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Physicochemical properties, phytochemicals and fat soluble vitamins of seed oil extracts from *Sesamum Indicum L.*

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Abstract
This research work presents the extraction and physicochemical, phytochemical and vitamins characterization of oil from seed of *Sesame indica*. The oil was extracted by soxhlet apparatus using n-hexane as the solvent. Results from the physicochemical analysis showed Oil content (49±1.41 %), acid value (0.48±0.04 mg KOH/g), free fatty acid (0.24±0.02 mg KOH/g), Peroxide value (7.73±0.39 mEq./Kg), Saponification value (190±1.41 mg KOH/g), Iodine value (109.5±9.19 gI_2/100g), Specific gravity (0.919±0.01), five phytochemicals tested include alkaloid, tannin, flavonoid, total phenols and saponins had the following quantities 134.80 ± 0.28, 14.12 ± 1.32, 62.14 ± 0.10, 192.49 ± 4.45 and 40.82 ± 1.21 mg/g respectively. Vitamin analysis showed vitamin A was 10.59 ± 0.14 (mg/g), Vitamin D content was 12915.2 ± 104.3 (µg/100g) and Vitamin E content was 132.5 ± 0.31 (µg/100g). This study showed that the sesame seed oil is a good source of vitamin and could be useful as food supplement and industry.

Keywords: seed oil, micronutrients, food, supplement, extraction, phytochemicals

Introduction

Essential plant oils have been used medicinally at different periods in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer. The compositions of essential oils which made them useful are the phytochemicals such alkaloids, tannins, flavonoids and phenolic compounds. Vitamins on the other hand, are defined as organic substance required in small amount for the maintenance and growth of living organisms. Their deficiency may lead to certain specific diseases or symptoms which can be cured by the administration of that specific vitamin only. Vitamins are highly essential to human body except vitamins D, K and biotin as they cannot be synthesized in the body. Vitamin D is synthesized in the body by irradiation of sterols in the skin by UV rays. Many plants and microorganisms except humans and some other animals synthesize vitamins. Hence they need to be supplied through diet to the human body. Most of the vitamins are present in required quantities in the fresh and natural foods available both plants and animals sources. Vitamins are required in tiny amounts because of their inactivation in the body they play a catalytic role in many metabolic reactions of the cells and act as coenzymes or part of coenzymes and enzyme systems. Certain vitamins act as hormones and exert their action at intracellular receptor sites like Vitamin A and D [1].

Sesame indica is a flowering plant in the genus *Sesamum*, also called *benne*. Numerous wild relatives occur in Africa and a smaller number in India [2]. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods or buns. The world harvested 4.2 million metric tonnes of sesame seeds in 2013, with India and China as the largest producers. Sesame seed is one of the oldest oil seed crops known, domesticated well over 3000 years ago [2]. Sesame has many species, most being wild and native to sub-Saharan Africa. *Sesame indicum*, the cultivated type, originated in India and is tolerant to drought-like conditions, growing where other crops fail [3].

Sesame has one of the highest oil contents of any seed. With a rich, nutty flavor, it is a common ingredient in cuisines across the world. Like other nuts and foods, it can trigger allergic reactions in some people. Sesame seed is considered to be the oldest oilseed crop known to humanity [4]. The genus has many species, and most are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. *Sesame indicum*, the cultivated type, originated in India [5]. Sesame (*Sesamum indicum* L.*)* is one of the most important oilseed crops worldwide, and has been cultivated in Korea since ancient times for use as a traditional health food. Sesame seeds are used in the making of tahin (sesame butter) and halva, and for the preparation of rolls, crackers, cakes and pastry products in commercial bakeries [6]. There are numerous varieties and ecotypes of sesame adapted to various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information. Two studies that used morphological characters to group genotypes into clusters found a w ide genetic diversity in Indian sesame genotypes [7]. Multivariate analysis based on morphological characters provides genetic information that will allow the breeder to improve populations by selecting from specific geographic regions. So, aim of the present study was to exmain the proximate composition and physicochemical analyses on the seed and

Sesame oil [8]. The major objective of this study is to evaluate the properties, quantitative estimation of phytochemicals and fat-soluble vitamins of Sesame indica obtained from Owerri, Imo state.

2. Material and method

Materials and reagents that were used for this analysis include; electric weighing balance, centrifuge, ethanol, Soxhlet extractor, Whatman filtter paper, trichloroacetic acid (TCA), n-hexane, water bath, 0.5N alcoholic potassium hydroxide, acetic anhydride chloroform reagent, ethanol 0.5% 1 1-dipyridyl, 0.2% ferric chloride, distilled water.

2.1 Source of raw material

Seeds of *Sesame indica* were bought from the main market in Owerri, Imo State and were immediately transported to the biochemistry laboratory of the Imo State University Owerri for standard biochemical analysis.

2.2 Preparation of the sample

The seeds were thoroughly washed with clean water separately accordingly, based on how they were collected. They were sun dried until a constant weight was achieved. They were then grounded using electric blender and were stored in a well labeled air-tight container for analysis.

2.3 Oil extraction

Oil extraction was performed with a Soxhlet apparatus using n-hexane as the solvent. 100 g of powdered seeds was extracted for 6 hours and then the solvent was evaporated by using a rotary evaporator at 40°C. The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at –18 ^{0}C until analyzed.

The percentage oil yield was determined using the following expression:

Oil content (%) = $(W_1 / W_2) * 100$

W_1 = weight of oil, W_2 = weight of sample. The extracted oil was kept in a clean container and later used for the analysis.

2.4 Analytical Procedure

2.4.1. Physicochemical Characterization

The physicochemical characterization was determined as described in previous reports [9-10].

2.4.2 Methods of determining the phytochemicals

The quantitative phytochemicals were determined as described in previous reports [11-12].

2.4.2.1. Alkaloid Content

The 5 g of the oil extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. All samples were analyzed in triplicates.

Alkaloid (mg/g) = Weight of residue/weight of sample

2.4.2.2. Total Flavonoid Content

The 2.5 g of the oil extract was mixed with 25 mL of 80% aqueous methanol. The whole solution was filtered through the whatman filter paper. The filtrate was transferred to a crucible and evaporated into dryness over a water bath and weighed. All samples were analyzed in triplicates. Flavonoid (mg/g) = Weight of residue/weight of sample

2.4.2.3. Total Saponin Content

The 5 g of the oil extract was introduced into a conical flask and 25 mL of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 hour with continuous stirring at about 55 ^{o}C. The concentrate was transferred into a 250 mL separatory funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The 15 mL of n-butanol was added, and then 2.5 mL of 5% aqueous sodium chloride was added. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight.

Saponin (mg/g) = Weight of residue/weight of sample

2.4.2.4. Total Tannin Content

The tannin content of the sample was determined using the Folin-Ciocalteu phenol reagent. The 0.1 mL of the oil was added with 7.5 mL methanol. The 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% sodium carbonate solution were also added. The mixture was diluted to 10 mL with distilled water. The mixture was well shaken, kept at room temperature for 30 min and the absorbance was measured at 725 nm. Blank was prepared with distilled water. A set of standard solutions of tannic acid was read against a blank. Total tannin content was determined as mg of tannic acid equivalent per gram of the sample using the equation obtained from a standard tannic acid calibration curve $y = 0.021x + 0.343$. All samples were analyzed in triplicates

2.4.2.5. Total Phenolic Content

The total phenolic content of the *Sesamum indicum* seed oil was determined by Folin-Ciocalteu spectrophotometric method (McDonald et al., 2001). The 0.1 mL of Folin-Ciocalteu reagent was added to 2 mL of the oil. The mixture was allowed to stand for 15 min. Then, 5 mL of saturated sodium carbonate (Na2CO3) was added. The mixture was allowed to stand for 30 min at room temperature and the total phenolic content was determined spectro-photometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of mg of gallic acid equivalent per gram of the sample using the linear regression equation obtained from the standard gallic acid calibration curve $y = 0.006x + 0.038$. All samples were analyzed in triplicates.

2.4.3. Vitamin

The vitamins in the seed oil were determined by the official methods of the Association of Official Analytical Chemists (AOAC).

2.4.3.1. Determination of Vitamin A (Retinol)

Vitamin A was determined by calorimetric method. 1g of the sample and standard was mixed with 3ml of ethanol. 50% KOH (Potassium Hydroxide) solution was added to it and boiled gently

for 30 minutess under reflux wash with distilled water after boiling, vitamin A was extracted with 3 x 50ml of diethyl ether. The extract was evaporated to dryness at low temperature and was dissolved in 10ml isopropyl alcohol. 1ml of standard vitamin A solution prepared and that of the dissolved extract were transferred to a separate cuvettes and their respective absorbance were read in a spectrophotometer at 325nm with the reagent blank at zero.

Calculation

$$\text{Con of vit A in Sample} = \frac{\text{Abs of sample}}{Abs\ of\ std}\ x \frac{\text{conc. of standard}}{1}$$

2.4.3.2. Determination of Vitamin D

0.5g of the sample was dissolved in 5ml water, 20ml of ethanol, 1m sodium ascorbate solution and 3ml of 60% potassium hydroxide (KOH). Heat under reflux condenser for 30 minutes, cool rapidly with running water. Transfer the liquid to a separating funnel. 5ml of petroleum ether was added to the sample, shake vigoursly for 30 seconds. Allow to stand until the two layers separated. The lower aqueous was then separating and was pour in a beaker, while the upper petroleum ether layer was pour in another beaker. The extraction was done like two to three times intil two layers are cleared. The final volume of the petroleum ether was noted. The absorbance of the yellow colour was read in spectrophotometer of 450nm for vitamin D.

Calculation:

$$amount\ of\ vitamin\ D = \frac{A_{450}\ x\ vol\ of\ sample\ x\ 100\ x\ 4}{weight\ of\ sample}$$

2.4.3.3. Determination of Vitamin E (Tocopherol)

This was determined by Filter – Mayer method with association of vitamin chemists. 1g of sample was mixed 10ml of 10% ethanoic sulfuric acid and boiled gently under reflux for 30 minutes. It was transferred to a separating funnel and treated with 3 x 30ml diethyl ether and recovering ether layer each time. The ether extracted was transferred to a desiccator and dried under low temperature for 30 minutes, the evaporated at room temperature. The dried extract dissolved in 10 ml of pure ethanol, 1ml dried extract and equal volume of standard vitamin E were transferred to separate tubes. After autoclave at 120^0C for 30 minutes cooled and inoculated with 2ml the test organism for 8 hours. The absorbance was measured at 660nm and the level of pyridoxine was evaporated from the standard curve.

$$Conc.\ of\ vitamin\ E = \frac{abs\ of\ sample}{abs\ of\ std}\ x\ \frac{conc.\ of\ std}{1}$$

3.0 Result and discussion

The physicochemical characterization result for the extracted oil is presented in Table 1. The oil contents, acid value, free fatty acid, peroxide value, saponification value, iodine value and specific gravity are presented. The percentage yield of the extracted seed oil was 49.0±1.41 %, and is comparable with that obtained by Mohammed and Hamza [14] which was 48-50 %. Ibrahim [11] explained that high percentage yield of the white *Sesamum indicum* seeds oil shows that the processing of the oil for industrial or edible purposes can be of economic importance. According to Hwang [15], *Sesamum indicum* seeds have higher oil content than most of the known oil seeds. Fatty acids are usually in the triglyceride form and tend to get hydrolyzed into free fatty acids [9, 10, 13]. Therefore, there is a direct relationship between acid value and the free fatty acid content [10, 13]. These mean that higher acid value will cause higher free fatty acid and thereby decreasing the oil quality [13]. AOAC stated 0.6 mg KOH/g as the maximum accepted level for vegetable oil. The current study showed lower acid and free fatty acid values, indicating that the oils may be good for use. Similar studies have reported similar acid values for sesame oil grown in Jigawa State, Nigeria [14].

Table 1: The physicochemical characteristics of the oil extract

Physicochemical parameters	Concentration (mg/g)
Oil content (%)	49±1.41
Acid value (mg KOH/g)	0.48±0.04
Free fatty acid (mg KOH/g)	0.24±0.02
Peroxide value (mEq./Kg)	7.73±0.39
Saponification value (mg KOH/g)	190±1.41
Iodine value (gI_2/100g)	109.5±9.19
Specific gravity	0.919±0.01

Value reported as Mean ± SD for triplicate analysis n = 3

The peroxide value is an indicator of the level of lipid peroxidation or oxidative degradation. It is a useful indicator of the early stages of rancidity occurring under mild conditions and a measure of primary lipid oxidation products [9,13]. The obtained peroxide value (7.73±0.39 mEq./Kg) is lower than the recommended a limit of 10 mEq./Kg of Standard Organization of Nigeria (SON) and Nigerian Industrial Standard (NIS) [13]. The peroxide value is comparable to value reported elsewhere [14]. Saponification value, Iodine value and specific gravity obtained in the current study is comparable to other studies on oil [9,13,14]. The iodine value (109.5±9.19 gI_2/100g) is high, indicating that it is semi dry oil. Thus, the oil will not attract high interest in the paint and coatings industry unless it undergoes dehydration before use [14].

The quantitative estimation for phytochemicals is presented in Table 2. Information on the phytochemical constituents of materials of plant origin is generally required for the discovery of novel drugs. The phytochemicals tested include alkaloid, tannin, flavonoid, total phenols and saponins. The quantities of these chemicals were 134.80 ± 0.28, 14.12 ± 1.32, 62.14 ± 0.10, 192.49 ± 4.45 and 40.82 ± 1.21 mg/g respectively. The results of the phytochemical screening showed compounds with different therapeutic effects. Ibrahim [11] reported similar phytochemicals in his study on sesame indica obtained from the market in Argungu town, Argungu local government area of Kebbi State, Nigeria.

Table 2: The quantitative phytochemicals present in the oil

Phytochemicals	Concentration (mg/g)
Alkaloid	134.80 ± 0.28
Tannin	14.12 ± 1.32
Flavonoid	62.14 ± 0.10
Total phenols	192.49 ± 4.45
Saponins	40.82 ± 1.21

Value reported as Mean ± SD for triplicate analysis n = 3

It has been reported that plants used for medicinal purposes are very rich in variety of bioactive compounds. Alkaloids play some

important metabolic role in living organisms, causing some physiological changes and are involved in protective function in animals, thus are used in making medicines. They have been shown to have important pharmacological functions such as anticancer, psychedelics and antimalarial, analgesic, antispasmodic and bactericidal, antioxidant and stimulating activities. Tannins, which are polyphenols, are importance because of their physiological potentials. They have been reported to exhibit antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antivirus, antidiarrheal, antihaemorrhoid, and antimalarial activities. Saponins are reported to exhibit broad range of pharmacological actions, such as ability to heal wounds and inflamed mucous membranes. It also has anti-hyper cholesterol and haemolytic effects [12]. The extract is rich in flavonoids, which are the most common polyphenols found in human diet and which have been implicated in many human diseases including lipid lowering, hepatoprotective, antiinflammatory, antioxidant, antimalarial and antimicrobial activities by acting as antioxidant [12,16].

The result in Table 3 is that of vitamin analysis carried out on the *sesame indica* oil.

Table 3: Vitamin composition of *Sesame indica oil*

Vitamins	Mean ± SD
Vitamin A (mg/g)	10.59 ± 0.14
Vitamin D (µg/100g)	12915.2 ± 104.3
Vitamin E (µg/100g)	132.5 ± 0.31

Value reported as Mean ± SD for triplicate analysis n = 3

The present study has shown the presence of vitamin A, vitamin D and vitamin E in the *Sesame indica* seed oil. The result obtained from the table 3 shows that the content of all the vitamins were taken in triplicate and their standard deviation were calculated for all the vitamins. Vitamin A was 10.59 ± 0.14 (mg/g), Vitamin D content was 12915.2 ± 104.3 (µg/100g) and the Vitamin E content was 132.5 ± 0.31 (µg/100g). This results suggest that the *Sesame indica* seed oil if consumed in sufficient amount would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition. The highest occurring vitamin from this research made was vitamin D, followed by vitamin E. The concentration of vitamin D in sesame indica shows a seasonal variation, which is related to the amount of sunlight available to convert 7-dehydrocholesterol in the animal's skin to cholecalciferol. Other plant sources are the lipid fraction of nuts, fruits, vegetables and cereal grains. Among the whole grains, maize has the highest total vitamin E content of about 8 mg/100 g. Animals store vitamin E in their fatty tissues, thus the main animal sources of the vitamin are high fat products such as eggs, butter, cheese and liver. The naturally occurring form of vitamin E in food is the free alcohol. Commercial vitamin supplements often contain vitamin È esters such as tocopheryl acetate or tocopheryl succinate. Approximately half of the human dietary vitamin A intake is derived from oil of plant seeds and vegetables containing α-, β- and γ-carotenes. *Sesamum indicum* L. seed oil is of unsaturated type and contains mainly the fatty acids oleic and linoleic. The oil can be classified in the oleic-linoleic acid group. High unsaponifiable matters content guarantees the use the oils in cosmetics industry.

4. Conclusion

This study showed that the sesame seed oil is a good source of phytochemicals and vitamins with good physicochemical properties. Overall, *Sesamum indicum* seed oil has nutritional, pharmacological benefits and properties that showed it could be used in the industry.

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