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# Proximate Composition and Physicochemical Properties of *Hyptis spicigera* Seed and Oil

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## ABSTRACT

The plant *Hyptis spicigera* is a medicinal plant used traditionally to treat ailments such as headaches, and colds, and has been used as insects' repellent. The seeds contain significant amount of lipids. There appears to be few reports of the chemical composition ofthe seeds of *H. spicigera*. Therefore, proximate composition (moisture, ash, protein, fibre, lipid, and carbohydrate contents) of the seeds of local *H. spicigera* and physicochemical characteristics (colour, odour, pH, refractive index, acid value, iodine value, peroxide value, and saponification value) of Soxhlet extracted oil were determined in. The results showed the seeds to have moisture (3.50±0.5%), ash (5.33±0.76%), lipids (59.00±0.73 %), protein (20.56±0.22 %), carbohydrate (7.36±0.14%), fibre (4.25±0.78 %) and significant amounts (g/100 g protein) of 10 essential amino acids and vitamin E (1.32±0.05mg/g). The seed oil was liquid at 25 $^{o}$C (room temparature), amber with unobjectionable odour, pH of 6.32±0.21, RI 1.73±0.80, acid value 10.53 ± 0.13 mg/g, iodine value of 11.07 ± 0.14 g$I_2$/g, peroxide value of 50 ± 1.0 mEq/kg and saponification value of 176.4 ± 10.7 mgKOH/g. The results suggest *H. spicigera* seed contains an appreciable amount of essential amino acids and its oil could be consumed, used in industry for soap making and biodiesel production.

**Keywords**: *Hyptis spicigera*, Seed and oil, Proximate composition, Physicochemical properties.

## INTRODUCTION

*Hyptis spicigera* of *Lamiaceae* family commonly known as black baniseed or black sesame is locally called 'Bunsurun fadama' or 'doddoyan daji' in Hausa language. It is an erect aromatic herbaceous annual plant with tiny brown seeds.The plant has close to 400 species and is found around Senegal to western Cameroon, possibly native to Brazil, now widely naturalized in tropical Africa including Nigeria, and also found in Asia (Takayama *et al*., 2011; de Almeida *et al*., 2015). *H. spicigera* has been reported to have reinvigorating, embalming, and repellent effect, and treats skin diseases, cough, bronchitis, gastric ulcer, migraine, headaches, pains, colds, and catarrh (Onayade *et al*., 1990; Takayama *et al*., 2011). It is used locally in Nigeria for many purposes (undocumented) such as treatment of headacheand fever by sniffing it, hot water bath (aerial parts), spices (leaves), etc.The oil has been reported to have a gastroprotective effect (Pinheiro *et al*., 2015; Takayama *et al*., 2011) and anti-inflammation (Simões *et al*., 2017).It has been established that plants display variation in the concentration of their bioactive phytochemicals as a result of factors such as climate, geographical location, season, and nature of the soil (Chadwick and Marsh, 2008; Muraina *et al*., 2008). Hence, there could be variation in the chemical composition of local *H. spicigera* in the northern parts of Nigeria when compared with that from other parts of the country and any other place where the plant can be found. There appeared to be little literature on the evaluation of the physicochemical characteristics of local *H. spicigera.* Driven by the rise in the interest in naturally occurring compounds in plants and their extracts, there is continued rapid growth in the exploration of plants especially those known to be used traditionally for health-promoting purposes such as treatment of cardiovascular diseases, cancers, diabetes, etc. (Newman and Cragg, 2012). Therefore, in this study, some physical and chemical characteristics of the seeds of local *H.spicigera* and its extracted oil were determined to serve as a base-line for further studies.

## MATERIALS AND METHODS

### Reagents and Chemicals

All chemicals used in the study were of 90-98%purity, and of analytical grade. Sulphuric acid, sodium hydroxide, boric acid, N-hexane, hydrochloric acid, carbon tetrachloride, potassium iodide, Starch indicator, phenolphthalein, potassium iodide, petroleum ether, and acetic acid were all purchased from ABBACHEM Trading's Limited, Nigeria.

### Sample Collection and Seed Preparation

The dried *H. spicigera* plant was obtained from Badariya village, Kalgo Local Government, Kebbi State. The seeds were removed from the plants by mechanical shaking and crushing the flower and then further separated by winnowing. The dried seeds were washed, dried and then ground into powder. The powder was kept at room temperature until analysed.

### Extraction of Oil

Oil was extracted from *H. spicigera* seed powder by Soxhlet using n-Hexane as the solvent according to the method of Association of Official Analytical Chemists (AOAC, 2005). Fifty grams (50 g) of the powder was packed into a weighed thimble, which was introduced into the Soxhlet system. About 250 mL of the solvent (n-Hexane) was poured into the flask. The set up was damped and heated on a heating mantle. The extraction processes were carried out in six hours at the temperature of n-Hexane (60°C). Solution obtained from the extraction was regained through a reflux process for 15 minutes after which the content was evaporated by increasing the temperature to 70°C for about half an hour to obtain brownish oil at room temperature. Percentage yield of the oil was calculated by dividing the weight of oil obtained by the weight of the sample (50g) multiplied by

100, which served as the percentage crude lipid. The pH of the oil was determined using a digital bench top pH meter (pH/mV/ISE) and was then subjected to further analysis.

**Proximate Composition of *Hyptis spicigera* Seeds**

**Determination of Moisture Contents**

The moisture content of the *H. spicigera* seed powder was determined by air-oven method, according to a method described previously (Ghatak and Panchal, 2010) with slight modification. Five grams (5g) of the powder was weighed in previously dried, weighed and tared dish. The dish was heated for 1 hour at 105$^{o}$C. The dish was then removed, cooled in a desiccator and then weighed. The dish was heated again for another 1 hour, allowed to cool down before being weighed again. This was repeated until the change in weight between observations was less than 1mg. The moisture content (percentage by weight) was calculated by

$\frac{W1}{W}$ X 100

Where W1 = weight loss (g) of the sample on heating; W = weight of the sample (g) taken.

**Determination of Fibre Content**

Crude fibre was determined according to AOAC, (2005). Two grams (2 g) of the powderwas weighed and extracted three times with light petroleum ether by stirring, settling and decanting.The air-dried extracted sample was transferred to a dry 100 mL conical flask, 80 mL of 0.1275 M sulphuric acid was measured at room temperature and brought to its boiling point. This was boiled in a fume cupboardfor 30 minutes, while a constant volume was maintained, the flask was rotated every few minutes in order to mix the contents and remove particles from the sides. Buchner funnel was fixed to a perforated plate and to the funnel a filter paper was also fixed to cover the holes in the plate. The mixture was poured immediately into the prepared funnel. The funnel was adjusted so that filtration is completed within 10 minutes after which the insoluble matter was washed with warm water several times. It was then transferred back to the conical flask and 0.313 M sodium hydroxide (80 mL) was added. The mixture was boiled for 30 minutes, allowed to stand for 1 minute and then filtered immediately. The insoluble material was then transferred to the filter paper then washed with 1% hydrochloric acid and washed again with boiling water until free from acid. The residue was washed twice with ethanol and three times with ether, the insoluble matter was transferred to a dried weighed crucible and dried at 100°C to a constant weight the crucible and its content was placed on a heating mantle in a fume cupboard to burn off the organic matter. It was transferred to a muffle furnace at 550°C for 3 hours. After cooling, the fibre content was then determined by using the relationship:

Crude fibre (%) = $\frac{w_1 - w_2}{w_3} \times 100$

Where $W_1$= weight of sample extraction + filter paper, $W_2$= weight of $w_1$ after ashing, $W_3$= weight of sample used.

**Determination of Ash Content**

Ash content was determined according to AOAC (2005) method. A clean dried crucible was weighed as ($W_1$). Two grams (2g) of the sample was placed into the crucible and weighed ($W_2$). The sample was placed in the muffle furnaces set at 550-600 $^{o}$C. The ash was covered with Petri-dish and placed in a desiccator prior to weighing. This was then weighed as ($W_3$). The percentage ash content was calculated as follows

Ash (%) = $\frac{Weight\ of\ ash}{Weight\ of\ sample}$X 100 = $\frac{w_3 - w_1}{w_2 - w_1} \times 100$

**Determination of Crude Protein Content**

Crude protein was determined by Kjeldahl method. Briefly, 2 g of the sample powder were weighed and transferred into Kjeldhal's digestion flask into which 1 g catalyst and 20 mL concentrated sulphuric acid were added. The mixture was heated for 1 hour until the liquid becomes clear. The digest was cooled down and made alkaline with 15 mL 40% NaOH. Ammonia was steamed and distilled into 2% boric acid (10 mL) with 5 drops of methylene red indicator for 15 minutes. The Distilled ammonia was then titrated with 0.02M Hydrochloric acid. The % crude protein was calculated as follows

Protein (%) = % Nitrogen x 6.25

% Nitrogen=

$$\frac{(vol\ of\ HCl\ X\ its\ normality - vol\ of\ NaOH\ X\ its\ normality) X\ 1.4007}{Weight\ of\ sample}\ x\ 100$$

6.25 = specific factor

**Determination of Amino Acids Content**

The amino acid profile was determined using the method described by Benitez (Benitez, 1989). Exactly 500 mg of the sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator. Finally, the prepared sample was loaded into the Applied Biosystems PTH Amino Acid Analyzer according to manufacturer instructions.

**Determination of Carbohydrate Content**

The total carbohydrate was determined by difference according to AOAC (2005) and was calculated as % carbohydrate = 100 - (% moisture + % Ash + % Fat + % Protein + %Fibre)

**Determination of Vitamin E**

The total vitamin E of *H. spicigera* seed was determined according to previously described method (Rutkowski *et al*., 2005) with slight modification. One gram (1 g) of sample was dissolved in 10 mL distilled water and allowed to dissolve for 1 hour. Then, 1 mL of the solution was transferred into centrifuge tube I and 0.5 mL of anhydrous ethanol was added and shaken vigorously for 1 minute after putting a tight stopper. Exactly 3 mL of xylene was then added and shaken vigorously for

another 1 minute. The test tube was then centrifuged at 1500 g for 10 minutes. Simultaneously, 0.25mL solution of bathophenanthroline was measured into test tube II and 1.2 mL of supernatant of test tube I was added and mixed. $FeCl_3$ (0.25 mL) was added and mixed, then 0.25 mL $H_3PO_4$ was added and mixed again. A standard solution (1mL) was similarly prepared but deionized water was added instead of anhydrous ethanol at the beginning of the analysis without centrifuging. Absorbance of the test sample Ax and that of standard at 530 nm were recorded. Vitamin E concentration was calculated as

$$\text{Vitamin E (mg/g)} = \frac{Ax}{As} x Cs$$

Ax = absorbance of sample, As absorbance of standard, Cs= concentration of standardin mg/g.

**Physicochemical Characteristics of Extracted *H. spicigera* Seed Oil**

**Determination of Refractive Index**

The refractive index of the oil was determined using Abbe refractometer at 25 $^oC$ (AOAC, 2005). Few drops of the oil were placed, ensuring complete cover and avoiding bubbles, on the polished surface of the measuring prism. The illuminating prism was brought into contact with the measuring prism and reading was taken. The procedure was repeated thrice and readings were taken.

**Determination of Acid Value**

Acid value was determined according to AOAC method. Two grams (2g) of the oil was weighed, dissolved in 50 mL of neutral solvent and the transfered into a 250 mL conical flask. Few drops of phenolphthalein were added and the contents titrated against 0.1 N potassium hydroxide. The mixture was shaken gently until a pink colour which persists for fifteen seconds was obtained. The acid value was then calculated as

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titrate value x Normality of KOH}}{\text{Weight of the sample (g)}} x\ 56.1$$

**Determination of Iodine Value**

Iodine value of the oil was determined according to the AOAC method. The oil sample (250 mg) was weighed into an Iodine flask and 10mL of chloroform was dissolved, 25 mL of iodine solution was added then drained in a definite time, mixed, and allowed to stand in a dark cupboard for about 30 mins with occasional shaking. Afterwards, 10 mL of 15% KI was added and shaken thoroughly then 100 mL freshly boiled and cooled water was added then free Iodine on the stopper was washed down. The mixture was then titrated against 0.1 N sodium thiosulphate until the yellow solution turned colourless. A few drops of 1% starch (as indicator) were added and again, it was titrated until the blue colour disappeared completely. Iodine value was calculated by

$$IV = \frac{12.69\ X\ N\ (V2 - V1)}{W}$$

Where N = normality of sodium thiosulphate, V2= volume (mL) of sodium thiosulphate for blank, V1= volume (mL) of sodium thiosulphate consumed by sample and W= weight (g) of sample, 12.69 is a constant based on grams of iodine in 0.1 mol iodine solution.

**Determination of Peroxide Value**

Peroxide value of the oil was determined according to AOAC (1990). One gram of the extracted oil was weighed into a clean dry tube and 1 g of potassium iodide was added to 20 mL of solvent mixture of glaciated acetic acid and chloroform (3:2 volume/volume, [v/v]). The tube was placed into boiled water, the liquid boiled rigorously within 30 seconds. The content was transferred quickly into a conical flask containing 20 mL of 5% potassium iodide solution. Each time the tube was washed twice with 25 mL of water and collected into the conical flask. The resulting solution was then titrated against 0.1 M sodium thiosulphate solution until the yellow colour disappeared after which 0.5 mL of 1% starch solution was added, shaken vigorously then titrated carefully until the blue colouration disappeared. A blank without the oil was also determined. PV (meq/Kg) was calculated as follows:

$$\text{PV (meq/Kg)} = \frac{M\ x\ (V - V0)\ x\ 1000}{\text{Weight of sample (g)}}$$

Where M is the molar concentration of sodium thiosulphate, V is the volume of titrant for sample and $V_0$ volume of titrant for blank.

**Determination of Saponification Value**

Saponification value of the oil was determined according to AOAC (2005). Briefly, 4 g of oil was weighed into a flask into which 50 mL of alcoholic KOH was added. A blank was prepared by taking 50 mL of alcoholic KOH without sample. The reaction was refluxed using a condenser on a water bath at 100 $^oC$ for 1 hour. The mixtures were allowed to cool, 1 mL phenolphthalein indicator added then titrated against 0.5 N HCl until the pink colouration disappeared. The number of mL of 0.5N HCL used were noted.

The saponification value was calculated from the titre value as follows:

Saponification value (mg KOH/g) =

$$\frac{28.05\ \times\ (\text{titre value of blank} -\ \text{titre value of sample})}{Weight\ of\ sample\ (g)}$$

**Statistical Analysis**

Data are presented as mean ± SD of triplicate determinations and descriptive statistics was conducted using Microsoft Excel (2016).

**RESULTS AND DISCUSSION**

The proximate composition, vitamin E and amino acid contents of *H. spicigera* seed are presented in Tables 1 and 2. The moisture content of the seed of *H. spicigera* was lower than those reported for most seeds. Moisture content has been reported to influence the storage longevity of seeds (Vertucci and Roos, 1990). Such that, the lower the moisture, the more likely the seed to survive

storage over a long period (Ellis *et al*., 1991) and could suggest high oil contents. Ash content of *H. spicigera* seed was 5.33±0.76% and is higher than values previously reported for different sesame cultivars (Paroha *et al*., 2014) suggesting the mineral contents of *H. spicigera* seeds as noted in this study could be higher than previously reported. Interestingly, the lipid content (59.00±0.73%) of *H. spicigera* seed was found also to be higher than most previously reported (Ladan *et al*., 2010, 2011; Conti *et al*., 2011). The higher lipid obtained could be due to variation in extraction method specie of the plant, climate, and geographical location.

**Table 1:** Proximate and vitamin E composition of *H. spicigera* seed

| Parameter | *Hyptis spicigera* seed |
|---|---|
| **Moisture (%)** | 3.50±0.50 |
| **Ash (%)** | 5.33±0.76 |
| **Lipid (%)** | 59.00±0.73 |
| **Fiber (%)** | 4.25±0.78 |
| **Protein (%)** | 20.56±0.22 |
| **Carbohydrates (%)** | 7.36±0.14 |
| **Vitamin E (mg/g)** | 1.32±0.05 |

Values are presented as mean ± SD. Samples were of 3 replicates

Hence, the locally sourced *H. spicigera* seed could be a good source of oil. The fibre content is low but higher than fibre contents of brebra (Andualem and Gessesse, 2014), rice (Dodevska *et al*., 2013), rye grain, wheat and barley (Grausgruber *et al*., 2004). Moreover, the low carbohydrate contents indicate the high oil and to some extent protein contents as major energy source in *H. spicigera* seed. However, the carbohydrate content compares with that found in brebra seed (11.92%) (Andualem and Gessesse, 2014) and but higher than that of cashew nut (1.4%) (Akinhanmi *et al*., 2008). The vitamin E content of *H. spicigera* seed was lower than that reported to be present in the oil (Ladan *et al*., 2010) (186.15 mg/mL) but was higher than that of soybean oil (1.13mg/g), rapeseed oil (0.95mg/g) and sunflower oil (0.75mg/g) (Xiao and Li, 2020) indicating the plant to be a rich source of vitamin E. The protein content, though lower than in some previous reports is within the range of 13.5-26.8% for protein-rich seeds such as quinoa (Ogungbenle *et al*., 2009), bambara groundnut (Yagoub and Abdalla, 2007), cowpeas (Ragab *et al*., 2004). Interestingly, the seed contains an appreciable amount the essential amino acids (Table 2).

Leucine (5.60±0.40 g/100g) had the highest concentration and tryptophan (0.68±0.07 g/100g) the least followed by methionine (0.91±0.20 g/100g). Among the nonessential amino acids, glutamic acid (9.54±0.44 g/100g) was the highest in concentration and cysteine the least. Essential amino acids formed 29.4 g/100g, while nonessential 30.6 g/100g. The essential to nonessential ratio was 0.96 and the total amino acid was 60 g/100 g. This total amino acids of *H. spicigera* seed are higher than that of soybean (36.62 g/100 g) and brebra (44.0 g/100 g) (Andualem and Gessesse, 2014) which are the commonly consumed legumes. Therefore, *H. spicigera* seed could serve as a potential source of protein. The Proximate compositions of *H. spicigera* seeds and oil were determined.

The physicochemical characteristics of *H. spicigera* seed oil are shown in Table 3. The extracted oil appeared amber in colour with a non-objectionable odour with pH 6.32±0.21, and refractive index (RI) of 3.73±0.80 (Table 3). Furthermore, the oil was liquid at 25 $^{o}$C which suggests the oil most likely contains more unsaturated fatty acids than saturated ones. Reports showed that oils with high amounts of unsaturated fatty acids are healthier than those containing predominantly saturated ones (Dietary Guidelines Advisory Committee Reports, 2015; Nicolosi, 1997). The observed pH of *H. spicigera* oil was 6.32±0.21, hence is weakly acidic. pH is an important quality indicator for edible oils and most oils are weakly acidic except for olive oil which is the most alkaline oil. The refractive index (RI) of oils reflects their purity, fatty acids molecular weight, chain length, unsaturation and degree of conjugation (Nichols and Sanderson, 2003). RI value changes with a change in triglycerides content of oil rather than fatty acids (Shahidi, 2005). The RI obtained in this study (1.73±0.80) is higher than previously reported 1.39 (Usman *et al*., 2020) and 1.43 (Rai *et al*., 2013) for *Hyptis suaveolens* and 1.50 for *H. spicigera* (Ladan *et al*., 2010) seed oils. The predominant fatty acids in *H. spicigera* seed oil as reported by Ladan *et al*. (2010) were linoleic, stearic, and 1-undecanol. Therefore, even though the fatty acid profile was not determined, the oil in this study could contain a higher amount of these fatty acids on its triglycerides, thus the high refractive index.

The acid value, an important marker of oil rancidity is due to the hydrolysis of triglycerides. The value obtained was 10.53 ± 0.13mg KOH/g which is above the permissible limit of 0.6mg KOH/g (Alimentarius, 1999) making the oil at risk of rancidity within a short period of storage. However, the high acid value could be due to high triglycerides as suggested by the high refractive index. The iodine value (IV) measures the relative degree of unsaturation in fatty acids. The IV of *H. spicigera* seed oil is lower than previously reported at 81.22g $I_2$/100g (Ladan *et al*., 2010) for *H. spicigera* and 115.80 g $I_2$/100g for *H. suaveolens* (Rai *et al*., 2013). *H. spicigera* seed oil is considerably less saturated than coconut oil (IV 7.7-10.5). Even though, the lower IV indicates higher saturation of oil, the oil obtained was liquid at room temperature which on the other hand indicates the presence of polyunsaturated fatty acids. Moreover, the lower IV suggests the oil may not be easily susceptible to oxidation posed by higher IV from high unsaturation. The peroxide value (PV), a measure of oxidation products of oil was higher than 1.95 mEq $H_2O_2$ reported by Ladan *et al*. (2010). However, the high PV may be explained by high triglycerides as reflected by high RI. The saponification value on the other hand was higher than

that of *H. spicigera* seed oil previously reported (Ladan *et al*., 2010). This suggests the oil could be suitable for soap-making purposes, making it of economic importance.

**Table 2:** Amino acids composition of *H spicigera* seed

| Amino Acids | Concentration: g/100g protein |
|---|---|
| **Essential** | |
| **Arginine** | 4.64±0.14 |
| **Leucine** | 5.60±0.40 |
| **Lysine** | 3.02±0.43 |
| **Isoleucine** | 3.14±0.33 |
| **Phenylalanine** | 3.37±0.38 |
| **Tryptophan** | 0.68±0.07 |
| **Valine** | 3.51±0.30 |
| **Methionine** | 0.91±0.20 |
| **Histidine** | 1.53±0.36 |
| **Threonine** | 3.00±0.50 |
| **Nonessential** | |
| **Proline** | 3.35±0.39 |
| **Tyrosine** | 2.58±0.56 |
| **Cysteine** | 0.60±0.04 |
| **Alanine** | 3.41±0.35 |
| **Glutamic acid** | 9.54±0.44 |
| **Glycine** | 2.90±0.51 |
| **Serine** | 2.70±0.29 |
| **Aspartic acid** | 5.52±0.49 |

Values are mean ± SD of 3 replicates determination.

**Table 3: Physicochemical** characteristics of *H. spicigera* seed oil

| Parameters | Inference |
|---|---|
| **State at room temperature(25$^{o}$C)** | Liquid |
| **Colour** | Amber |
| **Odour** | Unobjectionable |
| **pH** | 6.32±0.21 |
| **Refractive index (RI)** | 1.73±0.80 |
| **Acid value (mg KOH/g)** | 10.53 ± 0.13 |
| **Iodine value ($gI_2$/g)** | 11.07 ± 0.14g |
| **Peroxide value (mEq/kg)** | 50 ± 1.0 |
| **Saponification value (mgKOH/g)** | 176.4 ± 10.7 |

Data presented as mean ± SD of triplicate determination.

## CONCLUSION

This study revealed that *H. spicigera* has an appreciable amount of essential amino acids hence, is a good source of protein. The oil quality is likely to deteriorate under long term storage due to its high acid value even though this could be addressed through refining. The physicochemical properties of the seed oil suggest the oil could be a potential raw material for soap making, biodiesel production and for domestic use. Further studies on the fatty acid profiles as well as vitamins and mineral contents are recommended to explore the nutritional benefits of *H. spicigera* seed oil.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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