EFFECT OF EXTRACTION SOLVENTS ON QUALITY OF VIETNAMESE TEA (Camellia sinensis O.Kuntze) SEED OIL

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Abstract. Tea (Camellia sinensis O. Kuntze) seed oil (TSO) is one of the high-quality vegetable oils, similar to olive oil with a high concentration of unsaturated fatty acids, especially essential linoleic acid and low content of saturated fat. Its solvent extraction method can obtain not only oil but also natural compounds from raw material. This study demonstrated the physicochemical properties of TSO originated from the Northern Midland of Viet Nam, particularly the Province of Phu Tho. The extraction method was used with ten solvents: n-hexane (Hx), dichloromethane (DCM), ethyl acetate (EtOAc), ethanol (EtOH), EtOH:DCM (v:v, 3:1, 1:1 and 1:3), EtOH:EtOAc (v:v, 3:1, 1:1 and 1:3), and then concentrated by rotary evaporator followed by chemical analysis to assess its properties. The oil content, sensory attributes, acid value, peroxide value, iodine value, saponification value and fatty acids were analyzed. The oil contents ranged between 15.55 and 19.06 %. Iodine value was in the range of 81.9 to 100.15 g I₂/100 g, peroxide value < 10 meq O₂/kg, acid value < 4 mgKOH/g, saponification value was between 216.43 to 173.34 mg KOH/g, unsaturated fatty acid was between 69.52 and 79.19 g/100 g TSO extracted with EtOH:EtOAc v:v 3:1 gave the highest antioxidant activity (determined by IC₅₀ index) and had high amounts of polyphenol, γ-tocopherol and carotenoid compounds (5.00 mgGAE/g dry weight; 807.9 mg/kg and 78.44 mg/kg, respectively). This study aims to identify the effect of solvent on the quantity and quality of oil extracted from tea seeds in terms of physicochemical and antioxidant properties.

Keywords: tea seed oil, oil quality, solvent extraction.

Classification numbers: 1.3.1, 1.4.5.

1. INTRODUCTION

Tea is the most widely consumed beverages in the world, with approximately two-thirds of the world population drinking tea [1]. It originally came from Southwest China and has now been widely cultivated in several countries and regions around the world, mainly in Asia, Africa and South America. Viet Nam is one of the most ancient home of tea. Tea plant not only provides a beverage with several health benefits derived from its leaves but also valuable vegetable oils from its seeds [2]. The popularity of tea beverages and increasing cultivation of
tea plants are associated with elevated yield of tea seeds. Extraction of tea seeds yields a high content of oils (30 - 32%) that are rich in unsaturated fatty acids, particularly linoleic acids and oleic acids [3]. These plant-derived fatty acids, especially linoleic acids, may address several health problems related to heart and skin [4, 5, 6]. Tea seed oils (TSOs), therefore, can be used for various purposes, ranging from cooking oils with a high smoke point and long shelf life, to dietary supplements, herbal medicine, or beauty products with antioxidant properties.

The extraction techniques and solvents are crucial to obtain the highest yield of oils from tea seeds while preserving the valuable natural substances within oils such as fatty acids and phenolic compounds. An ideal solvent for extracting oil from seeds has been under investigation for both scientific and industrial purposes [6]. Regarding the oil yield, non-polar solvents are more effective extraction solvents for plant-based oils than polar solvents [7, 8]. However, these non-polar solvents are not necessarily better at maintaining the quality of oils than polar solvents. Indeed, polar solvents can penetrate the cell membrane, hence extract more membrane-associated lipids and cytosolicpolar compounds such as tocopherols that prevent fatty acid oxidation and improve oils’ shelf-life. Therefore, extraction solvents can be combined to alter the polarity of solvents to obtain a higher yield and higher quality of oils. Hexane (Hx) and ethyl acetate (EtOAc) are effective non-polar and polar solvents, respectively, for oil extraction but are flammable and cause acute and chronic health conditions through inhalation, ingestion, and direct contact [8]. Dichloromethane (DCM) is an effective and less flammable alternative to hexane, but their potential damage to the human brain and central nervous system limits their use in industrial production of edible oils. Short-chained alcohols such as ethanol are also suggested to be safe and effective solvents, however, a high amount is required since they easily evaporate. This study analyzed the oil extraction process utilizing four single solvents: Hx, DCM, EtOAc, EtOH; and their combinations, EtOH:DCM (v:v, 3:1, 1:1 and 1:3), EtOH:EtOAc (v:v, 3:1, 1:1 and 1:3) with different polarity. The effects of solvent polarity on the quantity and quality of extracted TSO based on its fatty acid profiles and oxidation stability will also be discussed here. The quality of oils is evaluated based on the fatty acid profiles and the oxidation stability of oils.

2. MATERIALS AND METHODS

2.1. Materials

Tea seeds were harvested from mature tea plants in Phu Tho province. They were dried until reaching the 7 - 8 % moisture content. The pericarps were manually separated from the seeds. Desired tea seed kernels were milled using an electric blender (Retsch ZM200-2017, Germany).

2.2. Analytical methods

2.2.1. Oil extraction

Ten different single and mixed solvents, including Hx, DCM, EtOAc, EtOH, EtOH:DCM (v:v, 3:1, 1:1 and 1:3), EtOH:EtOAc (v:v, 3:1, 1:1 and 1:3) were used at the material: solvent ratio of 1:10. After 7-hour extraction at 35 °C ± 0.5°C, the extracted products were filtered and concentrated by rotary evaporation at 40 °C ± 0.5 °C using a rotary evaporator (Model R-3000, Buchi, Switzerland), then placed in an oven at 80 °C ± 0.5 °C for an hour to evaporate the residual solvent. The crude TSO extracts were allowed to cool at room temperatures in well-
desiccated desiccators before being transferred into well-labeled sample bottles and stored in a refrigerator at −20˚C ± 0.5˚C prior to analysis (Figure 1).

**Figure 1. The extraction process of tea seed oil.**

2.2.2. Oil content (%)

The oil content was determined by the mass of tea oils extracted from the oil-bearing material (milled seeds) used in the extraction. The same content of seed materials was extracted by the process described in Figure 1, hence differences between oil contents were solely dependent on the efficiency of single or mixed solvents used during extraction.

2.2.3. Analysis of iodine value (IV)

The Iodine value is the number of grams of I₂ absorbed by 100 g of oil and determined according to Wijs as indicated by AOCS method Cd 1-25 using the formula [9]:

\[
IV = \frac{12.69 \times (V_1 - V_2) \times C}{m},
\]

Where: IV: Iodine value (g I₂/100g oil); m: Weight (g) of oil; C: Concentration of Na₂S₂O₃ (mol/l); V₁: Volume (ml) of Na₂S₂O₃ used in the test; 12.69: Atomic mass of iodine; and V₂: Volume (ml) of Na₂S₂O₃ used in the blank.

2.2.4. Analysis of saponification value (SV)
The saponification value was determined according to AOCS method Cd 3 - 25 [9] with slight modifications. About 5 g of oil was weighed into a 250 ml round bottom flask. 50 ml of 0.5 M KOH was added to the sample. This mixture was boiled under reflux for 1 h. The hot soap solution was then titrated with 0.5 M HCl using phenolphthalein indicator. A blank sample was prepared in the same manner. The saponification values were calculated using the formula:

\[ \text{SV} = \frac{(V_1 - V_2) \times C \times 56.1}{m}, \]

where: SV: Saponification value (mgKOH/g oil); m: Weight (g) of fat; \( V_1 \): Volume (ml) of HCl used in the sample; C: Concentration (mol/l) of HCl; \( V_2 \): Volume (ml) of HCl used in the blank.

2.2.5. Analysis of peroxide value (PV)

The peroxide value was determined according to the IUPAC method 2.501 with slight modifications. Approximately 2 g of oil was weighed into a flask. 20 ml of solvent (2:1 v:v, glacial acetic acid: chloroform) was added to the sample followed by 1 ml of freshly prepared saturated KI solution to form a homogenous solution. After a few minutes, 30 ml of water was added, and the mixture was titrated with sodium thiosulphate solution (0.01 M) and a starch solution as the indicator. The peroxide values were calculated using the equation:

\[ \text{PV} = \frac{(V_2 - V_3) \times f \times N \times 1000}{m}, \]

where: PV: Peroxide value (meqO₂/kg oil); m: Weight of oil (g); \( V_1 \): Volume of \( \text{Na}_2\text{S}_2\text{O}_3 \) used in the test; \( V_2 \): Volume of \( \text{Na}_2\text{S}_2\text{O}_3 \) used in the blank; N: Concentration of \( \text{Na}_2\text{S}_2\text{O}_3 \), and \( F \): factor for concentration correction.

2.2.6. Analysis of acid value (AV)

AV was determined according to AOCS Official Method Cd 3d-63 [9] with slight modifications using the formula:

\[ \text{AV} = \frac{V \times C \times 56.1}{m}, \]

where: AV: Acid value (mgKOH/g oil); m: Weight (g) of oil; V: Titrant solution volume (ml) used in the titration of the sample; and C: Molarity alcoholic KOH solution (\( f = 1 \)).

2.2.7. Analysis of fatty acid methyl esters (FAME)

The fatty acid composition of tea seed oils was analyzed by gas chromatography-flame ionization detector (GC-FID) according to the Ce 1-62 Method of American Oil Chemists’ Society [9]. Oil samples were converted into methyl esters by vigorous shaking the oil solution in 1 ml n-hexane with 2 ml of methanol potassium hydroxide. The tube carrying the solution was placed in a water bath (60 °C) for 20 minutes and shaken for 1 hour. The solution was decanted during the final 5 minutes, and 1 μl of the upper layer was injected into the GC-FID (6890 N, Agilent, US) equipped with a DB-225 capillary column (30 m × 0.25 mm × 0.25 μm) under the following conditions: initial temperature = 35 °C (1 minute) → 180° (20 °C/min), final temperature = 220 °C (35 minutes), heating rate = 20 °C/min, detector temperature = 260 °C, injector temperature = 250 °C, pressure of nitrogen (carrier gas) = 42.12 psi. % fatty acid calculated by area of the peak.
\[ X = \frac{A_i}{A} \times 100, \]

where: \( X \): Acid content \( i \text{ (%fame)} \); \( A_i \): Peak area of acid \( i \) obtained in the sample; and \( A \): Total peak area of all acids in the sample.

### 2.2.8. Analysis of total carotenoid content

Carotenoids were photometrically analyzed with a UV–Vis spectrophotometer. Oils weighed 0.04 g were diluted in 5 ml petroleum ether/acetone (v:v 1:1). The absorption of petroleum ether/acetone (v:v 1:1) served as a blank value and was deducted from the absorption value of the samples. Carotenoid contents (mg/kg) were calculated according to the following formula:

\[ X = \frac{(A \times y \times 10^7)}{(A_{\text{\%1cm}} \times 1000 \times m)}, \]

where: \( X \): Carotenoid content (mg/kg); \( A \): Absorbance at 445 nm; \( y \): Volume of extracting solution (ml); \( A_{\text{\%1cm}} \): Average absorption coefficient 2500 of the carotenoid molecule; and \( m \): Weight of the sample (g).

### 2.2.9. Analysis of total polyphenols content

Implement a method based on the method Folin-Ciocalteu [10]. 0.50 ml of the diluted sample was added into 2.5 ml of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 2 ml of saturated sodium carbonate solution (about 75 g/L) was added. The absorbance of the mixture was measured at 760 nm after incubation for 2 h at room temperature. Gallic acid was used as a reference standard. The total polyphenol content was calculated following this formula:

\[ X = \frac{OD \times c \times V}{a \times m} \times 1000, \]

where: \( X \): Total polyphenol content (mg gallic acid equivalents (GAE)/kg dry weight); \( OD \): Absorbance at 760 nm; \( c \): Dilution of extract (times); \( V \): Volume of extracting solution (ml); \( a \): Coefficient of gallic acid standard curve equation (\( y = ax + b \)); and \( m \): Dry weight of sample (g).

### 2.2.10. Analysis of tocopherol content

Tocopherol content in test portions of oil is determined by saponification of all-rac-alpha-tocopherol, partitioning with an organic solvent, separation from product matrix, and quantification by liquid chromatography by mode references by AOAC Official Method 992.03 [9].

\[ A = \frac{H_{\text{sam}}}{H_{\text{std}}} \times C_{\text{std}}, \]

where: \( A \): Vitamin E content (mg/kg); \( H_{\text{sam}} \): Peak height of samples; \( H_{\text{std}} \): Peak height of std; and \( C_{\text{std}} \): Concentration of standard (mg/kg).

### 2.2.11. Analysis of oxidation resistance (DPPH free radical reduction ability)

Following the method of Thaipong et al. [11], 24 mg DPPH with 100 ml methanol was prepared by dissolving as the stock solution and then stored at -20 until needed. Mixing a 10 ml stock solution with 90 ml ethyl acetate to obtain the working solution. Ethyl acetate was used to
dilute the sample to 200, 100, 50, 25, 12.5 mg/ml. Oil samples, control samples (150 µl) were allowed to react with 2850 µl of the DPPH solution for 30 minutes in the dark. Then the absorbance was taken at 515 nm using the spectrophotometer. Results expressed in percent of free radical inhibition I (%): \((\frac{A_{control} - A_{sample}}{A_{control}}) \times 100\) when there is a percentage inhibiting free radicals to build the equation of the sample (the correlation between I % and sample concentration) has the form \(y = ax + b\). Since then, replacing I % by 50 % has obtained \(IC_{50}\) sample concentration, which inhibits 50 % free radicals.

2.3. Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using Minitab statistical package version 16 at p < 0.05. The least significant difference (LSD) test was used in a mean separation where statistically significant differences were recorded. Data were tabulated as means of triplicate determinations ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Oil content

The first purpose of this study is to assess the oil content Y% extracted from tea seeds using currently useful solvents and investigate possible correlations between Y% and solvent dielectric constant. All tea seed oil extracts were golden yellow and at the liquid form at room temperatures (Figure 2). Among all the single and combined solvents tested, extraction with EtOH:EtOAc solvent v:v 1:3 ratio showed the highest oil content (19.06 %) while EtOH:DCM solvent at v:v 3:1 ratio gave the lowest oil content (15.55 %) (Table 1).

<table>
<thead>
<tr>
<th>Solvents</th>
<th>OC (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>16.94 ± 0.04g</td>
<td>slightly dark yellow</td>
</tr>
<tr>
<td>EtOH</td>
<td>17.13 ± 0.02f</td>
<td>slightly dark yellow</td>
</tr>
<tr>
<td>EtOAc</td>
<td>18.20 ± 0.01d</td>
<td>slightly dark yellow</td>
</tr>
<tr>
<td>Hexane</td>
<td>17.6± 0.03e</td>
<td>bright yellow</td>
</tr>
<tr>
<td>EtOH:DCM v:v 1:1</td>
<td>16.6 ± 0.02b</td>
<td>bright yellow</td>
</tr>
<tr>
<td>EtOH: DCM v:v 1:3</td>
<td>18.9 ± 0.02b</td>
<td>bright yellow</td>
</tr>
<tr>
<td>EtOH: DCM v:v 3:1</td>
<td>15.55 ± 0.03i</td>
<td>dark yellow-brown</td>
</tr>
<tr>
<td>EtOH: EtOAc v:v 1:1</td>
<td>18.95 ± 0.02b</td>
<td>bright yellow</td>
</tr>
<tr>
<td>EtOH: EtOAc v:v 1:3</td>
<td>19.06± 0.06c</td>
<td>slightly dark yellow</td>
</tr>
<tr>
<td>EtOH: EtOAc v:v 3:1</td>
<td>18.76 ± 0.03c</td>
<td>slightly dark yellow</td>
</tr>
</tbody>
</table>

(Data with the different superscript letter within column differ significantly at 5 % significance level).

These results indicated that the extraction solvents had a significant influence on the quantity of oil extracted from tea seeds. The solute-solvent interactions were mainly determined
by the dielectric constant that is the direct measurement of the solvent polarity [12]. Polar solvents decrease the difference between the surface tensions on the phase boundary and improve phase separation. However, high solvent polarity could limit the solubility of lipids and accelerate the destruction of lipids associations with cell membranes or lipoproteins [12, 13].

Figure 2. Images of tea seed oil obtained by extraction using different solvents. 
1- Hex, 2- EtOH, 3- EtOAc, 4- DCM, 5- EtOH:EtOAc v:v 1:1, 6- EtOH:EtOAc v:v 1:3, 7- EtOH:EtOAc v:v 3:1, 8- EtOH:DCM v:v 1:1, 9- EtOH:DCM v:v 1:3, 10- EtOH:DCM v:v 3:1

3.2. Peroxide value

The variation among the peroxide values in different seed oils may depend on the extracting solvents. The highest PV (7.75 meq/kg) resulted from extraction with Hexane and the lowest PV (4.82 meq/kg) was observed in the sample extracted with the mixed EtOH:EtOAc (v:v 3:1) solvent (Table 2). High PV is associated with high levels of oxidative rancidity of oils [14]. According to International Olive Council standards, the PV should be less than 20 meq/kg oil. The obtained PVs of samples in this study were far lower than this, at less than 10 meq/kg, suggesting that our tea seed oils are high-quality (due to physicochemical quality following the Vietnam National Standard – TCVN 7597:2013).

3.3. Acid value

Similarly, the acid values of tea seed oils extracted by ten solvents were different, ranging from 2.17 - 2.86 g KOH/g oil (Table 2). According to 2013, CODEX STAN 210-1999, the acid value of vegetable oils should be lower than 4.0 mg KOH / g oil. The acid contents in our tested samples were much lower than this threshold level (Table 2). Similar to the peroxide value, the acid value is negatively correlated with the oil quality and the simplicity of oil preservation. The lowest acid content was reported in samples extracted with either DCM or EtOH:EtOAc v:v 3:1 solvents (Table 2).

3.4. Saponification value

Tea seed oil extracted from EtOH:EtOAc v:v 1:3 had the highest saponification values (216.43 mg KOH/g) and the lowest is 173.34 mg KOH/g with EtOH (Table 2). The saponification values of these tea seed oil samples were highly similar to those of corn oil, sunflower oil and soybean oil (Table 2). This harmonized with a study that previously demonstrated that tea seed oil (Indian), sunflower and olive oil extracts showed no significant differences in their SVs [15].

3.5. Iodine value
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Data from Table 2 indicated that tea seed oil extracted by the EtOH:DCM v:v 3:1 solvent had the highest iodine value (100.15 g I$_2$/100g), while the lowest iodine content 81.90 g Iod/100g was observed in samples extracted with EtOH:EtOAc v:v 1:3 solvent (Table 2). The iodine value is an indicator of unsaturation, thus explaining why tea seed oils predominantly consisting of monounsaturated fatty acids had a lower iodine number than other vegetable oils such as corn oil consisting of more polysaturated fatty acids does (Table 2) [16]. The degree of unsaturation is negatively and positively correlated with the melting point and oxidation, respectively. In other words, the iodine value may indicate the quality of tea seed oils.

Table 2. Physicochemical properties of tea seed oil extracted by different solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>PV (Meq/kg)</th>
<th>AV (mgKOH/g)</th>
<th>SV (mg/g)</th>
<th>Iodine (I$_2$/100 g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>6.67 ± 0.06$^d$</td>
<td>2.17 ± 0.06$^a$</td>
<td>201.47 ± 127$^b$</td>
<td>82.15 ± 0.24$^e$</td>
</tr>
<tr>
<td>EtOH</td>
<td>5.58 ± 0.15$^{bc}$</td>
<td>2.32 ± 0.02$^{ab}$</td>
<td>173.34 ± 1.48$^g$</td>
<td>84.75 ± 0.55$^e$</td>
</tr>
<tr>
<td>EtOAc</td>
<td>5.42 ± 0.22$^b$</td>
<td>2.32 ± 0.02$^{ab}$</td>
<td>179.35 ± 1.79$^f$</td>
<td>84.09 ± 0.57$^{ef}$</td>
</tr>
<tr>
<td>Hexane</td>
<td>7.75 ± 0.07$^d$</td>
<td>2.86 ± 0.01$^d$</td>
<td>181.54 ± 0.95$^{ef}$</td>
<td>96.17 ± 0.31$^b$</td>
</tr>
<tr>
<td>EtOH:DCM v:v 1:1</td>
<td>5.75 ± 0.08$^c$</td>
<td>2.24 ± 0.02$^a$</td>
<td>184.49 ± 0.55$^{de}$</td>
<td>90.74 ± 0.46$^c$</td>
</tr>
<tr>
<td>EtOH:DCM v:v 1:3</td>
<td>7.15 ± 0.09$^c$</td>
<td>2.24 ± 0.03$^a$</td>
<td>185.11 ± 0.94$^d$</td>
<td>87.40 ± 0.76$^d$</td>
</tr>
<tr>
<td>EtOH:DCM v:v 3:1</td>
<td>5.52 ± 0.12$^{bc}$</td>
<td>2.66 ± 0.02$^c$</td>
<td>201.65 ± 0.17$^b$</td>
<td>100.15 ± 0.34$^a$</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 1:1</td>
<td>5.71 ± 0.05$^{bc}$</td>
<td>2.30 ± 0.01$^{ab}$</td>
<td>193.21 ± 0.89$^c$</td>
<td>100.01 ± 0.87$^a$</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 1:3</td>
<td>5.77 ± 0.05$^c$</td>
<td>2.47 ± 0.16$^b$</td>
<td>216.43 ± 0.52$^a$</td>
<td>81.90 ± 0.87$^f$</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 3:1</td>
<td>4.82 ± 0.03$^a$</td>
<td>2.17 ± 0.01$^a$</td>
<td>192.52 ± 0.66$^c$</td>
<td>82.85 ± 0.31$^g$</td>
</tr>
<tr>
<td>Corn*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>190</td>
</tr>
<tr>
<td>Sunflower*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>190</td>
</tr>
<tr>
<td>Soybean*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>189</td>
</tr>
<tr>
<td>Limit*</td>
<td>20</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(Data with the different superscript letter within column differ significantly at 5 % significance level)

* according to TCVN 7597:2013.

3.6. Fatty acid profile

The saturated fatty acid (SFA) and unsaturated fatty acid (UFA) profiles of tea seed oil extracted by different solvents showed differences. Unsaturated oleic acid and linoleic acid and saturated palmitic acid were dominant fatty acids in tea seed oils (Figure 3).
Figure 3. Fatty acid compositions (%) of tea seed oils extracted by different solvents.

The degree of unsaturation in vegetable oils may profoundly determine their quality, hence the chosen extracting solvents should preserve the high levels of unsaturated fatty acids in tea seed oils. The effect of solvent polarity index on the levels of SFAs and UFAs was illustrated in Figure 3. Solvents with distinct polarity resulted in different fatty acid profiles of the final oil products. The highest saturated fatty acid contents were observed in samples extracted by EtOH, EtOH:EtOAc v:v 1:1, and EtOH:EtOAc v:v 1:3 (Figure 3). The remaining samples demonstrated a similar pattern in fatty acid composition, with a lower level of saturated acids and higher level of unsaturated acids, which predominantly consisted of monounsaturated (oleic acid) and fewer polyunsaturated (linoleic acid) (Figure 3). This agreed with previous studies that examined the impact of extracting solvents and techniques on the fatty acid contents of oils derived from animals and plants [17], palm oil [14], pistachio oil [18]; and peach almond oil [19].

In terms of nutritional values, both oleic acids and linoleic acids have been shown to have anti-inflammatory effects and protective roles against cardiovascular diseases [5, 20]. Because the polyunsaturated structure of linoleic acids reduces the oxidative stability, hence the quality of oils during cooking, they have been largely replaced by more stable fatty acids such as oleic acids in recently developing oils. Further research on the long-term benefits and possible risks of these fatty acids is crucial to determine the optimal composition of fatty acids in dietary oils.

3.7. Antioxidant activity

Vegetable oil stability is also influenced by the abundance of antioxidant compounds. The levels of antioxidant compounds (total phenols, total tocopherol, total carotenoid content) and IC₅₀ values of TSO extracted from different solvents are shown in Table 3.
Table 3. Content of antioxidant compound in tea seed oil extracted by different solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>TPC (mgGAE/kg dry weight)</th>
<th>IC₅₀ (mg/ml)</th>
<th>Total carotenoids content (mg/kg)</th>
<th>Total tocopherol content (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>3023.3 ± 5.8és</td>
<td>143.33 ± 0.01bh</td>
<td>100.20 ± 0.58a</td>
<td>694.93 ± 2.22és</td>
</tr>
<tr>
<td>EtOH</td>
<td>5280.1 ± 34.6as</td>
<td>83.70 ± 0.01bh</td>
<td>78.44 ± 0.49es</td>
<td>706.27 ± 1.01es</td>
</tr>
<tr>
<td>EtOAc</td>
<td>4536.7 ± 40.4cs</td>
<td>108.37 ± 0.02cs</td>
<td>60.52 ± 0.37ds</td>
<td>770.03 ± 5.68cs</td>
</tr>
<tr>
<td>Hexane</td>
<td>1363.3 ± 5.8bs</td>
<td>140.26 ± 0.01es</td>
<td>59.68 ± 0.13es</td>
<td>65.84 ± 4.50es</td>
</tr>
<tr>
<td>EtOH:DCM v:v 1:1</td>
<td>2630.1 ± 26.5fs</td>
<td>125.56 ± 0.03es</td>
<td>79.70 ± 0.11b</td>
<td>701.67 ± 2.86es</td>
</tr>
<tr>
<td>EtOH:DCM v:v 1:3</td>
<td>2083.3 ± 5.8gs</td>
<td>139.91 ± 0.01es</td>
<td>80.72 ± 0.21b</td>
<td>674.53 ± 5.17fs</td>
</tr>
<tr>
<td>EtOH:DCM v:v 3:1</td>
<td>3056.9 ± 20.7es</td>
<td>118.68 ± 0.05js</td>
<td>69.58 ± 0.51js</td>
<td>745.6 ± 6.19gs</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 1:1</td>
<td>4510.1 ± 10.1cs</td>
<td>133.00 ± 0.02fs</td>
<td>77.96 ± 0.43cs</td>
<td>786.1 ± 1.78bs</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 1:3</td>
<td>4003.3 ± 5.8ds</td>
<td>120.13 ± 0.01ds</td>
<td>62.80 ± 0.50c</td>
<td>625.28 ± 4.23bs</td>
</tr>
<tr>
<td>EtOH:EtOAc v:v 3:1</td>
<td>5003.3 ± 20.8hs</td>
<td>70.80 ± 0.06es</td>
<td>78.44 ± 0.01c</td>
<td>807.9 ± 1.13as</td>
</tr>
</tbody>
</table>

(Data with the different superscript letter within column differ significantly at 5% significance level)

γ-Tocopherols have the strongest antioxidant activity among vitamin E homologs in vivo [19, 21]. The higher the polyphenol content, the more oxygen resistance, and the higher antioxidant activity. They also enhance the oxidation stability in oils by preventing polyunsaturated fatty acids from peroxidation [5, 22]. Due to their nutritional and quality-promoting effects, many efforts have been made to analyze the impact of solvent characteristics on the tocopherol levels in extracted oils. According to our study, samples extracted with EtOH had the highest tocopherol content, followed by those extracted by EtOH:EtOAc v:v 3:1, EtOAc, and EtOH:EtOAc v:v 1:1 (Table 3).

4. CONCLUSIONS

The quality of oils is dependent on the balance between their nutritional values and stability. This study has indicated that extracting solvents with different polarity significantly influenced the physiochemical properties and antioxidant activities of tea seed oils. Suitable extracting solvents for oil processing should be carefully chosen to satisfy the purposes and requirements of final oil products. All parameters, including oil content, saturation degree, oxidation stability and antioxidant compound levels, that determine the oil quality are influenced by the utilization of extracting solvents.
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