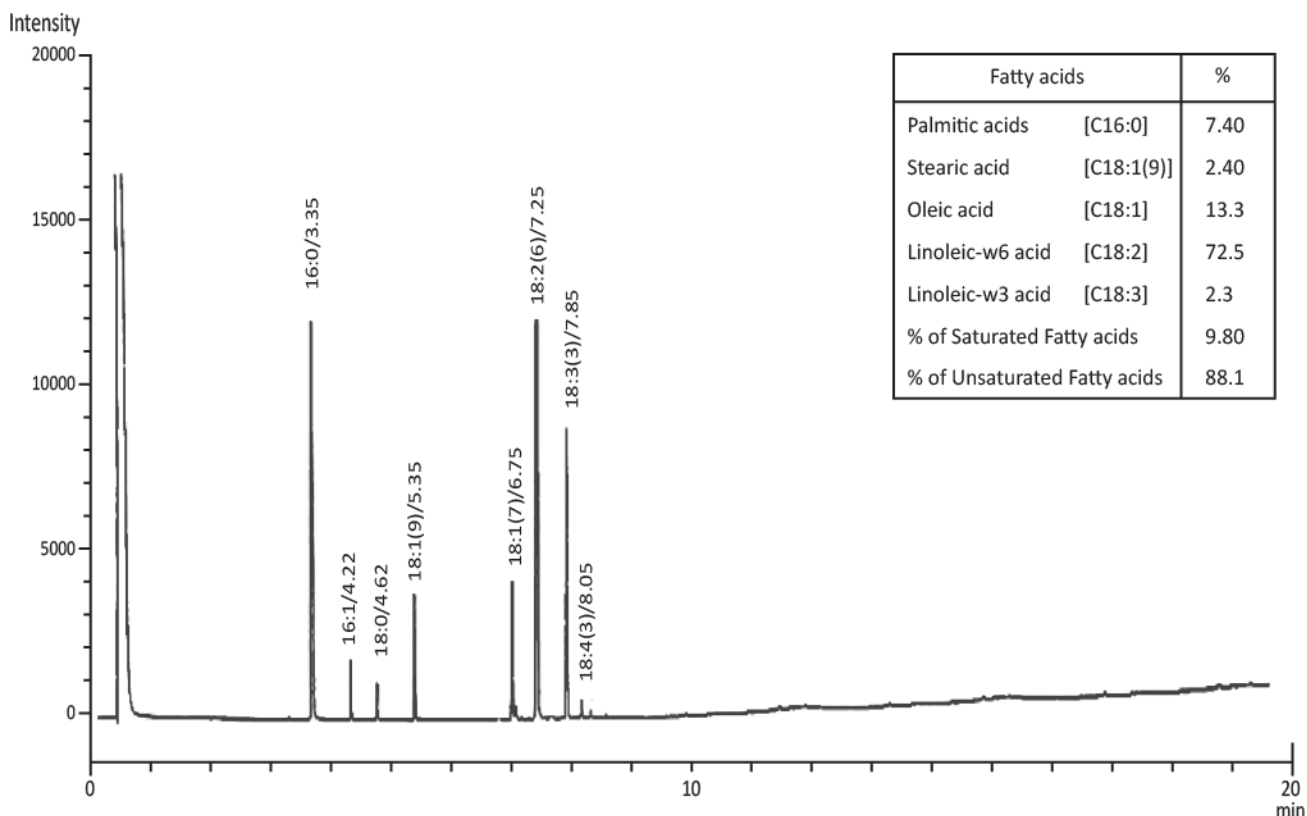| Fatty acids		%
Palmitic acids	[C16:0]	7.40
Stearic acid	[C18:1(9)]	2.40
Oleic acid	[C18:1]	13.3
Linoleic-w6 acid	[C18:2]	72.5
Linoleic-w3 acid	[C18:3]	2.3
% of Saturated Fatty acids		9.80
% of Unsaturated Fatty acids		88.1

Fig. 1. Gas chromatography (GC) of seed oil from *S. bigelovii* collected from Al Jubail, Eastern Province, Saudi Arabia. Data are presented as the mean ± SE (n = 3).

Table 1. Physico-chemical properties from *Salicornia bigelovii* seed oil in comparison with other *Salicorina* species.

| Properties	*Salicornia bigelovii*	*Salicornia brachiata* Eganathan *et al*., 2006	*Salicornia fruticosa* Elsebaie *et al*., 2013	*Salicornia europaea* LIU *et al*., 2005
Oil content (%)	29	22.4	26.4	27.8
Iodine value (mg I_2 g^{-1})	86.3 ± 2.45	19.01 ± 0.57	84.5 ± 2.77	85.4 ± 2.81
Acid value (mg KOH g^{-1})	1.76 ± 0.05	9.2 ± 0.38	1.84 ± 0.09	1.79 ± 0.06
Saponification value (mg KOH g^{-1})	189.3 ± 1.22	547.5 ± 2.82	195.6 ± 1.33	197.3 ± 1.39
Cetane number (CN)	51.24 ± 0.66	----	----	----
Higher heating value (HHV)	40.37 ± 0.54	-----	-----	-----

Minor components not included in table
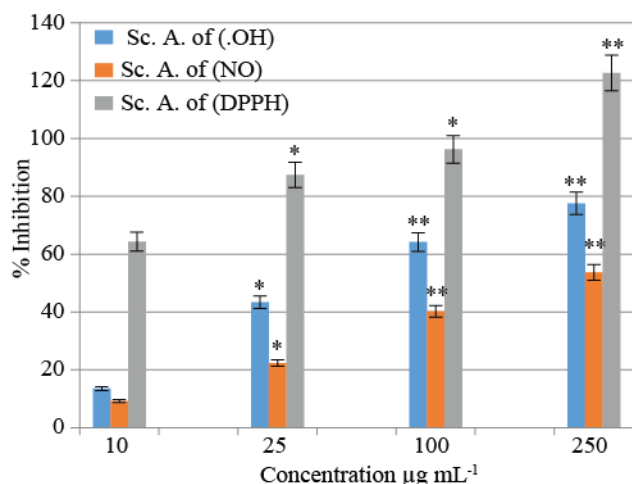
Fig. 2. Antioxidant capabilities [Sc.A. of (·OH), hydroxyl radical scavenging assay; Sc.A. of (NO), nitric oxide (NO) scavenging activity and Sc.A. of (DPPH), radical scavenging effects of the extract on DPPH free radical] of seed oil from *S. bigelovii* collected from Al Jubail, Eastern Province, Saudi Arabia. Data are presented as the mean $\pm$ SE (n = 3). *Significantly correlated (p≤0.05).

Discussion

Iodine value gives a reasonable quantification of unsaturation if the double bonds are not conjugated with each other or with carbonyl oxygen (Allen, 1955). According to our results, the extracted oil was rich in polyunsaturated fatty acids, principally oleic and linoleic acid, which has therapeutic importance and, additional precisely, the oil components include a small quantity of C18:3 linolenic-ω3, which might resulted in a better oil stability than marketable oils (El-mallah *et al*., 1994). For example, soyabean oil comprises up to 6.8% of linolenic-ω3, and it is less stable due to fast oxidation in comparison with *Salicornia bigelovii* seed oil, which has only 2.3% of this fatty acid. Our results are consistent with the results of (El-mallah *et al*., 1994) who analysed the seed oil of *Salicornia* SOS-7 cultivated at the Egyptian border of Red Sea. The lower values of saturated acids remained in *Salicornia bigelovii* seed oil (9.80%), represents main measurable variation in comparison with commercial oils. Moreover, our results are consistent with those reported in another studies (Eganathan *et al*., 2006, in *Salicornia brachiate* seed oil (16.5%) and (Weber *et al*., 2007) in chenopod *Suaeda*

fruticosa (17.0%). Concentrations of palmitic acid (21.8-29.4%) were found in seeds of salt fat and coastal dune halophytes (*Arthrocnemum macrostachyum*, *Haloxylon stocksii*, *Alhagi maurorum*, *Cressa cretica* and *Halopyrum mucronatum*) from Asia (Weber *et al*., 2007). The seed is rich in oil (30%) and protein (35%) with a high content of polyunsaturated fatty acids(88.1%). In addition to its value in human diet, the oil can be used for the production of biodiesel (Anwar *et al*., 2002). It hence appears to be a potentially valuable new biodiesel seed crop for the costal deserts; however the cost of the production may increase due to the need of annual planting. Our results are consistent with the results obtained by Martinez-Garcia, 2010, who reported that *S. bigellovii* could be considered as a potential food and biofuel crop for integrated aquaculture-agriculture systems. Its saponification and iodine values are quite close to that of semi dry oils such as corn, cotton seed and sunflower oils. The above result are in accordance with Glenn *et al*., 1998, who observed high saponification value in *S. bigellovii*. Another studies compatible with our findings (Anwar *et al*., 2002; Eganathan *et al*., 2006 and Hongshan *et al*., 2010).

The properties of biodiesel depend on the type of oil used for the trans-esterification process. Cetane number is one of the most significant properties to specify the ignition quality of any fuel. The cetane number of biodiesel fuels is considerably influenced by their fatty acid methyl ester composition (Gopinath *et al*., 2010). The cetane number is one of the best important properties to identify the ignition value of any fuel. The cetane number of esters of vegetable oils (Biodiesel) is greater than those of both vegetable oils diesel fuel (Marvin, 1999). As indicator of ignition quality, the cetane number is a prime indicator of fuel quality in the realm of diesel engines. It is conceptually similar to the octane number used for gasoline. Generally, a compound that has a high octane number tends to have a low cetane number and vice versa. The cetane number of a fuel is related to the ignition delay time, i.e., the time that passes between injection of the fuel into the cylinder and onset of ignition. The shorter the ignition delay time, the higher the cetane number and vice versa. Standards have been established worldwide for cetane number determination, for example ASTM D613 in the United States. This study showed that all seed oil extracted from *S. bigellovii* had lower ·OH radical scavenging activity than the positive control, but the highest concentrations of the seed oil

showed significantly higher ·OH radical scavenging activity. The ·OH is an extremely reactive free radical formed in biological systems, which may lead to serious damage, such as damaging the biomolecules of living cells. The ·OH has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this radical species is thought to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Bloknina *et al.*, 2003). This study, therefore, confirmed that seed oil from *S. bigelovii* was active scavengers of hydroxyl radicals.

Higher concentrations of seed oil extracted from *S. bigellovii* collected from Al Jubail, Eastern Province, Saudi Arabia had significant great ability of ·NO scavenging activity. There are two possible pathways for the formation of phenoxyls from the reaction of ·NO radical, the first mechanism, involves H-atom abstraction to produce HNO and phenoxyl radicals. The second mechanism, is also possible in which phenols reduce ·NO by single electron transfer to produce the phenol radical cation, with subsequent loss of a proton to form phenoxyl radical. Reaction of ·NO with phenolic groups may prevent accumulation of ·NO in the reaction system (Moncada and Higgs, 2006). In the present study, the extracts might be involved in competition with oxygen to react with nitric oxide and thus inhibit generation of the mentioned anions. As a result, the present data suggest that the seed oil of *S. bigellovii* might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO.

According to our results in the present work, the seed oil extracted from *S. bigellovii* exhibited a marked DPPH scavenging activity (Fig. 2). As a result, the new findings showed that the increase of seed oil concentration resulted in a highly significant decrease in the concentration of DPPH due to the free radical scavenging effect of the oil. Since the hydrogen donating of the tested concentration was comparable to Vitamin. C, it was obvious that the seed oil could serve as hydrogen donors, and subsequently ending the radical chain reaction. This means that the new data are indicative of the hydrogen donating ability of the *S. bigellovii* seed oil antioxidants, such as phenolic and polyphenolic compounds. They can be clarified on the bases of other studies (Conforti *et al.*, 2005) which relate the hydrogen donating ability using DPPH method to the presence of phenolic and polyphenolic compounds. In the presence of hydrogen donors, DPPH· is oxidized and a stable free radical is formed from the scavenger.

Conclusion

Cultivating halophytes in saline lands appear as better feedstock for biofuel production Seeds of *S. bigelovii* has the advantage of being sustainable feedstock for biofuel production. However, considering the energy demand and the quantity of biomass likely to be available for conversion.

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