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# Characterization, stability, and antioxidant activity of *Salicornia herbaciea* seed oil

**Dubok Choi*, Geum-Sook Lim**, Yu Lan Piao***, On-You Choi****,*****, Ki-An Cho****,*****, Choon-Bae Park******, Young-Cheol Chang*******, Young-Il Song********, Myung-Koo Lee*, and Hoon Cho***,†**

*Department of Pharmacy, College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
**Interdisciplinary Program of Perfume and Cosmetics, Chonnam National University Graduate School, Gwangju 500-757, Korea
***Department of Biochemical Polymer Science & Engineering, Chosun University, Gwangju 501-759, Korea
****Biochem Lab., Shinchon E&C R&D, Gwangju 500-010, Korea
*****Department of Medicine Management, Chodang University, Jeonnam 534-800, Korea
******Department of Information & Communication Engineering, Wonkwang University, Iksan 570-749 Korea
*******Biosystem Course, Division of Applied Sciences, Muroran Institute of Technology, 27-1 Mizumoto, Muroran 050-8585, Japan
********Leaders in Industry University Cooperation (LINC), Chosun University, Gwangju 501-759, Korea
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**Abstract**−We investigated the physicochemical properties, chemical composition, stability and antioxidant activity from seed oil of *Salicornia herbaciea* grown in Korea. The density, refractive index, acid value, peroxide value, iodine value, saponification value, and unsaponifiable matter of oil were 0.91 mg/mL, 1.48 at 20 °C, 1.89 mg KOH/g oil, 10.20 mEq/kg oil, 1.08 g I/g oil, 216.21 mg KOH/g oil, and 2.60%, respectively. The major fatty acids were linoleic acid (43.73%), oleic acid (19.81%), arachidic acid (13.52%), and palmitic acid (11.84%), respectively. The oil contained high levels of $\alpha$-tocopherol (249.2 mg/kg oil), followed by $\delta$-tocopherol (89.3 mg/kg), and $\gamma$-tocopherol (75.6 mg/kg oil). The oil was found to have high levels of $\beta$-sitosterol (94.5 mg/kg oil) and stigmasterol (65.7 mg/kg oil), respectively. The total phenol, chlorophyll and $\beta$-carotene content of oil was 15.2, 94.5, and 8.2 mg/kg oil, respectively. The oil had good oxidative stability during 60 days of storage in a dark area at 50 °C. The maximum degradation rates of the oil were observed at 242.3 °C (9.5%/min), 382.6 °C (5.2%/min), and 440.7 °C (1.3%/min), respectively, where the rate of the weight decrease increased to a maximum up to this point. The ABTS radical scavenging activity of the oil was increased from 50.2 to 71.8% when the oil concentration extracted by methanol was increased from 100 to 300 μg/mL. This study suggests that *S. herbaciea* seed oil has potential use in functional foods, cosmetics or pharmaceuticals.

Keywords: *Salicornia herbaciea*, Seed Oil, Fatty Acids, Stability, Antioxidant Activity

## INTRODUCTION

Vegetable oils are important not only for nutritional purposes, but also as raw materials for a wide range of industrial products, which include fuels, skin care products, pharmaceuticals, high pressure lubricants and alkyd resins for paints [1]. Because the constituents of non-traditional vegetable oils have unique chemical properties, they are important and might augment other edible oil sources. Some species of newer sources of edible oils are important because they can be used for both health benefits and the production of formulations because they contain phytochemicals with significant biological activities [2].

*Salicornia* plants are wild and annual halophytes growing in the salt marshes or the vicinity of coastal areas on Asia, North America, and Middle East [3]. The Salicornioideae family comprises approximately 15 genera and 80 species. The commonly found *Salicornia* species are *S. ramosissima*, *S.indica*, *S. brachiata*, *S. bigelovii*, *S. herbacea*, *S. perenis*, *S. fragilis*, *S. nitens*, *S. pusilla*, and *S. disarticulate* [4]. *Salicornia* species are rich in dietary fiber and many bioactive substances, such as phytosterols, polysaccharides and phenolic compounds mainly flavonoids and phenolic acids [5,6]. In particular, *S. herbacea* is also known as "Tungtungmadi" in Korea and is distributed in tidelands on the southern and western coast of Korea. This species grows approximately 10-40 cm high and its stem appears deep green that changes to red in the fall [5]. It has been used as a seasoned vegetable and a folk medicine to treat a range of diseases, such as hypertension, diabetes, hemorrhoids and cancer [6] *S. herbacea* was reported to be a nutrient-rich dietary source containing a large number and concentration of minerals, particularly magnesium, calcium, and potassium, as well as essential fatty acids and dietary fibers [7]. Some studies have suggested that extracts of *S. herbacea* inhibited the tyrosinase activity [8], scavenged free radicals, and showed skin whitening effects [9]. The growth inhibitory and immunomodulatory effects of *S. herbacea* have been reported from experiments with cultured cancer cells and macrophages [10]. *S. herbacea* also showed preventive effects on diabetes, arteriosclerosis, hyperlipidemia and fatty liver *in vivo* [11]. Recently, *Salicornia* plants have emerged as a potential candidate for seawa-

†To whom correspondence should be addressed.
E-mail: hcho@chosun.ac.kr

ter crops, because the seeds are available for oil production from *Salicornia* seeds and the seed meal can be eaten by some animals and fishes for livestock. *Salicornia* seeds are round or ellipsoid with green and opaque colors [12]. Glenn et al reported that *S. bigelovii* is an important halophyte used as a conventional oilseed crop for direct seawater irrigation in coastal areas of arid regions. It was shown to be a promising species because it colonizes through the prolific production of seed composed of 30% oil (similar to safflower oil), 35% protein and less than 3% salt. The seeds produce oil that is high in polyunsaturated fat [13]. Eganathan et al. also reported the oil concentration and its physical and physio-chemical parameters in the seed oil of *S. brachiata*, which used as a vegetable by mangrove-dependant village people [14]. Previously, we reported the manufacture method from *Salicornia* seed oil and the manufacture method of biodiesel using its oil [14,15]. Although many studies have examined the seed of *Salicornia* species, the characterization, stability and antioxidant activity of seed oil of *S. herbaciea* grown in Korea have not been reported.

In this study, we examined the physicochemical properties, chemical composition, stability and antioxidant activity from seed oil of *S. herbaciea* grown in Korea.

## MATERIAL AND METHODS

### 1. Sample Preparation

*Salicornia herbacea* seed grown in Henam coastal area at Jeonnam, South Korea in 2011 were obtained. The sample was dried naturally in a cool, windy and dry location. The seed was ground finely in a grinder and subsequently garbled to a 0.05-0.1 mm particle size. The oil was extracted from the seed by using 30 g of seed and 300 mL of hexane at room temperature, followed by solvent removal at 50 °C with a rotary evaporator.

For oil extraction by methanol from the oil extracted by hexane, 10 g of oil was dissolved in 10 mL of hexane. The hexane layer was extracted sequentially with 20 mL of methanol (70%). The methanol extract was pooled and evaporated in a rotary evaporator to remove the solvent. All the oils were prepared in duplicate, and the oils were analyzed for their antioxidant activity.

### 2. Proximate Composition and Physicochemical Properties

Proximate composition of seed and physicochemical properties of the oil were determined by the official and tentative methods of the American Oil Chemists Society [16].

### 3. Fatty Acid Composition

Oil (15 mg) was methylated with 3 mL of HCl in methanol at 95 °C for 1 hr. The fatty acid methyl esters (FAME) were extracted with 2 mL of hexane and dried over sodium sulfate. One microliter of the FAME was analyzed by gas chromatography (GC) equipped with a flame ionization detector, and an AOC-20i automatic injector. A SP-2560 capillary column was used. The oven temperature was programmed as follows: 120 °C for 5 min, increased to 240 °C at 4 °C/min, and held at that temperature for 25 min. The injector and detector temperatures were 260 °C. The carrier gas was helium, the flow rate was 30 mL/min, and the split ratio was 1/50.

### 4. Tocopherol Composition

The tocopherols were analyzed by high performance liquid chromatography (HPLC). Oil (100 mg) and ascorbic acid (50 mg) were placed in a test tube. 5 mL of ethanol (90.2%) and 0.5 mL of KOH (80%) were added to the test tube and vortexed for 30 sec. The test tube was flushed with nitrogen, capped, and incubated in a water bath for 30 min at 70 °C with periodical vortexing. The tubes were placed in an ice bath for 5 min, and 3 mL of deionized water and 5 mL of hexane were then added and vortexed for 30 sec followed by centrifugation for 10 min at 1,0000 g at room temperature. The upper hexane layer was transferred to another test tube. The aqueous layer and residue were re-extracted by repeating the same procedure. The upper hexane layers from both extractions were pooled and evaporated to dryness under a nitrogen stream. One milliliter of mobile phase was added to the tube and vortexed for 30 sec to re-dissolve the extract, and then transferred to an HPLC sample vial. A sample was injected into the LC-Si column. The chromatographic separation was performed by isocratic elution of the mobile phase constituting of ethyl acetate/acetic acid/hexane (1/1/98, v/v/v) at a flow rate of 1.5 mL/min. Detection was monitored at 295 nm.

### 5. Sterol Composition

Oil (100 mg) was placed in a test tube with 2 mL of KOH (2 M) in ethanol, sealed, heated at 85 °C for 1 hr, and cooled in cold water, and 2 mL of distilled water and 5 mL of hexane were added. The unsaponifiable matter was extracted three times with hexane, and the combined hexane fractions were washed three times with ethanol (10%) until the washing solution was neutral. The hexane phase was dried with an anhydrous sodium sulfate. The residue after evaporation was then dissolved in 5 mL of hexane, and 1 μL of the product was analyzed using a Hewlett-Packard (HP 6890) GC with a capillary column (25 m length×0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor). Helium was used as the carrier gas with a flow rate through the column of 1.2 mL/min. The temperature of the injector and detector was set to 280 and 290 °C, respectively, with an oven temperature of 260 °C.

### 6. Polyphenol

The polyphenol content was determined using Folin-Ciocalteu's method. A diluted sample solution (1 mg/mL, 80 μL) was incubated with 100 μL of $Na_2CO_3$ (2%) at room temperature for 5 min. The mixture was then added to 20 μL of Folin-Ciocalteu's reagent (50%). After 30 min, the absorbance at 750 nm was analyzed using a microplate reader (Spectra Max 250; Molecular Device, Sunnyvale, CA, USA).

### 7. Chlorophyll and Carotenoid

Chlorophyll and carotenoid compounds were determined at 670 and 470 nm, respectively, in cyclohexane using the specific extinction values. Chlorophyll (mg/kg oil)=$(A670\times10^6)/(613\times100\times d)$. Carotenoid (mg/kg oil)=$(A470\times10^6)/(2000\times100\times d)$, where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

### 8. Infrared Analysis

A (Tensor 27, Bruker) spectrum Fourier transform infrared (FT-IR) spectrophotometer detector was used to collect the FT-IR spectra of the oil with a resolution of 4 $cm^{-1}$ at 64 scans. The data interval provided by the instrument for a resolution of 4 $cm^{-1}$ was 1 $cm^{-1}$. Duplicate spectra were collected for the same sample. All spectra were recorded in the range of 500-4,000 $cm^{-1}$ and processed using the computer software program, Spectrum (Tensor 27).

### 9. Thermal Analysis

Thermogravimetric curves of the oils were obtained using a model SDT 2960 simultaneous thermal analyzer. The tests were carried out using approximately 10 mg of each sample in an aluminum pan in a

synthetic air atmosphere; the flow rate was 110 mL/min, the heating rate was 10 °C/min, and the heating ramp ranged from 50 to 800 °C.

**10. ABTS Radical Scavenging Activity**

The experiment was conducted using ABTS decolorization assay, which involves the generation of an $ABTS^+$ chromophore by the oxidation of ABTS with potassium persulfate. The ABTS radical cation was generated by adding a 7 mM stock solution of ABTS and 2.45 mM potassium persulfate together in 10 mL of distilled water, and was left to stand overnight in the dark at room temperature. The absorbance was maintained in the 0.65-0.75 range with ethanol prior to the assay. Several concentrations of oils were prepared and added to 800 μL of an ABTS solution. The solutions were left to stand for 8 min. A control and blank were also performed simultaneously. The experiments were carried out in triplicate, and the mean values were used for further analysis. The absorbance was read at 734 nm using a multiplate reader. The $ABTS^+$ scavenging capacity of oil was compared with that of trolox, which was used as a standard. A standard curve was prepared by measuring the decrease in absorbance of the $ABTS^+$ solution at different concentrations of trolox and samples over a period of 7 min. The trolox equivalent antioxidant capacity (TEAC) of oil represents the concentration of trolox solution with the same antioxidant capacity. The TEAC values were determined as follows:

$$\Delta A_{trolox} = (A_{t=0\ min\ trolox} - A_{t=7\ min\ trolox}) - \Delta A_{solvent\ (0\text{-}6\ min)}$$

$$\Delta A_{trolox} = m\ [Trolox],$$

$$TEAC_{oil} = (\Delta A_{oil}/m) \cdot d$$

where ΔA is the reduction of absorbance, A is the absorbance at a given time, m is the slope of the standard curve, [Trolox] is the concentration of trolox and d is the dilution factor.

**11. Statistical Analyses**

The experimental data was evaluated by analysis of variance (One-way ANOVA) and the significant differences among the means of the three replicates ($P<0.05$) were determined using Duncan's multiple range test on SPSS 9.0 for Windows.

## RESULTS AND DISCUSSION

**1. Proximate Chemical Analysis of *S. herbaciea* Seed, Stem, and Root**

To investigate the proximate composition of *S. herbaciea* seed, stem, and root, moisture, oil, protein, ash, and carbohydrate contents were measured. The proximate compositions of *S. herbaciea* seed, body, and root are shown in Table 1. The moisture of *S. herbaciea* seed was similar to those of its stem and root. The oil contents of *S. herbaciea* seed, stem, and root were 29.4, 2.0, and 1.8%, respectively. Anwar et al. reported that oil content ranged from 27.2 to 32.0% of *S. bigelovii* seeds from five coastal areas of Pakistan on the Arabian Sea, and oil concentration was high in those seeds harvested from extreme coastal environments and where the irrigation water from seawater wells was supplemented with nitrogen fertilizers [17]. Eganathan et al. investigated solvent systems using petroleum ether and hexane for oil extraction from *S. brachiate* seed. About 10.5% of oil yield was obtained when the seed was extracted with petroleum ether, whereas hexane was used, the maximum yield of oil was obtained, 22.4% [3]. The maximum oil content of *S. europaea* seed was about 28% [18]. In the case of seeds of the South American glasswort, *Sarcocornia ambigua*, the maximum oil content was about 13.0% [19]. Weber et al. analyzed oil content from various halophytes seeds such as *Arthrocnemum indicum*, *Alhaji maurorum*, *Cressa cretica*, *Halopyrum mucronatum*, *Haloxylon stocksii* and *Suaeda fruticosa* obtained from a salt marsh located at Manora creek near Sandspit at Karachi, Pakistan to determine their potential as a source of edible oil and the oil content was varied from 22 to 25% [20]. The oil content of *S. herbaciea* exceeds those of conventional oilseed crops, cotton (15.0-22.0%) and soybean (17-21%) grown in the United States, Brazil, and Asia [12], but is slightly lower than that of safflower seeds (31.9-36.7%) [17]. The protein content of *S. herbaciea* seed, stem, and root was 23.1, 9.8, and 14.9%, respectively. *S. herbaciea* seed was found to be a rich source of protein, which could be added to chicken diets as a source of calories and protein and may replace soybean meal for the local poultry industry. The ash content of *S. herbaciea* seed, stem, and root ranged of 6.8 to 18.2%. In the case of carbohydrate content of *S. herbaciea* seed, stem, and root, they were 30.2, 68.3, and 53.8%, respectively.

**Table 1. Proximate composition of *S. herbaciea* seed, stem, and root**

| Components | Seed | Stem | Root |
|---|---|---|---|
| Moisture (%) | 10.5±0.73 | 10.7±0.65 | 11.3±0.51 |
| Oil (%) | 29.4±0.92 | 2.0±0.09 | 1.8±0.03 |
| Protein (%) | 23.1±1.11 | 9.8±0.67 | 14.9±0.88 |
| Ash (%) | 6.8±0.43 | 9.2±0.84 | 18.2±1.89 |
| Carbohydrate (%) | 30.2±1.00 | 68.3±3.21 | 53.8±2.10 |

**2. Physicochemical Properties of *S. herbaciea* Seed Oil**

The physicochemical properties of *S. herbaciea* seed oil were examined by measuring the density, refractive index, acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, and free fatty acid. Table 2 lists the results of the physicochemical properties. The density, refractive index, acid value, and iodine value of oil were 0.91 mg/mL, 1.48 at 20 °C, 1.89 mg KOH/g oil, and 1.08 g I/g oil, respectively. The peroxide value is a widely used measure of primary lipid oxidation, indicating the amount of peroxides formed during oil oxidation. Food lipid oxidation products, such as peroxides, free radicals involved in their formation and propagation, malonaldehyde and several cholesterol oxidation products have been reported to promote atherosclerosis and coronary heart disease [21]. The peroxide value was 10.2 mEq/kg oil, which was lower than that of *S. brachiate* seed oil cultivated in India (46.9 mEq/kg oil) [12]. A high saponification value indicated that oils are normal triglycerides and quite useful in the production of liquid soap and shampoo industries. The saponification value of *S.*

**Table 2. Physicochemical properties of *S. herbaciea* seed oil**

| Physicochemical parameters | Result |
|---|---|
| Density (mg/mL) | 0.91±0.01 |
| Refractive index (20 °C) | 1.48±0.10 |
| Acid value (mg KOH/g oil) | 1.89±0.13 |
| Peroxide value (mEq/kg oil) | 10.20±0.51 |
| Iodine value (g I/g oil) | 1.08±0.06 |
| Saponification value (mg KOH/g oil) | 216.21±8.93 |
| Unsaponifiable matter (%) | 2.60±0.16 |
| Free fatty acid (%) | 0.96±0.03 |

*herbaciea* seed oil was 216.21 mg KOH/g oil, which was lower than that of *S. brachiate* seed oil cultivated in India (547.5 mg KOH/g oil). On the other hand, it was higher than those of *S. bigelovii* seed oil (183.7 mg KOH/g oil)) cultivated in Pakistan and safflower oil (189.0 mg KOH/g oil). This suggests that *S. herbaciea* seed oil could be good for soap or shampoo production and the manufacture of lather shaving creams. The unsaponifiable matter of oil is a small portion of oil that is extracted with an organic solvent after the oil is saponified by the alkali. These minor substances of the oil contained in unsaponifiable matter have antioxidant and other health benefits in animals and humans, and are useful for softening the skin and cosmetics because effective on dry and damaged skin [22]. The unsaponifiable matter of seed oil includes tocopherols, sterols, triterpenic alcohols, hydrocarbons, aliphatic alcohols, and waxes [23]. The unsaponifiable matter of *S. herbaciea* seed oil was 2.6%, which was higher than that of soybean oil (1.6%), sunflower seed oil (1.6%), *S. bigelovii* seed oil (1.78%), and safflower seed oil (1.4%). The formation of free fatty acids might be an important measure of the rancidity of foods. The free fatty acid content, resulting from the hydrolysis of triacylglycerides and the further decomposition of hydroperoxides, is one of the most important indicators of oil deterioration during heating. Free fatty acid formation might also be promoted by a reaction of the oil with moisture [24]. The free fatty acid content of *S. herbaciea* seed oil was found to be 0.96%. The quantity of free fatty acids was reported to affect the formation of soap or during bio-diesel manufacture and a free fatty acid content <1% is recommended [25].

**Table 3. Fatty acid composition of *S. herbaciea* seed oil**

| Fatty acids | Content (%) |
|---|---|
| Caprylic acid (C8:0) | 0.25±0.01 |
| Capric acid (C10:0) | 0.03 |
| Lauric acid (C12:0) | 0.04 |
| Myristic acid (C14:0) | 0.13 |
| Pentadecanoic acid (C15:0) | 0.02 |
| Palmitic acid (C16:0) | 11.84±0.45 |
| Heptadecanoic acid (C17:0) | 0.14±0.02 |
| Stearic acid (C18:0) | 3.07±0.05 |
| Arachidic acid (C20:0) | 13.52±0.47 |
| Heneicosanoic acid (C21:0) | 0.89±0.05 |
| Behenic acid (C22:0) | 2.52±0.13 |
| Tricosanonic acid (23:0) | 0.03 |
| Lignoceric acid (C24:0) | 1.53±0.09 |
| Palmitoleic acid (C16:1) | 0.01 |
| cis-10-Heptadecenoic acid (C17:1) | 0.01 |
| Elaidic acid (C18:1n9t) | 0.02 |
| Oleic acid (C18:1n9c) | 19.81±0.86 |
| cis-11-Eicosenoic acid (C20:1) | 0.32±0.04 |
| Erucic acid (C22:1n9) | 0.04 |
| Nervonic acid (C24:1) | 0.22±0.01 |
| Linolelaidic acid (C18:2n6t) | 0.18±0.05 |
| Linoleic acid (C18:2n6c) | 43.73±1.23 |
| cis-11,14-Eicosadienoic acid (C20:2) | 0.18 |
| cis-13,16-Docosadienoic acid (C22:2) | 0.55±0.01 |
| Linolenic acid (C18:3n3) | 0.35±0.02 |
| cis-11,14,17-Eicosatienoic acid (C20:3n3) | 0.23±0.01 |
| Docosahexaenoic acid (C22:6n3) | 0.12 |
| Eicosapentaenoic acid (C20:5n3) | 0.22±0.01 |

### 3. Fatty Acid Composition of *S. herbaciea* Seed Oil

Table 3 lists the fatty acid composition of *S. herbaciea* seed oil. The main fatty acids of *S. herbaciea* seed oil were linoleic acid, oleic acid, arachidic acid, and palmitic acid, which comprised approximately 88.9% of the total fatty acids. Linoleic acid is indispensable for the healthy growth of human skin. It can be transformed by the organism into series of long fatty acids chains, which are the precursors of eicosanoids [26]. Dietary fat rich in linoleic acid, apart from preventing cardiovascular disorders, such as coronary heart diseases and atherosclerosis, also prevents high blood pressure [27]. Among the many fatty acids, the linoleic acid content was the highest, 43.73%, which was approximately 96% of polyunsaturated fatty acid. This linoleic acid content was higher than that of groundnut oil ( 29.85%), am brette seed oil (39.4%), cotton seed oil (29%), cuphea viscosissima seed oil (2-5%), diplotychea painter seed oil (4%), dukudu seed oil (39%), fig seed oil (30-35%), *heteranthus epilobiifolia* seed oil (5%), kapok seed oil (7-35%), lemon seed oil (2-5%), mango seed oil (3-6.45%), palm oil (5-21%), rapeseed oil (11-30%), and *S. branthta* seed oil (25.6%) [23]. The EPA and DHA content of *S. herbaciea* oil was 0.12 and 0.22%, respectively, which was higher than that of other vegetable oils. Among the monounsaturated fatty acids, the oleic acid content was the second highest, 19.81%, comprising approximately 97% of monounsaturated fatty acid content. On the other hand, the content of other monounsaturated fatty acids was below 0.5%. The oleic acid content was higher than that of acacia minhassai seed oil (14-17%), *Salicornia bigelovii* (11.3-16.83%), coconut oil (5-10%), niger seed oil (4-10%), and parsley seed oil (12-15%) [23]. The total unsaturated fatty acid content was 65.99%, in which the monounsaturated and polyunsaturated fatty acids comprised 20.43 and 45.56% of the total fatty acids, respectively. The total unsaturated fatty acid content of *S. herbaciea* seed oil was higher than that of palm oil (48.5-53.7%) and coconut oil (6.0-10.4%) [23]. The total saturated fatty acid of *S. herbaciea* oil is 34.01%, which makes it strongly resistant to oxidative rancidity. Moreover, various reports suggested the preventive effects of lauric and myristic acids on the development of prostatic hyperplasia because of their reductase inhibitory activity [29,30]. Among the saturated fatty acids of *S. herbaciea* seed oil, arachidic acid and palmitic acid were the highest, 13.52 and 11.84%, respectively, which was approximately 39.81 and 34.8% of saturated fatty acid. The arachidic acid content was approximately 10 times higher than that of peanut oil [23]. The stearic acid and behenic acid content was 3.07 and 2.52%, respectively. The lauric acid and myristic acid levels were 0.04 and 0.13%, respectively.

The polyunsaturated fatty acid (PUFA)/saturated fatty acid (SFA) ratio is generally used to evaluate the nutritional value of oil. Chang and Huang [31] examined the effects of monounsaturated fatty acids (MUFA) and the ratio of the sum of polyunsaturated and monounsaturated fatty acids to saturated fatty acids (PUFA+MUFA/SFA) on the plasma and liver lipid concentrations in rats. They concluded that the prerequisites for maintaining a low plasma and liver lipid concentration are the low MUFA/SFA ratio and high PUFA/MUFA ratio, and the PUFA+MUFA/SFA ratio did not exceed 2. In

**Table 4. Tocopherol composition of *S. herbaciea* seed oil**

| Tocopherols | Content (mg/kg oil) |
|---|---|
| $\alpha$ | 249.2±3.25 |
| $\beta$ | - |
| $\gamma$ | 75.6±2.62 |
| $\delta$ | 89.3±3.01 |

**Table 5. Sterol composition of *S. herbaciea* seed oil**

| Sterols | Content (mg/kg oil) |
|---|---|
| $\beta$-Sitosterol | 94.5±4.65 |
| Stigmasterol | 65.7±2.95 |
| Spinasterol | 15.8±1.23 |
| Campesterol | 15.9±1.04 |
| Cholesterol | 2.5±0.01 |

the present study, the MUFA/SFA value was 0.60, PUFA/SFA was lower (1.34) and PUFA/MUFA was quite high (2.23) and PUFA+ MUFA/SFA was 1.94 as the MUFA content was quite low in *S. herbaciea* seed oil. *S. herbaciea* seed oil was found to exhibit cholesterol and triacylglycerol lowering effects.

**4. Tocopherol, Sterol, Polyphenol, Beta-caroten, and Chlorophyll Content of *S. herbaciea* Seed Oil**

Tocopherols are the major lipid-soluble, membrane-localized antioxidants in humans that improve the stability of vegetable oil. Tocopherols in vegetable oil are believed to protect polyunsaturated fatty acids from peroxidation [28]. Table 4 lists the tocopherol content of *S. herbaciea* seed oil. The total tocopherol content of *S. herbaciea* seed oil was 309.8 mg/kg oil, which consisted of $\alpha$-tocopherol (249.2 mg/kg oil), $\gamma$-tocopherol (75.6 mg/kg oil), and $\delta$-tocopherol (89.3 mg/kg oil). $\beta$-Tocopherol, however, was not detected in the *S. herbaciea* seed oil. The $\alpha$-tocopherol content of *S. herbaciea* seed oil was higher than those of *S. bigelovii* seed oil (49 mg/kg oil), soybean oil (99 mg/kg oil), palm oil (89 mg/kg oil), grape seed oil (38 mg/kg oil), groundnut oil (178 mg/kg oil), almond Kernel oil (228 mg/kg oil), low erucic acid rapeseed oil (202 mg/kg oil), apricot Kernel oil (15 mg/kg oil), barley oil (16 mg/kg oil), basil seed oil (52 mg/kg oil), coconut oil (8 mg/kg oil), basella rubra seed oil (138 mg/kg oil), camellia oleifer seed oil (107 mg/kg oil), and brassica chinensis seed oil (140 mg/kg oil) [17,23]. The $\gamma$-tocopherol content of *S. herbaciea* seed oil was 75.6 mg/kg oil, which was higher than that of palm oil (18 mg/kg oil) and sunflower seed oil (11 mg/kg oil) [32]. The tocopherol composition and total content of the body and root oil of *S. herbaciea* were similar to those of seed oil (data not shown). The $\delta$-tocopherol content, which exhibits the strongest antioxidant potency than either $\delta$-, or $\gamma$-tocopherol, was 89.3 mg/kg oil, which was higher than those of sunflower oil (0.6 mg/kg oil), cottonseed oil (3.3 mg/kg oil), groundnut oil (7.6 mg/kg oil), and low erucic acid rapeseed oil (9 mg/kg oil), but was lower than the levels in maize oil (54 mg/kg oil) and soybean oil (421 mg/kg oil) [32].

Sterols are the key components of the unsaponifiable matter of vegetable oils and fats. This fraction, which has a complex composition, can reach more than 10% in certain plants. Sterol analysis provides rich information about the quality and identity of the investigated oil and can be considered a fingerprint [33,34]. Recently, it was proposed that these profiles could be used to classify virgin olive oils according to their fruit variety and suggested that sterols have anti-inflammatory antibacterial, antifungal, antiulcerative and antitumoral activities [35]. Sterols also help to reduce the total plasma cholesterol and LDL cholesterol levels, and as a result these compounds are being considered as ingredients in functional foods [36]. Table 5 lists the sterol content of *S. herbaciea* seed oil. The total sterol content of *S. herbaciea* seed oil was 194.4 mg/kg oil, which consisted of $\beta$-sitosterol (94.5 mg/kg oil), stigmasterol (65.7 mg/kg oil), spinasterol (15.9 mg/kg oil), campesterol (15.9 mg/kg oil), and cholesterol (2.5 mg/kg oil), respectively. In vegetable oils, the most common forms of sterols are $\beta$-sitosterol, campesterol and stigmasterol [37]. Stiti et al. [38] examined the sterolic composition of olive oil and observed that $\beta$-sitosterol was the major sterol with a percentage range from 74.8 to 88.7%, followed by D5-avenasterol (4.1-19%) and campesterol (1.9-2.9%). The $\beta$-sitosterol content of *S. herbaciea* seed oil was the major sterol, constituting 48.6% of the total sterols and the next major component was stigmasterol (2.95%). On the other hand, in the case of the *S. herbaciea* body and root oil, stigmasterol was the major sterol, comprising 48.7% of the total sterols. The next major component was $\beta$-sitosterol (18.9%) (data not shown). Cholesterol, which is specific to animal lipids, is present at low levels in most vegetable oils. The cholesterol content of *S. herbaciea* seed oil was 1.3% of the total sterols, which is relatively higher than those of olive and soybean oils (0.3%) but lower than that in palm and tomato seed oils (2.30 and 20%), respectively [27]. These results indicate that the sterols of *S. herbaciea* seed oil are most effective as antioxidants, and suggest a synergistic effect of the sterols with other antioxidants.

Table 6 shows the total polyphenol, $\beta$-carotene, and chlorophyll content of *S. herbaciea* seed oil. The polyphenol content of *S. herbaciea* seed oil was 15.2 mg caffeic acid equivalents/kg oil. This value is similar to that of cold pressed soybean oil, higher than that of sunflower, rapeseed, corn, grape seed, hemp, flax, hemp, flax, rice bran and pumpkin oils, and lower than that of rice bran and pumpkin oils [39]. A higher content of phenolic compounds is released from the seeds when the oil is extracted at higher temperatures and pressures. In the case of *S. herbaciea* seed oil, approximately 15% was increased when obtained by pressing the seeds at 100 °C for 30 min and a high pressure compared to that of the room temperature pressed oil (data not shown). Several studies have also reported that the phenolic content of maize, tree nut and commercial Spanish virgin olive oils was 250-370, 168-783 and 300-500 mg gallic acid equivalents/kg oil, respectively [40]. $\beta$-Carotene is beneficial for the long-term storage of oils because it is a secondary or preventive antioxidant acting as a singlet oxygen quencher [41]. Furthermore, $\beta$-carotene has been shown to be a precursor of retinoic acid, which enhances the gap junction intercellular communication, and increases the im-

**Table 6. Polyphenol, $\beta$-carotene, and chlorophyll content of *S. herbaciea* seed oil**

| Items | Content (mg/kg oil) |
|---|---|
| Polyphenol | 15.2±2.1 |
| $\beta$-Carotene | 94.5±4.33 |
| Chlorophyll | 8.2±1.4 |

**Table 7. Oxidative stability of *S. herbaciea* oil**

| | Storage time (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 |
| Polyphenol (mg/kg oil) | 152.18±7.23 | 99.67±4.42 | 98.91±5.10 | 84.25±2.84 | 9.12±0.28 |
| Peroxide value (mEq/kg oil) | 15.26±0.45 | 20.82±1.31 | 25.74±1.25 | 37.13±2.47 | 45.60±1.86 |
| $\alpha$-Tocopherol (mg/kg oil) | 249.25±12.31 | 156.73±7.48 | 12.20±1.16 | 0 | 0 |
| $\gamma$-Tocopherol (mg/kg oil) | 75.67±2.56 | 68.62±2.56 | 53.77±2.30 | 45.15±3.65 | 34.02±1.52 |
| $\delta$-Tocopherol (mg/kg oil) | 89.39±3.18 | 89.28±4.37 | 89.25±4.89 | 89.20±5.31 | 88.12±5.88 |

munological function [42]. The $\beta$-carotene content of *S. herbaciea* seed oil was 94.5 mg/kg oil, which was higher than those of palm seed oil (55.1 mg/kg oil) and seed oil of *Dalbergia odorifera* [21]. In the case of chlorophylls, it was 8.2 mg/kg oil.

**5. Stability of *S. herbaciea* Seed Oil**

To research the oxidative stability of *S. herbaciea* seed oil, polyphenol, peroxide value and tocopherol contents were measured during 60 days storage in a dark area at 50 ℃. Table 7 lists the results of the oxidative stability of *S. herbaciea* seed oil. The polyphenol content did not decrease after 30 days of storage. On the other hand, it started to decrease after 45 days and decreased to 9.12 mg/kg after 60 days of storage, showing an approximately 40% decrease compared to its initial value. In the case of oil extracted at high temperatures and pressures, an approximate 65.2% decrease in the polyphenol content was noted after 60 days of storage (data not shown). The peroxide value increased slowly after 30 days of storage. On the other hand, after 60 days, it increased sharply to 45.60 mEq/kg oil, which was an approximately 3.0 fold increase compared to its initial value. In the case of oil extracted at high temperatures and pressures, the level increased after 60 days of storage. The peroxide value also increased when stored at high temperatures (data not shown). Okogeri and Tasioula-Margari [43] reported that during the storage of virgin olive oil under diffused light at temperatures between 6 ℃ and 18 ℃, an almost 60% decrease in the total phenol content was observed after six months, indicating rapid degradation. At the same time, the peroxide value increased from 3.6 to 17 mmol $O_2$/kg. In the same study, after 12 months storage in the dark, a slower rate of reduction was observed (a decrease of 50% of total phenols) with an increase in the peroxide value to 13 mmol $O_2$/kg. Tsimidou et al. [44] reported that the loss of phenolic compounds in virgin olive oil stored in closed bottles in the dark at 20 ℃ was observed in parallel with an increase in the peroxide level. Morello et al. reported a significant decrease in the phenol content of virgin olive oil after 12 months storage in the dark at room temperature with the subsequent loss of oxidative stability [45]. The total tocopherol content of *S. herbaciea* seed oil decreased with increasing storage time, and after 60 days storage the amount measured was 123.3 mg/kg. Specifically, the $\gamma$-tocopherol content of *S. herbaciea* seed oil decreased to 34.02 mg/kg after 60 days storage, indicating an approximate 34.02% decrease compared to its initial value. The $\alpha$-tocopherol content decreased sharply from 249.2 to 12.2 mg/kg oil when the storage time was increased to 45 days. After 45 days storage, it had decomposed completely. The $\delta$-tocopherol content was unchanged during storage. The lower stability of $\alpha$-tocopherol compared to $\gamma$-tocopherol was attributed to $\alpha$-tocopherol reacting faster with peroxy radicals formed in the autooxidation process [46]. This suggests that $\alpha$-tocopherol is consumed first, followed by $\beta$-tocophenol and $\gamma$-tocopherol, and finally $\delta$-tocopherol, which is more stable and consumed more slowly. Koski et al. [47] reported that the content of $\alpha$-tocopherol of rapeseed oil decreased from 200 mg/kg in fresh oil to zero within 7-11 days of storage at 60 ℃ in the dark, whereas 5-10% of the initial value of approximately 600 mg/kg $\gamma$-tocopherol was still present after two weeks storage. Morello et al. [48] reported that $\alpha$-tocopherol was totally absent in olive oil after 12 months storage at room temperature in the dark. At lower temperatures, a slower rate of reduction of $\alpha$-tocopherol in virgin olive oil was observed. In particular, the $\alpha$-tocopherol content decreased to 40% after 12 months storage in the dark between 6 ℃ and 18 ℃.

In nature, fatty acids exist as free or esterified substances. Oils are formed by triglycerides, which are the most frequent lipids in nature. Glycerol and three fatty acids constitute triacylglicerides. Many medicines and foods contain fatty acids and often are subjected to thermal treatment during processing or storage. Therefore, knowing the thermal stability profile of the oils is very important in industry. The thermal stability of oils depends on their chemical structure. Fig. 1 shows TG and DTG curves of *S. herbaciea* seed oil and soybean oil. The thermal decomposition of *S. herbaciea* seed

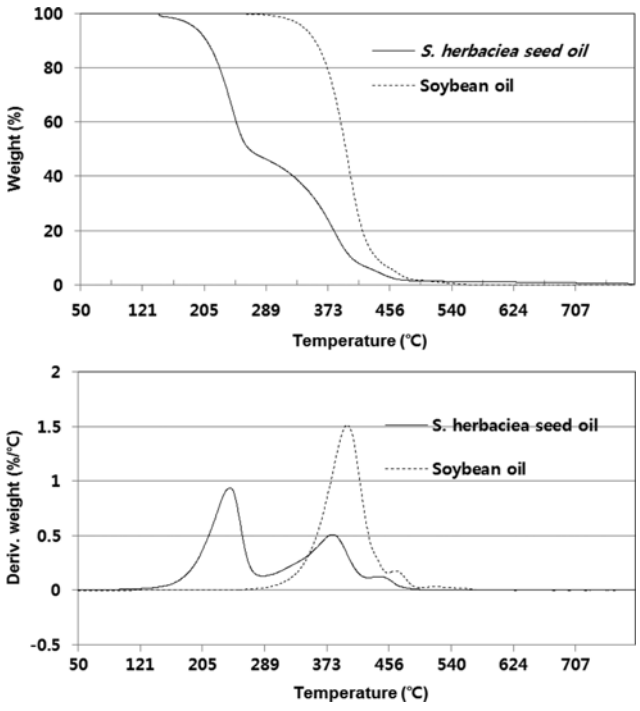


**Fig. 1. TG and DTG curve of *S. herbaciea* seed oil and soybean oil.**

oil began at 145 °C with approximately 0.2% mass loss, probably due to the impurities in oil. The initial decomposition temperature of *S. herbaciea* seed oil was 145 °C, which was lower than that of soybean oil (275 °C). On the other hand, the final decomposition temperature of *S. herbaciea* seed oil was 759 °C, which is higher than that of soybean oil (572 °C). The *S. herbaciea* seed oil revealed four mass loss steps. The first showed 47.5% mass loss from 145 to 259 °C. The second and third mass losses occurred between 260 °C and 398 °C, and the third mass loss between 399 °C and 487 °C was 40.3 and 10.9%, respectively, which were attributed to the volatilization or decomposition of the triacylglycerols initially from unsaturated fatty acids, saturated fatty acids and short-chain fatty acids. The last one with 1.2% of mass loss in the range from 487 to 759 °C was representative of the decomposition of the polymers formed during the oxidation process [49]. The maximum degradation rates of *S. herbaciea* seed oil occurred at 242.3 °C (9.5%/min), 382.6 °C (5.2%/min) and 440.7 °C (1.3%/min), where the rate of weight decrease increased to a maximum up to this point. The slower weight decreases were observed at higher temperatures. On the other hand, in the case of soybean oil, they were 15.7%/min at 399 °C and 1.84%/min at 458.3 °C, respectively. The three distinct steps in the degradation under oxygen correspond to the decomposition of unsaturated fatty acids, saturated fatty acids and the oxidation of carbon residue [50]. The principal means of vegetable oil decomposition is the oxidation of fatty acids components. Therefore, the mass loss due the temperature effect is compensated for by the absorption and reaction of fatty acids with atmospheric oxygen [51]. The thermal stability results can be related to the degree of unsaturation of the fatty acids that constitute the oils. Normally, a lower level of saturation is conducive to a lower thermal stability. This can be explained by the lower boiling point of the unsaturated fatty acids compared to their saturated equivalents.

**6. Infrared Absorption Spectroscopy**

Fig. 2 shows the FTIR spectra of *S. herbaciea* seed oil and soybean oil. For *S. herbaciea* seed oil, the main FTIR peaks were observed at 3412.6, 3009.1, 2926.1, 2854.7, 2672.9, 1744.4, 1721.8, 1710.4, 1653.4, 1464.7, 1418.1, 1377.9, 1282.4, 1243.5, 1168.6, 1097.7, 943.1, and 722.6 $cm^{-1}$. The peak at 3,412.6 $cm^{-1}$ was assigned to the carboxylic OH group, indicating the presence of some free fatty acids in the oil [45]. The peaks at 3,009.1, 2,926.1, 2,854.7, 1,464.7, 1,377.9, and 1,243.5 $cm^{-1}$ were attributed to -C-H symmetric, -C-H asymmetric, and -C-H bending stretching in the organic fatty acids, respectively. The peaks at 1,744.4, 1,721.8, 1,168.6, and 1,097.7 $cm^{-1}$ were assigned to -C-O and -C=O bonds stretching, respectively, and at 1,710.4 $cm^{-1}$ C=O group was assigned to free fatty acids [52]. The *cis* -C=C stretching mode of the unconjugated olefins normally shows moderate to weak absorption at 1,653.4 $cm^{-1}$. The peak at 722.6 $cm^{-1}$ was attributed to the *cis*-CH=CH-bond present in unsaturated fatty acids. A peak at 943.1 $cm^{-1}$ indicated the absence of *trans* CH=CH-from the fatty acids. A peak at 2,672.9 $cm^{-1}$, probably due to Fermi resonance of the carbonyl group, was also observed. A peak at 1,418 $cm^{-1}$ can be attributed to rocking vibrations of C-H bonds of *cis*-disubstituted olefins. The spectra of soybean oil and *S. herbaciea* seed oil were similar with slight differences in the frequencies at which the maximum absorbance was generated in each oil. These differences were attributed to the different fatty acid composition, number of chain lengths, and the degree and position of the double bonds in the triacylglycerols.

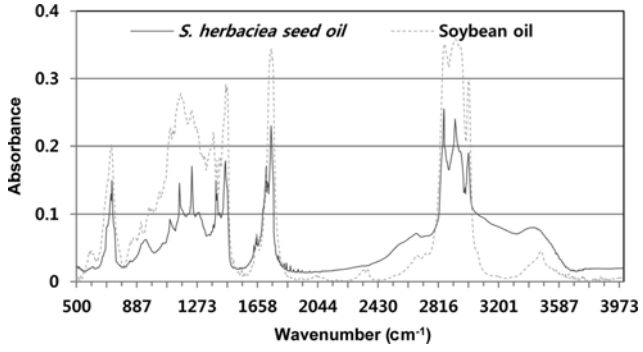


**Fig. 2. FTIR spectra of soybean oil and *S. herbaciea* seed oil.**

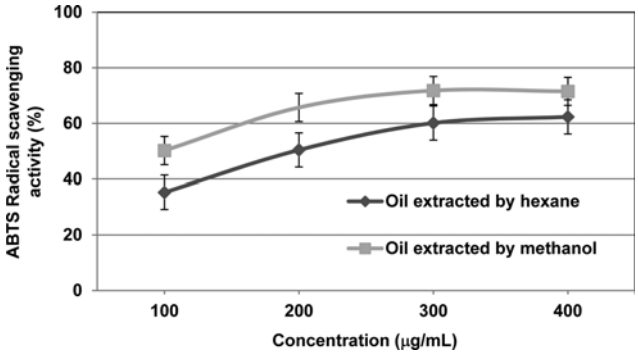


**Fig. 3. ABTS radical scavenging activity of *S. herbaciea* seed oil.**

**7. Antioxidant Activity of *S. herbaciea* Seed Oil**

To evaluate the antioxidant activity of *S. herbaciea* seed oil, the ABTS radical scavenging activities were measured (see Fig. 3). The ABTS radical scavenging activities of oils increased with increasing oil concentration by 300 mg/mL, irrespective of the type of oil. In particular, when the oil concentration extracted by hexane was increased from 100 to 300 mg/mL, the ABTS radical scavenging activity increased from 35.3 to 60.1%, but did not increase above 400 mg/mL. On the other hand, when the oil concentration extracted by methanol from oil extracted by hexane was increased from 100 to 300 mg/mL, the ABTS radical scavenging activity was increased from 50.2 to 71.8%, but did not increase above 400 mg/mL. This suggests that *S. herbaciea* seed oil can be used as a source of natural antioxidant additives in the food industry.

## CONCLUSION

We examined the physicochemical characterization, stability, and antioxidant activity of *S. herbaciea* seed oil for industrial applications. This oil has relatively high quality in terms of its high content of unsaponifiable matter and unsaturated fatty acids (oleic acid and linoleic acid). Among various tocopherols, $\delta$-tocopherol was the most stable and $\alpha$-tocopherol was the least stable during storage. This oil can also be used to improve the quality, stability and safety of food products. Furthermore, it can be used as a stock for biodiesel production. This study suggests that *S. herbaciea* seed oil is a source of powerful antioxidants, such as phenols and tocopherols, which could be used against diseases related to oxidative stress, der-

matological applications, cosmetics, as well as supplements in the food industry. The large-scale production of this oil and its availability on the world market can decrease the high demand for conventional vegetable oils and reduce the upward pressure on their prices. *S. herbaciea* seed is expected to have a potential positive socioeconomic impact in the future. However, further research will be needed to examine the effect of the oil extraction process on the other chemical compounds and their potential biological activities.

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