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Analysis of Fatty Acid Composition and Physicochemical properties of Seed oil of Salvia schimperi

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Abstract

The indigenous weed plant named as *Salvia schimperi* Benth. (Locally called Dibirik) (Family Lamiaceae) grows in dried highland areas of Shewa and Tigray, Ethiopia. The leaf and seed of the plant are traditionally useful. The leaves of the plant are smoked for evil eye and insect repellent whereas the seed of the plant is important medicinally for treating bacterial infections causing diarrhoea, and stomach ache. It is obvious that seeds are rich in oil. Although the seed of *S. schimperi* contains oil, its chemical composition and property are not yet known. This study focused on determination of the seed oil content, physicochemical properties and the fatty acid composition of *S. schimperi* seed collected from Debre Berhan town using Gas Chromatography-Mass Spectrometer (GC-MS). The isolated golden seed oil (15%) was characterized, and its GC-MS analysis revealed the presence of methyl oleate (32.8%), methyl linolenate (21.6 %), methyl stearate (30.9%), and methyl palmitate (8.8%) as main components. This fatty acid composition and the experiential physicochemical properties of the seed oil revealed its possible importance as complementary source of oil for human consumption.

Keywords: Salvia schimperi, Dibirik, Fatty acid, GC-MS

1. Introduction

Salvia schimperi (Family Lamiaceae) is an indigenous weed plant to Ethiopia usually grows in dried highland areas of Shewa and Tigray. Its fruits are traditionally used for the treatment of diarrhoea and other bacterial disease [1,2]. In Ethiopian folk medicine, aqueous decoction of its leaves is applicable for treatments of various ailments including diarrheal and bacterial infections. In Eritrea, people use the leaves to protect themselves from insect biting [2,3]. There are few reports from locally grown *S. schimperi* on the flower and the leaf part of the species [1,2] whereas no reported chemical work was found on its seed oil althogh the seed is traditionally consumed for the treatment of complaints causing diarrhea and stomach ache. Therefore, this study focused mainly on the analysis of the physicochemical properties and fatty acid composition of *S. schimperi* seed oil extracted by soxhlet extractor using hexane.

2. Materials and Methods

2.1 Sample collection

Seeds of *S. schimperi* (Dibirik Amh) (500 g) (Fig 1) were purchased from Debre Berhan town about 130 km North East of Addis Ababa, Ethiopia on April, 2018 from local market and stored in paper bags under room temperature. The picture of the plant was taken from the field where it grows up around Debre Berhan town.

Figure-1 Picture of the leaf and seed of *Salvia schimperi* (Photo: Authors)

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2.2. Apparatus, Instrument, and Chemicals

Soxhlet appataus (500 mL), Electric grinder, rotary evaporator, cotton cloth thimble, Abbe's refractometer, pH meter, three necked round bottomed flask (250 mL), heating mantle, Agilent gas chromatograph equipped with a mass spectrometer detector and Agilent automatic injector spectrometer (Agilent Technologies, 7890A GC-MS, USA) were used in the study.All chemicals (potassium hydroxide, anhydrous sodium sulphate, saturated sodium chloride, hydrochloric acid, phenolphthalein, sodium thiosulfate, buffer solutions (pH 4, 7 and 10) and analytical grade solvents (methanol, hexane, ethanol, chloroform, ethyl acetate) were used without further actions.

2.3 Extraction and Methylation of the seed oil

The collected seed (105 g) was powdered using electric grinder found at the Department of Chemistry, Debre Berhan University. Ground *S. schimperi* seeds (100 g) were extracted using Soxhlet apparatus with hexane (Fig 2) for 3 h and the solvent was removed by rotary evaporator under reduced pressure and the seed oil was stored in vials until used.

Figure-2 Extraction of the seed oil using Soxhlet apparatus

Part of the seed oil (4 g) was placed into three-naked round bottom flask, and 40 mL of 2% methanolic potassium hydroxide was added and shaken. After refluxing the mixture for 1h at 60^0C, it was allowed to cool and the organic layer was separated several times by separatory funnel in the presence of hexane (100 mL). The organic layer was dried with anhydrous sodium sulphate, filtered and the solvent was removed by rotary evaporator to yield the fatty acid methyl esters (FAME) of the seed oil (1.8 g) [4]. The FAME was labelled and submitted to the Department of Chemistry, Addis Ababa University for analysis by Gas-Chromatography coupled to Mass Spectroscopic (GC-MS). The remaining seed oil was subjected to physicochemical analysis.

2.4 Analysis of FAME

Analysis of FAME was carried out using Agilent technologies 7820A GC and 5977E MSD system equipped with auto-sampler. Chromatographic separations were conducted using capillary column with 30 m length, 0.25 mm internal diameter and 0.25 µm column phase thickness. Injection mode was split-less with helium as carrier gas and 1 µL of the sample was injected at a constant flow rate of 1 mL/min to the inlet heated to 275°C. Initial oven temperature was 60°C with 2 min hold time then heated to 200°C with ramp of 10°C/min and 3°C/min to 240°C. Conditions used for the mass spectrometer were a temperature of 230°C for the ion source and 150°C for quadruple, scan range 40– 650 m/z, operating in positive electron impact mode with ionization energy of 70 eV. Chromatographic and mass spectral data were processed by the instrument built in software (MS Mass Hunter; Agilent Technologies, USA). The components were identified from the chromatograph library (NIST-18).

2.5 Physicochemical Analysis of the seed oil

Determination of Moisture Content: Ground seed sample (5 g) was put in oven at 103 ± 2^0C until a constant mass and its percentage was expressed as the mean deference of the seed before and after oven drying against the initial mass of the sample [5].

$$Moisture\ \% = \frac{w_1 - w_2}{w_1} * 100\%$$

Where: w_1= Original weight of the sample before oven drying and w_2 = Weight of the sample after drying.

Determination of specific gravity: The specific gravity of the seed oil was determined by using clean and dry vial of 1 mL capacity. It was weighed (w_0) and then the vial was filled with the oil, and reweighed to give (w_1). Similarly, the water was filled into the cleaned and dried vial instead of seed oil and weighed again to give (w_2). Then specific gravity was determined by the following formula [6].

$$Sp.gr = \frac{w_1 - w_0}{w_2 - w_0}$$

Determination of pH: The seed oil (1 g) was poured into a clean dry 25 mL beaker mixed with 6.5 mL of hot distilled water in the beaker and stirred slowly. It was then cooled in a cold-water bath to 25°C. The pH

meter was standardized with buffer solutions at pH 4, 7 and 10 and the electrode was immersed into the sample read and recorded three times in order to take the average value [7].

Determination of Saponification Value (SV): The seed oil (0.5 g) was added to 250 mL conical flask and mixed with 50 mL of 0.5 N ethanolic KOH. Similarly, blank solution was prepared in another conical flask. The content was refluxed for 45 min while stirring. After completion, 2 drops of phenolphthalein indicator were added and titrated against 0.5 N HCl to the end point. SV was calculated by using the following formula [8].

$$V = \frac{56.1 * N(V_b - V_s)}{W_s}$$

Where:
N= concentration of HCl
V_b = the volume of HCl for the blank
V_s = the volume of HCl for the sample
W_s = weight of the sample

Determination of acid value (AV) and free fatty acid value (FFA): To the seed oil (2 g) in 250 mL round bottom flask, 10mL of ethanol was added and then the mixture was heated to 70 ^oC for 3 min. This solution was titrated after adding 1 drop of phenophtelain indicator to it against 0.1 N NaOH until the appearance of pink colour. Similarly, blank solution without the seed oil was prepared and titrated against NaOH (0.1 N) till the appearance of pink colour. Acid value was calculated using the following formula [7,8].

$$AV = \frac{56.1 * N * V}{W_s}$$

Where:
N = normality of NaOH
V = volume of NaOH used
W_s = weight of the oil sample

The free fatty acid value (FFA) of the seed oil was calculated by dividing the acid value by two.

Determination of Refractive Index of the Seed oil: Refractive index was measured by using Abbe's refractometer. The sodium-lamp was turned on. Then the double prism of the refractometer was opened and both of the glass surfaces were cleaned using cotton and 97% ethanol. A thermometer was placed near to the refractometer. One drop of the seed oil sample was transferred into the cleaned and dried glass slide of the refractometer by pipette. The refractometer scale knob was turned to get a clear interface between the illuminated and the dark regions. The dark region was adjusted to be in line with the intersection of the cross. Index of refraction was read out from telescope scale. Temperature was also noted from the thermometer put aside. This was repeated three times and the mean value was recorded as refractive index [9].

Determination of iodine value (IV): Seed oil (0.3 g) dissolved with 10 ml chloroform in 250 ml conical flask was mixed with 20 ml of reagent, prepared by mixing 5 g iodine in 100 ml of 97% ethanol and 6 g of Mercuric chloride in another 100 ml 97% ethanol, and then plugged with stopper. Then the mixture was placed in dark at room temperature for 30 min and 15 ml of 10% KI solution and 100 ml distilled water was added. The solution was titrated against 0.1 N $Na_2S_2O_3$ solution using 2 ml of starch solution until the color of the solution turned to blue. Similarly, the blank solution was prepared in another conical flask and titrated against 0.1 N $Na_2S_20_3$ solution. IV was determined as follows [8].

$$IV = \frac{12.69 * N(V_o - V)}{p}$$

Where:
N = normality of $Na_2S_2O_3$
p = weight of the oil sample (g)
V = volume of $Na_2S_2O_3$ solution used for the sample (mL)
V_0 =volume of $Na_2S_2O_3$solution used for the blank (mL)

Data Analysis
The experimental data of the triplicate measurements were reported as mean ± RSD.

3. Results and Discussion

In this study, the oil extracted from *S. schimperi* seed was investigated for its physicochemical properties, and fatty acid constituents using the known procedures.

3.1. Physicochemical Properties of Salvia schimperi Seed Oil

The seed oil was analysed based on the protocols listed above and the actual values were calculated using the respective equations. Ground seeds of *S. schimperi* was found to possess 8% water (Table 1). The yield of the golden coloured oil (15 g) isolated from the powdered seed (100 g) using Soxhlet extractor in hexane for 3 h (15%) was comparable with some of the conventional oil seed crops such as cotton (15-24%) and soybean (17-21%) [10], but it is lower than that of seed of *S. hispanica* (27.0-33.0%) [11,12] and Niger (40%) [13].

Table-1 Physicochemical properties of the seed of *S. schimperi*

Content	Value
Moisture content of the seed powder (%)	8.0
Seed oil content (%)	15.0
Specific gravity (g/mL)	0.8774
Refractive index at 20°C	1.460
pH	6.60
Saponification value (mg KOH/g oil)	173.91
Acid value (mg KOH/g oil)	1.964
Free fatty acid value (mg KOH/g oil)	0.982
Iodine value (g I/100 g oil)	16.92

The seed oil recorded a specific gravity of 0.8774 and refractive index of 1.460 (Table 1). The specific gravity of most seed oils varies from 0.86 to 0.98. The value measured is in the acceptable range indicating the loosely bound nature of the molecules in the seed oil compared to other seed oils reported [14]. Experimental value of the refractive index of the seed oil is also lower than the *S. hispanica* and other edible oils like sunflower and safflower [12[, and therefore the oil has lower possible rancidity.

Saponification value (SV) is defined as the weight of potassium hydroxide, in milligrams, needed to saponify one gram of seed oil. It is an indicator of the size of the fatty acid chains esterified to produce glycerol, and gives a measure of the average length of the fatty acid chain that makes up an oil. Since oil with high SV (greater than 200) is only useful for industrial purposes rather than nutritional significance, the experimental seed oil (SV= 173.9 mg) (Table 1) is nutritionally important rather than making soap. This SV is slightly lower than that reported for the common edible oils such as sunflower (188-194 mg KOH/g), safflower (186-198 mg KOH/g), soybean (189-195 mg KOH/g) and virgin olive oil (184-196 mg KOH/g) [12] and much lower than seed oil of *S. hispanica* (193-222.6 mg KOH/g) [12,15].

Acid value is the mass of KOH (mg) that required to neutralize one gram of oil. The free fatty acids (FFA) present in oil impart unpleasant odour to edible oil. The acid value was found to be 1.964 mg KOH/g which indicated its edible nature. The amount is much lower than the value reported for palm oil with average acid value of 19.3 [16].

Iodine value (IV) is defined as the number of grams of iodine absorbed by 100 grams of oil indicating the degree of unsaturation. The IV of oil does not indicate the position of the double bonds or amount of olefinic carbon but rather it provides an overall status of unsaturation of the oils [8]. The iodine value of the seed oil (16.92 g/100g oil) was found low and has advantages in shelf life. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid level and a decrease in total unsaturation of the oil [17]. Therefore, the seed oil understudy is recommendable for cooking.

3.2. Fatty Acid Compositions in Salvia schimperi Seed oil

The seed oil was transesterified to obtain FAME, and the GC-MS analysis (Table 2, Fig 3) revealed that oleic acid (**1**) (32.8%), linolenic acid (**2**) (21.6%), stearic acid (**3**) (30.9 %), and palmitic acid (**4**) (8.8%) (Fig 4) were the most abundant components. The first two unsaturated fatty acids and the latter saturated fatty acids are important components of edible oils which showed the possible use of the plant seed oil for cooking.

Table-2 Major Fatty acid composition of seed oil analysed by GC-MS

Constituents	Retention time	Area %
Methyl palmitate	11.4621	8.8307
Methyl linolenate	13.3829	21.5678
Methyl oleate	13.4602	32.8445
Methyl stearate	13.6610	30.9195

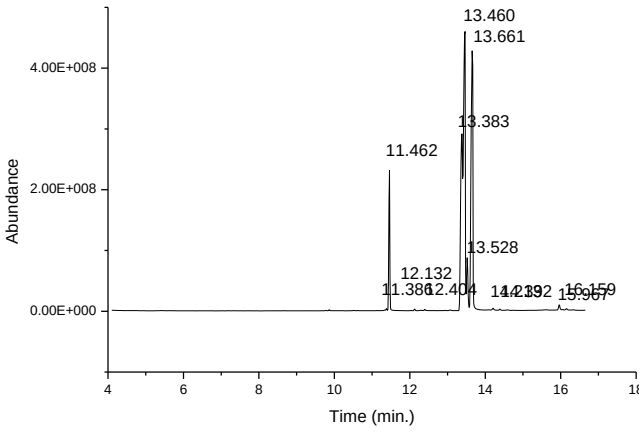

Figure 3. GCMS chromatogram of transesterified seed oil

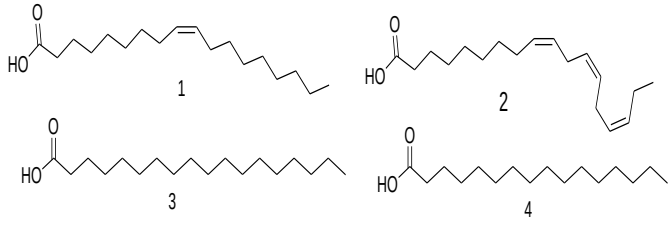

Figure. 4. Structures of major constituents of seed oil of *S. schimperi*

Conclusion

In this study, the seed oil of *S. schimperi* was extracted, physically characterized and chemically investigated using GC-MS. The most abundant fatty acids found in the seed oil totaling 94.1% includes oleic acid (C18:1) (32.8 %), linolenic acid (C18:3) (21.6%), stearic acid (C18:0) (30.9%), and palmitic acid (C16:0) (8.8%). Therefore, the observed physicochemical properties and the fatty acid composition of the extracted seed oil confirmed that *S. schimperi* seed is a possible source of edible oil besides its medicinal value.

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