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Phytochemicals and Mineral Elements Composition of White *Sesamum indicum* L. Seed Oil

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Abstract: Some phytochemicals and mineral elements constituents of white *Sesamum indicum* seed oil were investigated. Oil was extracted from the white *Sesamum indicum* seed by the Soxhlet extraction method. The phytochemicals were analyzed using the standard methods of phytochemical analysis, and the elements were analyzed using an atomic absorption spectrophotometer by wet digest method. The oil has a percentage yield of 46.4%. The result of the qualitative phytochemical analysis revealed that the oil contains alkaloids, saponins, flavonoids, tannins, steroids, terpenoids, anthraquinone and phenols. Cardiac glycosides and phlobatannins are absent. The results of the quantitative analysis revealed that the oil contains alkaloids (132.80 ± 0.15 mg/g), flavonoids (59.20 ± 0.15 mg/g), saponins (42.80 ± 0.12 mg/g), tannins (17.01 ± 0.12 mg/g tannic acid equivalence) and total phenols (196.44 ± 3.47 mg/g gallic acid equivalence). The result of the mineral element analysis revealed that 100 g of the oil contains 9.40 ± 0.02 mg of zinc, 1.60 ± 0.04 mg of copper, 43.40 ± 0.03 mg of iron, 1.40 ± 0.02 mg of manganese and 0.00 mg of lead. These results showed that the white *Sesamum indicum* seed is a good source of oil. The extracted white *Sesamum indicum* seed oil contains an appreciable amount of phytochemicals. Therefore, this oil has pharmacological attributes. The white *Sesamum indicum* seed oil can serve as nutritional supplement for zinc, iron and copper, but a poor source of sodium, potassium and manganese.

Keywords: *Sesamum indicum*; sesame; seed; oil; phytochemicals; mineral elements.

1. Introduction

Plants are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human health (Aliyu *et al.*, 2008). The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Glombitza *et al.*, 1993; Mahabir and Gulliford, 1997). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are among others alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Essential plant oils have also been used medicinally at different periods in history (Watt, 1962). Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer (Gilman *et al.*, 1990).

Sesamum indicum belongs to the family Pedaliaceae. It is an annual crop grown between 1.6 and 3.3 ft high. It has opposite leaves 4 to 14 cm long. The flowers may vary in color with some being blue, white or purple (Faoun, 2012). The seeds are small in size ranging between 3 to 4 mm long by 2 mm wide and 1 mm thick. The seeds are oval in shape and slightly flattened (Putnam *et al.*, 2003). *Sesamum indicum* plant has received considerable attention around the world because of the nutritional and medicinal benefits of the seeds (Raghav *et al.*, 1990). It is an important oil seed crop cultivated in the tropics and temperate zones of the world (Biabani and Pakniyat, 2008). It is one of the oldest oil crops cultivated in Africa and Asia (Ali *et al.*, 2007). It is called 'sesame' internationally, while it is called 'benniseed' in West Africa, 'simsim' in East Africa and 'till' in India. Within Nigeria, it is called with different names in different localities. It is generally called '*ridi*' in the northern states (Aboje, 2011).

The oil, otherwise referred to as gingerly oil is an edible vegetable oil (Shanthasheela *et al.*, 2007) with very pleasant flavor and can be consumed without further purification (Lyon, 1972). It ranks second with regard to nutritional value after olive oil (Alpaslan *et al.*, 2001).

The *Sesamum indicum* has been widely used in the treatment of various infections caused by fungi, bacteria, viruses and parasites. Over 60% of people in Nigerian rural areas depend on traditional medicine for the treatment of their ailments. Plant derived medicines are widely used because they are cheap and widely available. White *Sesamum indicum* seed oil is also been used in traditional medicine. Therefore, this research aims at revealing the phytochemical and mineral element composition of the white *Sesamum indicum* seed oil in order to verify the claim of its medicinal potential. The mineral element analysis revealed some of its nutritional benefits.

2. Materials and Methods

2.1. Sample Collection and Preparation

White *Sesamum indicum* seeds were obtained from the market in Argungu town, Argungu local government area of Kebbi State, Nigeria. The seeds were cleaned, well dried and ground using a mortar and pestle. The powdered sample was then used for oil extraction.

2.2. Oil Extraction

The oil was extracted from the powdered *Sesamum indicum* seeds using a Soxhlet apparatus according to the method described by William (2007). The 150 mL of n-hexane was poured into the round bottom flask of the Soxhlet apparatus. Subsequently, 61.0 g of the sample was introduced into the thimble and fitted into the Soxhlet extractor. The apparatus was then assembled. The solvent in the set-up was heated to 50 °C and the vapor produced was subsequently condensed by flowing water in and out of the extraction set-up. This process of heating and cooling continued until sufficient quantity of sesame oil was obtained for about 4 hours. At the end of the extraction, the thimble was removed while the remaining solvent in the extractor was recharged into the round bottom flask for the process to be repeated.

The percentage oil yield was determined using the following expression:

$$\text{Oil content (\%)} = \frac{W_1}{W_2} \times 100$$

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.

2.3. Test for Tannins

The 5 drops of 0.1% ferric chloride was added to 2 mL of the oil extract, and a brownish green or blue-black coloration indicates a positive result (Sofowara, 1993).

2.4. Test for Phlobatannins

The 2 mL of the oil extract was boiled with 1% aqueous hydrochloride. Deposition of a red precipitate indicates a positive result (Trease and Evans, 1989).

2.5. Test for Saponins

The 2 mL of the oil extract was diluted with 2 mL distilled water. It was then agitated in a test tube for 5 min. About 0.1 cm layer of foam indicates a positive result (Mbatchou and Kosoono, 2012).

2.6. Test for Flavonoids

The 2 mL of 10% sodium hydroxide was added to 2 mL of the oil extract in a test tube. An intense yellow color was formed which turned colorless upon addition of 2 mL of dilute hydrochloric acid indicating a positive result (Mbatchou and Kosoono, 2012).

2.7. Test for Alkaloids

To 2 mL of the oil extract, 2 mL of 10% hydrochloric acid was added. To the acidic medium, 1 mL of dragendroff's reagent was added. An orange precipitate indicates a positive result (Mbatchou and Kosoono, 2012).

To 2 mL of the oil extract, 2 mL of mayer's reagent was also added. An orange precipitate indicates a positive result (Mbatchou and Kosoono, 2012).

2.8. Test for Steroids

The 2 mL of the oil extract was dissolved in 10 mL of chloroform, and then 10 mL of concentrated sulphuric acid was added by the side of the test tube. The upper layer turned red whereas, the sulphuric acid layer turned yellow with green fluorescence. This indicates the presence of steroids (Mbatchou and Kosoono, 2012).

2.9. Test for Terpenoids

The 2 mL of the oil extract was mixed with 2 mL of chloroform and 1 mL of concentrated H_2SO_4 was carefully added to form a layer. A clear upper and lower layer with a reddish brown interphase indicates a positive result (Mbatchou and Kosoono, 2012).

2.10. Test for Glycosides

The 2 mL of acetic acid was added to 2 mL of the oil extract. The mixture was cooled in cold water bath. The 2 mL of concentrated H_2SO_4 was added. Color development from blue to bluish green indicates the presence of glycosides (Sofowara, 1993).

2.11. Test for Anthraquinones

The 2 mL of the oil extract was boiled with 5 mL of 10% hydrochloric acid for 3 min. The 5 mL of chloroform was added. The 5 drops of 10% ammonia was further added. A rose pink coloration indicates a positive result (Harborne, 1998).

2.12. Test for Phenols

The 2 mL of the oil sample was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result (Harborne, 1998).

2.13. Determination of Total Alkaloid Content

The total alkaloid content was measured using Harborne, (1993) and Obadoni and Ochuko (2001). 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.

$$\text{Alkaloid (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}}$$

2.14. Determination of Total Flavonoid Content

The total flavonoid content was determined using the method of Harborne (1993). 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.

$$\text{Flavonoid (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}}$$

2.15. Determination of Total Saponin Content

This was measured using the method of Obadoni and Ochuko (2001). 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 °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. All samples were analyzed in triplicates.

$$\text{Saponin (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}}$$

2.16. Determination of 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.

2.17. Determination of 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 (Na_2CO_3) was added. The mixture was allowed to stand for 30 min at room temperature and the total phenolic content was determined spectrophotometrically 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.

2.18. Determination of Mineral Elements by Wet Digest Method

The elements were extracted from the oil by the wet digest method (Taiye and Asibey-beko, 2001; Vavidel and Janardhana, 2000). The digested sample was taken to the atomic absorption spectrophotometer (AAS) for measuring the concentration (ppm) of the elements Zn, Pb, Fe, Mn and Cu.

3. Results

The percentage yield of the extracted white *Sesamum indicum* seed oil was 46.4%, while the results of the qualitative phytochemical screening are presented in Table 1. The results of the quantitative phytochemical screening of the oil extract are presented in Table 2, and the mineral element composition of the white *Sesamum indicum* seed oil is presented in Table 3.

Table 1. Phytochemical constituents present in the white *Sesamum indicum* seed oil

Phytochemical	Result
Alkaloid	++
Flavonoid	++
Tannin	+
Saponin	++
Total Phenols	++
Anthraquinone	+
Cardiac glycoside	-
Terpenoid	+++
Steroid	+++
Phlobatannin	-

Note: +: Present in small concentration; ++: Present in a moderately high concentration; +++: Present in a very high concentration; -: Absent.

Table 2. Concentration of some phytochemical constituents present in the white *Sesamum indicum* seed oil

Phytochemical	Concentration (mg/g)
Alkaloid	132.80 ± 0.15
Saponin	42.80 ± 0.12
Flavonoid	59.20 ± 0.15
Tannin	17.01 ± 0.12
Total phenols	196.44 ± 3.47

Note: Values are presented as mean ± standard deviation of three replicates; mg/g - Milligram of phytochemical per one gram of white *Sesamum indicum* seed oil.

Table 3. Mineral element composition of white *Sesamum indicum* seed oil

Element	Concentration (mg/100 g)
Zinc (Zn)	9.40 ± 0.02
Potassium (K)	4.00 ± 0.01
Sodium (Na)	2.00 ± 0.01
Iron (Fe)	43.40 ± 0.03
Copper (Cu)	1.60 ± 0.04
Manganese (Mn)	1.40 ± 0.02
Lead (Pb)	ND

Note: Values are presented as mean ± standard deviation of three replicates; mg/100 g - Milligram of element per 100 g of white *Sesamum indicum* oil; ND - Not Detected.

4. Discussion

The percentage yield of the extracted white *Sesamum indicum* seed oil was 46.4%, and is comparable with that obtained by Mohammed and Hamza (2008) which was 48%. The seed oil content varies from 35 - 57% among the different varieties of *Sesamum indicum* seed (Tashiro *et al.*, 1990). The 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. *Sesamum indicum* seeds have higher oil content than most of the known oil seeds (Hwang, 2005). However, the oil has been ranked as 9th among the top thirteen (13) oil seed crops which make up 90% of the world population of edible oils (Adeola *et al.*, 2010). Variation in the oil yield may be due to the differences in climate, ripening stage, the harvesting time of the seeds and the extraction method used.

The result of the qualitative phytochemical analysis of the oil extract indicates that anthraquinones and tannins are present in small concentration. Alkaloids, flavonoids, saponins and phenols are present in moderately high concentration. Terpenoids and steroids are present in a very high concentration while phlobatannins and cardiac glycosides are absent.

The presence of terpenoids and steroids in large concentration in the *Sesamum indicum* seed oil may be related to their non-polar nature which obviously favors their increased concentration in the oil (Njoku *et al.*, 2010). The absence of cardiac glycosides in the oil may be related to their expected tendency to partition away from the oil due to their lipid insoluble nature (Njoku *et al.*, 2010).

From the results of the quantitative phytochemical screening, total phenolic content has the highest concentration of 196.44 ± 3.47 mg/g gallic acid equivalent (GAE). The concentration of flavonoids is 59.20 ± 0.15 mg/g. The presence of phenols and flavonoids indicates that the oil will be good for the management of cardiovascular diseases and oxidative stress because flavonoids and phenols are biological antioxidants. The presence of flavonoids in *Sesamum indicum* seed oil accounts for its antioxidant property as reported by Ramesh *et al.* (2005). The presence of flavonoids in *Sesamum indicum* seed oil also accounts for its use in inhibiting the replication of human colon cancer cells as reported by Salerno and Smith (1991). Flavonoids also provide protection against these diseases by contributing to the total antioxidant defense system of the human body. The natural *Sesamum indicum* seed oil has high stability due to the presence of high levels of these natural antioxidants (Lyon, 1972).

The second most abundant phytochemical in the *Sesamum indicum* seed oil is alkaloid with a concentration of 132.80 ± 0.15 mg/g. Alkaloids have been used as central nervous system stimulants, topical anesthetics in ophthalmology, powerful pain relievers, anti-puritic action, among other uses (Heikens *et al.*, 1995). This indicates that the white *Sesamum indicum* seed oil can be used as a component of topical or systemic pain relievers.

From the result, it was observed that the analyzed *Sesamum indicum* seed oil has a saponins concentration of 42.80 ± 0.12 mg/g. The presence of saponins in *Sesamum indicum* seed oil accounts for its use in maintaining high density lipoprotein cholesterol (HDL-C) levels and lower low density lipoprotein cholesterol (LDL-C) levels as reported by World Health Organization (WHO) in 1992. Saponins aid in reducing cholesterol levels by forming complexes with cholesterol and bile acids (Shereen, 2011) which prevent them from being absorbed through the small intestine, hence lowers the cholesterol level in the blood and liver. The presence of saponins in sesame may account for its use as an anti-cancer agent as reported by Philips *et al.* (2005). Saponins also serve as antioxidants as they prevent degeneration of DNA and also help to reduce colon damage and risk of cancer. According to Chavali and Campbell (1987), saponins are used as adjuvants in vaccines and oral intake of saponins has been used to help treat retroviral infection. They stimulate antibody production, inhibit viruses and induce the response of lymphocytes which are white blood cells that fight infection. The presence of saponins in *Sesamum indicum* seed oil makes it to be a good immune buster.

From the results, tannin has the least concentration of 17.01 ± 0.12 mg/g tannic acid equivalence. The presence of tannin in *Sesamum indicum* seed oil accounts for its antibacterial and antifungal properties as reported by Shittu *et al.* (2007). Tannin has antibacterial, antiviral, and astringent properties (Akiyama *et al.*, 2001; Liu *et al.*, 2004).

The presence of terpenoid in the white *Sesamum indicum* seed oil is comparable to the result obtained by Njoku *et al.* (2010) which also reveals a large presence of terpenoid in white *Sesamum indicum* seed oil. The presence of terpenoids in white *Sesamum indicum* seed oil also accounts for its use as an anti-diabetic agent as reported by Ramesh *et al.* (2005). Terpenoids also act as antibiotics to protect plants from pathogenic microorganisms (Aliyu *et al.*, 2008). This also accounts for the use of *Sesamum indicum* seed oil as an antibacterial against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans* as reported by Shittu *et al.* (2007). Terpenoid is a heart-friendly phytochemical constituent which helps to reduce diastolic blood pressure and lowers the sugar level in the blood (Hawkins and Ehrlich, 2006).

The presence of steroids in white *Sesamum indicum* seed oil is comparable to the result obtained by Njoku *et al.* (2010) which also reveals a large presence of steroids in white *Sesamum indicum* seed oil. Steroidal compounds are of importance and interest in pharmacy due to their relationship with compounds such as sex hormones (Okwu, 2001). Intake of estrogen alone or an estrogen-progesterone combination is effective and safe at reducing menopausal decline (Ryan *et al.*, 2008). The presence of steroids in the white *Sesamum indicum* seed oil is an indication that it contains compounds that are related to sex hormones.

The analysis of the mineral element composition of the white *Sesamum indicum* seed oil reveals the presence of zinc, iron, potassium, sodium, copper, lead and manganese. The most abundant element found in the white *Sesamum indicum* seed oil is iron with concentration of 43.20 ± 0.03 mg/100 g. Thus, white *Sesamum indicum* seed oil is a good source of iron.

The second most abundant element found in the white *Sesamum indicum* seed oil is zinc with concentration of 9.40 ± 0.02 mg/100 g. Therefore, the white *Sesamum indicum* seed oil can serve as a nutritional supplement for zinc. Zinc is an essential trace element which plays many biological roles.

The copper content of the white *Sesamum indicum* seed oil is 1.60 ± 0.04 mg/100 g. Therefore, the white *Sesamum indicum* seed oil can serve as nutritional supplement of copper especially for children. Copper is an essential constituent of several enzymes, such as cytochrome oxidase, catalase, tyrosinase, superoxide dismutase etc. Deficiency of copper causes demineralization of bones, demyelination of neural tissue, fragility of arteries, myocardial fibrosis etc. (Lippard and Jeremy, 1994).

The manganese content of the white *Sesamum indicum* seed oil is 1.40 ± 0.02 mg/100 g. Manganese functions as a cofactor for several enzymes, such as arginase, pyruvate carboxylase, isocitrate dehydrogenase, superoxide dismutase and peptidase (Nielsen and Forrest, 1999). Manganese is also required for the formation of bone, proper reproduction and normal functioning of the nervous system (Lippard and Jeremy, 1994). Hence, white *Sesamum indicum* seed oil can be a good source of manganese.

The sodium and potassium contents of the white *Sesamum indicum* seed oil are 2.00 ± 0.01 mg/100 g and 4.00 ± 0.01 mg/100 g, respectively. Therefore, the white *Sesamum indicum* seed oil is a poor source of sodium and potassium, and cannot serve as nutritional supplement for these elements. Hence, this oil can be recommended for hypertensive patients.

Lead has not been detected in the white *Sesamum indicum* seed oil, which indicates that the oil is safe for consumption.

5. Conclusions

The white *Sesamum indicum* seed is a good source of oil because it has high oil content. The phytochemical constituents present in the oil are generally moderate or in high concentration. Therefore, this has the advantage of inferring pharmacological attributes on the oil. The oil is a good source of iron, zinc and copper because the concentration of these elements in the oil meet up with the adequate quantity needed by the body daily. Thus, the white *Sesamum indicum* seed oil has both nutritional and pharmacological benefits and being free from lead, it is safe for human consumption.

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