Journal of Biosciences and Medicines, 2024, 12, 167-186
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Nutritional Composition, Physico-Chemical Properties and Phytochemical Analysis of Oil from *Cucumeropsis mannii* (Sefophe) Seeds from Takatokwane in Botswana

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How to cite this paper: Tibe, O., Thatitswe, S., Letswee, K.M., Desse, H.G., Gaba, T., Muzila, A. and Batlhophi, M. (2024) Nutritional Composition, Physico-Chemical Properties and Phytochemical Analysis of Oil from *Cucumeropsis mannii* (Sefophe) Seeds from Takatokwane in Botswana. *Journal of Biosciences and Medicines*, **12**, 167-186.
https://doi.org/10.4236/jbm.2024.1212014

Received: August 19, 2024
Accepted: December 7, 2024
Published: December 10, 2024

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Abstract

Botswana is well endowed with abundant vegetable oil producing plants which have not been fully exploited on the nutritional and phytochemical profile. The communities use these plants for food and medicine. Nutrient data (g/100g) obtained showed that *Cucumeropsis mannii* seeds had moisture 3.89, crude protein 9.07, ash content 3.20, crude fiber 21.26, total carbohydrate 18.14. The oil yield in the seeds was 44.4%. The high oil content is an indication that these seeds are a good source of oil. The mineral content (mg/100g) were as shown: Na (4.44), K (537.9), Mg (93.15), Ca (71.13), Mn (1.89), Fe (12.79), and Zn (5.55). Mineral analysis indicated that the seed contains important macro and micro minerals, which play a significant role in protection of the body from diseases. Oil from *Cucumeropsis mannii* seeds was also extracted and characterized using Official Methods of Analysis of the Association of Official Analytical Chemist (AOAC) methods for physicochemical parameters, phytochemical screening, and quantitative analysis. The results revealed that the saponification value (mg KOH/g), acid value (mg KOH/g), peroxide value (mg peroxide/kg), specific gravity, and refractive index were 200.11, 9.54, 1.59, 0.94, and 1.46, respectively. The high saponification value demonstrated that the oil may be suitable for soap making. The low acid value indicated that the oil was edible; while the low peroxide value indicated that the oil was less susceptible to oxidation and rancidity, deterioration, and off flavours at room temperature. With respect to phytochemicals, they were all present in the oil extracts. The total flavonoid content was 2.21 mg/g catechin equivalent. The

total phenol content was 1.72 mg GAE/g. The radical scavenging activity was 94.3 ± 10.8 VCEAC/100g. Phytochemicals are active biological compounds found in plant and they are source of medicinal agents which are beneficial to the human body.

Keywords

Cucumeropsis manni, Oil, Extract, Phenols, Antioxidant

1. Introduction

Botswana is well endowed with abundant vegetable oil producing plants which have not been fully exploited. Some plants can be domesticated and commercialised to contribute to the economy of this country. These plants can be used to address problems of food insecurity, poverty, and improve the socio-economic status of the communities where they are found [1]. The communities use these plants for food and medicine [2]. Edible oils, derived from vegetable seeds or crop seeds, have made an important contribution to the diet of people in many countries including Botswana, serving as a good source of essential fatty acids for human nutrition [3]. Currently, vegetable oil consumption in Botswana is based on grapeseed, soybean, sunflower, rapeseed, palm and olive oil. The demand for world's vegetable oil is growing rapidly due to increasing human population, emerging wars or conflicts, climate change, and the expanding oil-chemical industry, thus resulting in the need to search for newer and under-utilized non-conventional vegetable or crop oil sources [4] [5]. The fatty acids in vegetable oils are long, straight chain, saturated and unsaturated monocarboxylic acids. These fatty acids usually occur in nature as water-insoluble lipids (fats and oils). As a result, this study aimed for full exploitation of local natural resources as valuable sources of edible oil and its potential application in the food, cosmetic and pharmaceutical industries. Up to now, there is an inadequate exploitation of natural resources for industrial application and establishment of manufacturing industries. *Cucumeropsis mannii*, Mann's Cucumeropsis, a white seeded melon, is an annual, herbaceous, climbing crop, belonging to the *Cucurbitaceae* family. It has a light green and pubescent stem. The leaves are alternate, simple, acuminate, broadly cordate, deep green, with three regular lobes, evenly serrate. Flowers are mono-ecious and yellowish in colour. The fruits are yellow green, spherically elongated. The flesh is white and it is not edible. The seeds are white, flat, and smooth. Seed oil of *Cucumeropsis mannii* is rich in linoleic acid whereas the residue is fed to animals or used in the preparation of snacks [6] [7]. The seed oil consists of linoleic acid 64.9%, oleic acid 12.4%, stearic acid 11.8% and palmitic acid 10.9% [8]. [9] also confirmed that *C. mannii* seed oil possessed linoleic 62.14%, oleic 13.7%, stearic 8.3%, palmitic acid 10.6%. [8] stated that plant oils are good source of compounds that decrease the risk of chronic diseases due to free radical

scavenging activities. According to [10], kgengwe (*Citrulus lanatus*) seed powder, a melon, with balanced nutrient compositions was discovered to have anti-atherogenic properties, which could have been mediated through changes in the inflammatory pathways. Benefits of oils also include the repair of worn-out tissues, new cell formation as well as source of energy. Free radicals produced by metabolic processes of the body, are also created by exposure to x-rays, ozone, smoking, pollution, and industrial toxins, among other external factors [11]. Free radicals are highly reactive entities which are unstable and owing their reactivity to their unpaired electron in their outermost shell. Important cellular components like DNA or the cell membrane may be damaged, which impairs cell function and leads to lifestyle diseases like hypertension, diabetes, atherosclerosis, cancer, stroke, inflammatory joint disease, senile dementia, and degenerative eye disease. Antioxidants, however, can interact freely with free radicals safely [2], remain stable because of delocalized electrons, and terminate the chain reaction before important biomolecules are destroyed [12] Apart from food and medicine, research conducted by [13] has demonstrated that several melon seeds could be used as feedstock for biodiesel production and heavy metals removal agent. Although studies have been conducted on the nutritional, physicochemical and phytochemical properties of some melon species in the past, there is still paucity of information available on the physicochemical and phytochemical properties of oil extracted from *Cucumeropsis mannii* species that is peculiar to the environment of Takatokwane in Botswana. Therefore, this study aims to determine the nutritional composition of seeds, physicochemical and phytochemical properties of oil extracted from white melon seeds. It is, thus, extremely important to extract, characterize and identify the bioactive compounds (phytochemicals/antioxidants) from the seed oil and provide recommendations to enhance their contribution to sustainable diets, pharmaceuticals, and cosmetics.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh, unbroken and in-season seeds of *C. mannii* were collected from Takatokwane village located in the Kweneng West region of Botswana (Latitude—24,00252S 24˚0'9,0558"S, Longitude 24,31102E 24˚18'39,67632"E). Whole plant with melons from which seeds were sourced was identified and verified by a Botanist at the Botswana University of Agriculture and Natural Resources. The seeds were wrapped in a polyethene bag, submerged in ice cubes (to retain moisture) and brought to the university's Food Science Technology laboratory. They were then washed with distilled water and de-shelled (for those allotted for phytochemical profiling). Before analysis, the choice seeds were brought to oven-dried at a temperature of 50˚C to prevent degradation of phytochemicals and then crushed using a grinder. The pounded sample was sieved until 0.425 mm mesh passable. These were kept in a labelled, sealed container and left in a cool dry place awaiting proximate, mineral, physico-chemical and phytochemical analysis (**Figure 1**).

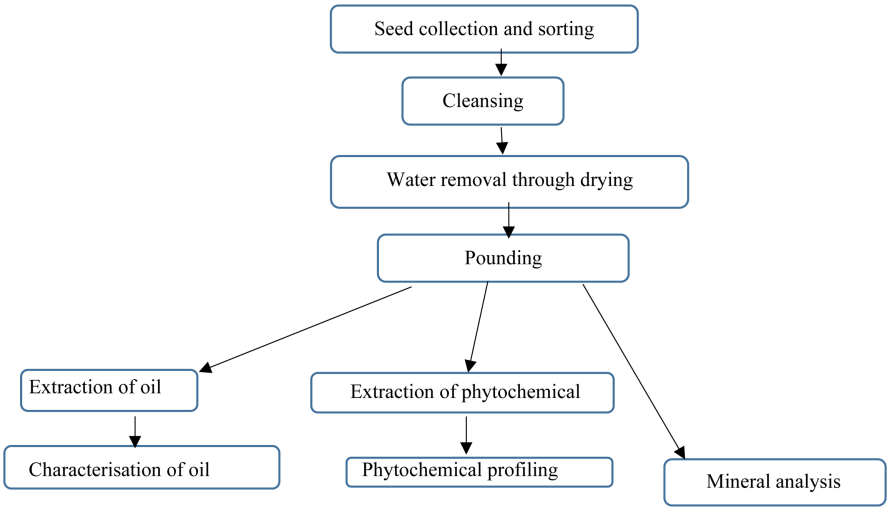

Figure 1. Sample collection and analysis.

2.2. Proximate Composition

The moisture and protein contents of the samples were analyzed according to Association of Official Analytical Chemists, Method Nos: 925.10, 923.03, 920.87 and 920.39, respectively) procedures [14]. All experimental setups were performed in triplicate.

2.3. Moisture Content

Moisture content was determined using the AOAC 925.10 method. 2 g of the seed powder (W_2) was weighed into clean dry pre-weighed crucibles (W_2) and placed in the oven at 100˚C for 3 hours. The seed powder was then cooled in a desiccator and reweighed and put back in the oven until a constant weight (W_3) was obtained. The moisture content was calculated by using equation 1 below;

$$\%\text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where, W_1 = weight of the empty crucible, W_2 = initial weight of the crucible + sample before drying and W_3 = final weight of the crucible + sample after drying.

2.4. Oil Yield

C. mannii seed oil was obtained from the milled seed powder by Soxhlet extraction process using n-hexane for 6 hours. After the extraction process, the oil was separated from the extraction solvent using rotary evaporator. The percentage yield of the oil was calculated using the equation below;

$$\%\text{Yield} = \frac{\text{mass of oil}}{\text{mass of sample}} \times 100$$

2.5. Ash Content

Ash content was determined according to the ASTM D 2866 method. Adequate

sample (2 g) was dried in the oven at 150˚C for 3 hours. The crucibles were ignited in the muffle furnace at 650˚C for 1 hour then cooled to room temperature in a desiccator. The pre-dried seed powder as weighed into the ignited crucibles then placed in the furnace at 650˚C. Ashing was considered to be complete when constant weight was achieved which was after 6 hours. Equation below was used to calculate the ash content;

$$\%\text{Ash} = \frac{W_1}{W_2} \times 100$$

Where, W_1 = weight of ash and W_2 = weight of sample.

2.6. Crude Protein Content

Crude protein content was determined by the Kjeldahl method according to the AOAC method 920.87. 0.5 g of the seed powder was weighed into the Kjeldahl digestion flask, 1 g of catalyst consisting of K_2SO_4 and $CuSO_4$ in the ratio of 10:1 and 15 mL of H_2SO_4 were added. The contents were placed in the digestor (DKL 20) and the conditions were set to 150˚C for 30 minutes then ramped to 350˚C until digestion was complete. Distillation was carried out using automatic distiller with sample diluted by water and 40% NaOH. The distillate was collected in 4% boric acid. Standardized 0.1 N HCl was used for titration and crude protein content was calculated using the following equation:

$$\text{Crude protein content} = \frac{(A-B) * N * 14.007 * 6.250}{W}$$

Whereby A is the volume of 0.1 N HCl used for sample titration, B is the volume of 0.1N HCl used for blank titration, N is the normality of HCl, W is the weight of sample (g), 14.007 is the atomic weight of nitrogen and 6.25 is the protein nitrogen conversation factor.

2.7. Crude Fibre Content

Fiber content was determined using the AOAC 2000, Method 962.09. The seed powder was defatted prior analysis by Soxhlet extraction using hexane since fat content was greater than 1%. 2 g of the dry defatted seed powder was transferred into a round bottom flask and then 200 mL of boiling 0.255 N H_2SO_4 was added. The contents were boiled for 30 minutes under reflux while rotating periodically to keep the solid from adhering to the sides. The sample was then sieved using 50 μm sieve and washed with near boiling distilled water. The residue was quantitatively transferred back into the round bottomed flask, boiled again under reflux using 0.313 N NaOH and filtered as above. 0.255 N H_2SO_4 was used to wash the residue and also with near boiling water. The mat and residue were transferred into pre ignited ashing dish and dried at 130˚C for 2 hours, after which the weight was taken. The sample was ignited at 600˚C in the muffle furnace until ashing was complete. The ashed sample was cooled in a desiccator and reweighed. % crude fiber was calculated using the equation below:

$$\%\text{Crude fiber} = \frac{W_1 - W_2}{W_3} \times 100$$

Whereby, W_1 = mass sample before ignition, W_2 = mass of sample after ignition and W_3 = original mass of sample.

2.8. Total Carbohydrate Content

Total carbohydrate (TC) was estimated as difference from proximate analysis percentages.

TC = 100 − %crude protein + %moisture + %crude fat + %crude fibre + %total ash.

2.9. Mineral Analysis

Individual minerals were quantified using approved methods of the AOAC International (2000). Seed powder samples (1.50 g and 0.50 g) of were weighed and digested using a microwave digestor. The digest was transferred into a 50 mL volumetric flask then filled to the mark with deionised water. Standard solutions of iron (Fe), manganese (Mn), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) were used. The concentration of Fe, Zn, Mn, Ca, Na, K and Mg were estimated using flame atomic absorption spectrophotometer (Agilent 280FS AA).

2.10. Physicochemical analysis of the oil

Acid, peroxide, saponification values and specific gravity were determined according to the method stated by Haki [10]. The refractive index was determined using a digital refractometer. All experimental setups were performed in triplicates.

2.10.1. Specific Gravity (Relative Density)

Specific gravity was conducted using a dry, density bottle size 25 mL which was weighed by a digital balance accurate to 0.001 g. The mass of empty, dry reagent bottle was recorded. It was then dried and filled with distilled water, subsequently oil filled with drying in between. Measurements per sample were triplicated. The specific gravity was calculated as:

$$\text{Specific gravity} = \frac{W_{b+o} - W_b}{W_{b+w} - W_b}$$

where,

W_{b+o} = the mass of density bottle filled with oil, g;

W_{b+w} = the mass of density bottle filled with water, g;

W_b = the mass of empty density bottle, g.

2.10.2. Acid Value

Acid value, an indication of the oil's free fatty acid content, was determined using ethanol, 0.1 mol/L NaOH and 0.5 mol/L KOH. Two (2) g of the oil sample was

dissolved in 50 mL of ethanol-ether (1:1 v/v) solution and then titrated with mixture against standard 0.4N ethanoic potassium hydroxide using phenophthalein indicator.

The acid value was evaluated from the equation:

$$\text{Acid value} = \frac{V \times C \times 56.1}{m}$$

where, V is the volume in ml of standard volumetric sodium or potassium hydroxide solution used. C is the exact concentration in moles per litre of the standard volumetric sodium or potassium hydroxide solution used. m is the mass in grams of the test portion.

2.10.3. Peroxide Value

Peroxide value, a measure of the oxidation and rancidity of the oil, was determined using chloroform, glacial acetic acid, saturated potassium iodide, sodium thiosulphate and starch as an indicator. 1.5 mL of oil sample extract was transferred into a conical flask and then chloroform plus 10 mL of glacial acetic acid was added. Potassium dichromate was dried at 100˚C for an hour, then 0.008527 M potassium dichromate was prepared. 25 mL of the standard solution was pipetted into a 250 mL conical flask, 5 mL of concentrated hydrochloric acid and 15 ml of 10% potassium iodide were added. The flask was stoppered immediately and allowed to stand for 5 minutes in darkness, then titrated with 0.01016 N sodium thiosulphate using starch as an indicator. The blank sample (0.20 mL) was run first.

Peroxide value was obtained from the following equation

$$\text{PV} = \frac{(V - B) \times N}{m} \times 100$$

where N = Normality (Molarity) of sodium thiosulphate, V = Volume (mL) of sodium thiosulphate used in titration of sample, B = Volume (mL) of sodium thiosulphate used in titration of blank, m = mass (g) of oil sample. Note: PV is expressed as (mEq O_2/kg).

2.10.4. Saponification Value

Saponification value, a measure of the soap content expressed in % m/m of sodium oleate, was determined using ethanol, potassium hydroxide, 0.4 N hydrochloric acid and phenolphthalein indicator. Ethanolic potassium hydroxide solution (0.4 N) was prepared by dissolving 22.44 g KOH in ethanol, then filled to the mark in a 1000 mL volumetric flask then left to stand for 24 hours. Two (2) g of oil sample was weighed into a 250 mL round bottomed flask with 0.4 N ethanoic potassium hydroxide, the mixture was boiled under reflux for an hour. A few drops of phenolphthalein indicator were added, and resultant mixture was titrated while still hot with 0.4 N hydrochloric acid until the end point was reached from a pink to clear colour. The saponification value was determined using the following equation:

$$\text{Saponification value} = \frac{56.1 \times M \times (B - V)}{m}$$

where,

M = the molarity of hydrochloric acid;

V = the volume of hydrochloric acid used in titration of sample;

B = the volume of hydrochloric acid used in titration of blank and m is the mass (g) of oil sample;

m = mass(g) of oil sample.

2.10.5. Colour

The seed oil sample (10 mL) was melted at 35˚C in a water bath and placed in a cuvette and analyzed using a Lovi bond—Tintometer. The colours of red, yellow, and blue units were adjusted until a perfect colour match is obtained.

2.10.6. Refractive Index

The refractive index of the seed oil was determined using the Rudolph J257 automatic refractometer at 20˚C. A drop of the oil sample was transferred into a glass slide of the refractometer. Water at 20˚C was circulated round the glass slide to keep its temperature uniform. After placing the oil sample into the refractometer, the machine displayed the readings. This was repeated and the mean value was noted and recorded as the refractive index.

2.11. Extraction for Phytochemical Qualitative Tests

Phytochemical extraction was conducted as described by [15]. The oil seeds were treated with various organic solvents for extraction and qualitative screening tests for phytochemicals. Ground oil seed sample (5 g) was dispersed into 50 mL of five solvents (water, methanol, ethanol, acetone and n-hexane). The solutions were left to stand for two hours at room temperature, then boiled at 60˚C for 30 minutes and the supernatant was filtered through Whatman filter paper No. 1. The filtrate was centrifuged at 2500 revolution per minute (rpm) for 15 minutes, and the filtrates were used for phytochemicals screening.

2.12. Qualitative Analysis of Phytochemicals

Qualitative chemical tests were carried out by using standard procedures to identify the phytochemical screening following the methodology of [15] as follows:

2.12.1. Terpenoids (Salkowski's Test)

Five ml of the extract was mixed with 2 ml of chloroform, and 3 ml of concentrated sulphuric acid was carefully added to form a layer. The formation of a reddish-brown coloration at the interface was a positive indicator for the presence of terpenoids [16].

2.12.2. Steroids

Steroids test was conducted according to Kumar *et al.* [17]. One ml of extract was dissolved in 10 ml of chloroform, and an equal volume of concentrated H_2SO_4 was

carefully added down the side of the test tube. The presence of steroids was confirmed by the change of the upper layer to a red color and H_2SO_4 layer to a yellow colour with green fluorescence.

2.12.3. Flavonoids

Two ml of extract was filtered using filter paper, then 5 ml dilute ammonia and 1 ml concentrated H_2SO_4 were added slowly and the development of a yellow color that disappears on standing was considered as an indicator for the presence of flavonoids [15].

2.12.4. Tannins

To a 2-ml of extract, 3 drops of 1% lead acetate were added and the formation of a yellowish precipitate was taken as an indication for the presence of tannins [15].

2.12.5. Coumarins

To a 2 ml of the extract, 3 ml of 10% NaOH was added and the formation of a yellow colour indicated the presence of coumarins [15].

2.12.6. Saponins (Foam Test)

To a 2 ml of the extract, 5 ml of distilled water was added. The mixture was shaken vigorously and observed for the appearance of a stable persistent froth on warming, as preliminary evidence for the presence of saponins [15].

2.12.7. Phenols (Ferric Chloride Test)

Two ml of the extract was treated with 5 % aqueous ferric chloride and observed for formation of deep blue or black colour to confirm the presence of phenols [18].

2.12.8. Fatty Acids

Half ml of extract was added to 5 ml of ether and allowed to evaporate on filter paper. Then the filter paper was dried and the appearance of transparency on filter paper was observed. This indicated the presence of fatty acids [15].

2.13. Quantitative Analysis of Phytochemicals

Quantitative chemical tests were carried out by using standard procedures as shown below:

2.13.1. Extraction of Sample

The seed powder extract was obtained using the method adopted from Montagner *et al*. [19] with modifications. 2.00 g of the seed powder was mixed with 25 ml of ethanol/water solution (30% v/v) then vortexed for 5 minutes. The mixture was incubated in a shaker for 15 minutes at room temperature then centrifuged at 2400 rpm for 10 minutes. The liquid phase was obtained by suction filtration then the extraction process repeated on the residue three times. The extract was added into a 100 mL volumetric flask and filled to the mark using the 30 % ethanol solution, it was covered with aluminium foil and stored at 4˚C [19].

2.13.2. Total Phenolic Content

Total phenolic content was determined using the *Folin-Ciocalteu* reagent method [20] with minor modifications. Gallic acid stock solution (1000 mg/L) was prepared and used for series of standard solutions (10 - 100 mg/L). 2 mL of each standard solution and the extract was mixed with 10 mL of 10 % (v/v in ethanol) Folin reagent, and the mixture was vortexed for 10 s. The mixture was left to stand in the dark for 60 s at room temperature then 10 mL of 7.5 % Na_2CO_3 solution was added to the mixture. The solution was heated at 50˚C for 90 s then kept for 1 hour in the dark at room temperature. The absorbance of the reaction mixtures was recorded at 510 nm and that of the blank solution were 2 mL of 30% (v/v) ethanol was used. For the extract, three samples were prepared. The results were expressed as milligram gallic acid equivalent per gram dry weight of the seed powder (mg GAE/g DW).

2.13.3. Total Flavonoid Content

The method used for determination of total flavonoid content was adopted from Xu and Chang [21]. 1000 mg/L (+)-Catechin solution was prepared and used for series of standard solutions (10 - 120 mg/L). 0.75 mL of each of the standard solution and that of the extract were mixed with 3.75 mL of deionized water and then 0.23 mL of 5 % $NaNO_2$ solution added. The solution wat allowed to stand for 6 minutes, after which 0.45 mL of 10% $AlCl_3{\cdot}6H_2O$ was added then allowed to stand for 5 minutes. 1.5 mL of 1 M NaOH was added to the mixture then thoroughly vortexed. The absorbance was measured at 510 nm and also that of the blank solution. For the extract, three samples were prepared. The results were expressed as mg catechin equivalent (CE) per gram of seed powder dry weight (mg CE/g DW).

2.13.4. Antioxidant activity

The antioxidant activity was determined using the scavenging effect on the stable 1,1-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity [22]. DPPH solution was prepared by dissolving 0.0200g of DPPH in ethanol, and the solution was prepared into a 500 mL volumetric flask. The series of ascorbic acid (AA) standard solutions were prepared from a 1000 mg/L stock solution. 17.10 mL of DPPH solution was added to 0.9 mL of each of the AA standard solutions and also on the extract in a test tube covered with aluminium foil. The contents were mixed then kept in the dark for 30 minutes and the absorbance was taken at 517 nm. The absorbance of the blank was also recorded. For the extract, three samples were prepared. The percentage of inhibition was calculated using Equation (1)

$$\%\text{Inhibition} = \frac{\text{ADPPH} - \text{AExtr}}{\text{ADPPH}} \times 100$$

where ADPPH is the absorbance of the control, and AExtr is the absorbance of the sample at a given concentration.

2.14. Experimental Design and Statistical Analysis

All the experiment of proximate, minerals and vitamin C, physicochemical

characterization and phytochemical analysis of oil were conducted in triplicates and the means ± standard deviation values were reported. Statistical analysis was carried out using one-way ANOVA with differences at $p < 0.05$ considered statistically significant.

3. Results and Discussion

3.1. Proximate Composition

The results of proximate composition of *Cucumeropsis mannii* are presented in Table 1. Results indicate that the moisture content in the seeds was low (3.89%). The moisture content in the seeds can influence the sensory attributes of a food product. Moisture content also determines shelf life. According to Eritsland [23], high moisture content in a food material can aid growth of microorganisms. Ash content is an indicator of mineral content. These are inorganic minerals which are left after decomposing the organic contents. Therefore, the ash content was 3.20% which suggested that the inorganic minerals were present in the seeds. A measure of 21.26% of crude fibre was reported. The crude protein and total carbohydrate content (g/100g) in the seeds were 9.07 and 18.14, respectively. Therefore, this suggests that the seeds have the potential of correcting protein deficiencies in the body such as marasmus, kwashiorkor and other infectious diseases. According to Mbuli-Lingudi *et al*. [24], proteins in the body play a significant role in repairing worn-out tissues. Lastly, the total carbohydrate content was 11.1% and this indicates that the three carbohydrates which are starch, sugars and fibres were present too.

Table 1. Proximate analysis of *Cucumeropsis mannii* seeds.

Parameters	Composition (g/100g)
Moisture content	3.89 ± 0.17
Crude protein content	9.07 ± 0.92
Crude fiber	21.26 ± 1.05
Ash content	3.20 ± 0.08
Total carbohydrate content	11.1

Mean of triplicates and standard deviation.

The minerals in the *Cucumeropsis mannii* seeds are shown in Table 2. These results indicate that these seeds are a good source of minerals such as potassium, sodium, calcium, iron, zinc and magnesium with important health benefits. Each mineral plays a different role in the human body. According to Byrd-Bredbenner *et al*., [25], calcium plays a significant role in the development and growth of strong bones and teeth. Magnesium is responsible for muscle contractibility and protein synthesis. Some of the functions of potassium are contraction of skeletal and cardiac muscles, while sodium is involved in nerve transmission and regulation of fluid balance. Iron is responsible for the production of haemoglobin which is required for

transport of oxygen in the body, while zinc is needed for the proper functioning of the immune system, antioxidant defense and DNA/RNA synthesis [26].

Table 2. Mineral Analysis of *Cucumeropsis mannii* seeds.

Mineral	Composition (mg/100g)
Sodium (Na)	4.44±0.16
Potassium (K)	537.9 ± 2.8
Magnesium (Mg)	93.15±0.16
Calcium (Ca)	71.13 ± 0.62
Iron (Fe)	12.79 ± 0.42
Zinc (Zn)	5.55 ± 0.088
Manganese (Mn)	1.89 ± 0.13
Mineral	Composition (mg/100g)

Mean of triplicates and standard deviation.

3.2. Physicochemical Properties

The results for physico-chemical properties are presented in **Table 3**.

Table 3. Physicochemical characteristics of *Cucumeropsis mannii* oil in comparison with literature values and soybean and groundnut parameters.

Parameter	Average values/observation	*C. mannii* (Literature values)[1]	Soybean oil (*G. max*)[1]	Groundnut (*A. hypogeae*)[1]
Colour	Pale yellow	Pale yellow	Golden yellow	Golden yellow
Odour	Agreeable	-	-	-
Physical state at room temperature	Liquid	Liquid	Liquid	Liquid
Oil yield (%)	44.4 ±1.14	57.26	14.51	10.54
Acid value (mg KOH/g)	9.54 ± 0.14	7.09	19.21	4.63
Saponification value (mg KOH/g)	200.11±2.92	220.19	228.19	211.37
Peroxide value (meq H_2O_2)	1.59 ± 0.07	20	5.18	2.15
Refractive index	1.46	1.35	1.4662	1.4622
Specific gravity	0.96	0.91	0.87	0.91

Sources: [9]; Mean of triplicates and standard deviation.

3.2.1. Oil Yield

The oil of *Cucumeropsis mannii* was golden yellow (**Figure 2**). The oil yield of the seeds was 44.4%. The percentage yield of the oil is in complete agreement with results from other studies [5] [6]. However, it is twice as much as oil from con-

ventional vegetable oils such as soybean and groundnut [27]. This significant amount of oil suggests that it can be used in the determination of produce for processing. Therefore, the white melon seeds are a good source of oil and can be used as alternative sources of oil for human consumption, ingredients for food product development, and industrial purposes. They can also be used for development of infant formulas and dietary supplements to prevent mineral deficiencies.

Figure 2. Oil obtained from *Cucumeropsis mannii* seeds.

3.2.2. Specific Gravity and Refractive Index

The specific gravity of the seed oil was 0.96, which is less than that of water (1.0) [28]. *Cucumeropsis mannii* oil, being a triacylglycerol oil, has a lower refractive index. The oil has a refractive index of 1.46 at 20˚C. These values fall within the range of the Codex Alimentarius (2005) Standards [29]. The refractive index of oil depends on the molecular weight of the oil, fatty acid chain length, and degree of unsaturation of oil. These values are in agreement to the values reported for other seed oils, that is, 1.4662 for soybean oil, and 1.4622 for groundnut oil. Values of refractive index for different oils generally vary between 1.447 and 1.482 [30]. These results were in complete agreement with those from other studies [30]. The higher measurements of the refractive index reported for the oil extracts revealed the necessity to purify the oils. In addition, the high values of oil refractive index also showed that the oil samples contain long chain fatty acids with a large number of carbon atoms.

3.2.3. Peroxide Value

The peroxide value of the seed oil was 1.59 ± 0.07 meq/Kg H_2O_2. This value was in agreement with the value of 2.95 meq/kg H_2O_2 [5] but lower than the value of 20.00 meq/Kg H_2O_2 reported by Olofinnade *et al.* [6]. The low peroxide value of the oil indicates that this oil is less liable to oxidative rancidity at room temperature [30] [31]. This rancidity leads to deterioration and off flavours of oils. Therefore, the peroxide value test helps to assess the spoilage level in oils. In addition, fresh oils with peroxide values less than 10 meq/Kg for refined oils are not likely to be affected by oxidative rancidity. On the other hand, the acceptable value for

cold pressed and virgin oils is up to 15 meqKOH/g. The value was very low indicating that the seed oils have very low oxidative rancidity. This demonstrates that they can be stored for a long time without significant changes to the seed oil identity. Therefore, the oil will not be susceptible to oxidation.

3.2.4. Saponification Value

The saponification value was 200.11 ± 2.92 mg KOH/g, which was slightly lower than in other studies [5]. A study conducted by Essien *et al*. [9] showed that the saponification value of *C. mannii* in Nigeria was 220.19 mg KOH/g. The saponification value was slightly higher than that of palm oil (196 - 205 mg KOH/g), olive oil (185 - 196 mg KOH/g), soybean oil (193 mg KOH/g) and linseed oil (193-195 mg KOH/g). A high saponification value indicates higher components of fatty acids with low molecular weight. The esters of the fatty acids of lower molecular weight require more alkali for saponification [32]. In addition, saponification value helps identify oil stability. According to Codex Alimentarius [29], the saponification value of oil ranges from 250 - 260 mg KOH/g. The high saponification value indicates better soap making, lather shaving creams and application in the cosmetic industries. Therefore, based on this property, the oil might be suitable for soap making. On the other hand, this property makes them useful as sources of essential fatty acids which are required in the human body.

3.2.5. Acid Value

The acid value of the *Cucumeropsis mannii* seed oil was 9.54 ± 0.14 mg KOH/g. This value is slightly higher than in other studies. However, in a study conducted by Nwoke *et al*. [5] the acid value in *C. manni* was 1.08 mg KOH/g. According to Opoku-Boahen *et al*. [32], the acid value was 7.09 mg I_2/g, suggesting good stability. Acid value is the amount of free fatty acid present in oil or the amount of base in milligrams required to neutralise the free organic acid present in 1 g of fat or oil. Acidity and oxidability of oils are attributed to free fatty acids.

Free fatty acids are able to speed up the oxidative decay of extracted oils by enzymatic and or chemical hydrolysis to form off volatile compounds. Acid value is an indicator of lipase efficacy. As a result, the lower the free fatty acids the higher the stability and the better the quality of oil [30] [33]. The low acid values of the seed oil demonstrates that the triglycerides were not hydrolysed, which indicates the good stability of the seed oils. The acceptable limit of acid value for edible oils is ≤10. Thus, from the results, *Cucumeropsis mannii* seed oil has failed the test of being edible with acid value of 15.70 mg KOH/g.

3.3. Phytochemical Screening

The results of qualitative phytochemical analysis in the oil extract of *Cucumeropsis mannii* are presented in **Table 4**. These results show that the oil extract, using n-hexane, contained coumarins, phenols, saponins, steroids and terpenoids. This demonstrates that this solvent was effective in extraction of these substances which are non-polar. However, some extracted substances in the oil

are polar in nature. Other studies have shown that phytochemicals are present in the oil extracts. Qualitative analysis of phytochemicals in a specific solvent provides information on its polarity and assist in the selection of an appropriate solvent for its separation, purification, and characterisation. This suggests that isolation, purification, and characterisation of phytochemicals from a plant material depends on the polarity of solvent used. In the current study, the solvents used were water, methanol, acetone, ethanol and n-hexane. Terpenoids and steroids were predominant in the hexane, water, methanol, ethanol, and acetone extracts. Flavanoids, tannins and phenols were not present in the solvents used for extraction, except in the water extract. Conventional extraction methods are still widely applied for extraction of bioactive (polyphenols) compounds utilizing water and polar organic solvents. However, there are factors affecting the extraction efficiency and their antioxidant activity, these include; the solvent used, solvent to sample ratio and or extraction time [19] [26]. Optimization of extraction of bioactive compounds have been reported in several studies [28] [34]. Phytochemicals are free radical scavenging molecules or compounds which possess antimicrobial properties, antioxidant, anti-inflammatory properties [35]. They are good sources of antioxidants and responsible for the treatment for gastrointestinal infections [35]. Phytochemicals also protect cells and DNA from damage that is phytochemicals help repair cells in the human body. The terpenoids are responsible for the aromatic (taste, smell) qualities of vegetable oils, while phenols are classified as secondary metabolites of plants that possess antioxidant properties. Tannins and flavonoids play different functions chelating agents for metal ion, antioxidant in biological systems and as protein precipitating agent. The growth of microrganisms such as yeast, viruses, bacteria, fungi can be inhibited by tannins. The antioxidant compounds reduce oxidative damage of cells that could result in diseases and also help in hormonal regulation because excess of hormones such as insulin and extrogen are linked with increased risk for breast and colon cancer. Saponins are used industrially as foaming agents such as surfacants and detergents, ore separation, emulsions for photographic films and cosmetics [35]. Steroids play a significant role in the pharmacy and their relationship with sex hormones.

Table 4. Qualitative phytochemical analyses of *Cucumeropsis mannii* seeds oil in triplicate.

	Terpenoids	Steroids	Flavonoids	Tannins	Saponins	Phenols	Fatty acids	Coumarins
Ethanol	+	+	−	−	−	−	+	+
Methanol	+	+	−	−	+	−	−	+
Acetone	+	+	−	−	−	−	+	−
Hexane	+	+	−	−	−	−	+	−
Water	+	−	−	−	−	+	−	+

+ = present and − = absent.

3.4. Total Phenolic and Flavonoid Contents

The results of total phenol and flavonoids are shown in Table 5. Total phenols in Sefophe (*Cucumeropsis manni*) seeds were high (1.72 mg GAE/g), compared to a report by Olofinnade *et al*. [6], which was 0.05, 0.03 and 0.10 mg GAE/g for the aqueous, petroleum ether, methanol extracts, respectively. The total phenolic compound content of the seeds of *C. manni* was higher than that reported for the wild melon, kgengwe (*Citrus lanatus*), which was 0.38 mg GAE/g [36]. The total flavonoids in *C. manni* were higher (2.21 mg/g catechin equivalent), compared to a study by Olofinnade *et al*. [6], which was 0.05, 0.03 and 0.12 mg Quercetin equivalent/g for the aqueous, petroleum ether, methanol extracts respectively [6]. Furthermore, the flavonoid content was higher than in wild melon, kgengwe (*C. lanatus*) in Botswana [36].

Table 5. Total phenolics, flavonoids contents and antioxidant activity of *Cucumeropsis manni* oil seeds collected from Takatokwane in Botswana.

Oil seed	Total phenols content (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH Vitamin C equivalent antioxidant capacity (VCEAC)
Cucumeropsis manni	1.48 ± 0.11	2.21 ± 0.11	94.3 ± 10.8 VCEAC/100g

Data presented as mean ± SD. GAE: gallic acid equivalent; CE: catechin equivalent.

3.5. Antioxidant Activity

The antioxidant activity of the seed oil was 94.3 ± 10.8 vitamin acid equivalent antioxidant activity (VCEAC)/100g (Table 5). The antioxidant behaviour of the oil seed could be attributed to the higher levels of their phenolic and flavonoid content. The relatively high antioxidant activity from the seed oils suggests an investigation of potential *in vivo* efficacy should be undertaken. *C. manii* seed oil are thus a good source of phenols and antioxidants and their cultivation and consumption could be important for the prevention of lifestyle-related diseases. The mechanism behind the scavenging of free radicals is well understood. Phenols, due to their hydroxyl groups, can donate electrons to the free radicals and remain stable, due to delocalized electrons, and as such inhibit reaction of free radicals with DNA and other important biomolecules [37]. Other studies attribute the preventative properties of total phenols and antioxidants to their ability to quench singlet oxygen or scavenge reactive oxygen species, hence impede the movement of radical reactions from one cell to another [34] [38]. Nowadays food that is rich in antioxidants is recommended to prevent these Western degenerative diseases. As reported in the previous studies, free radicals can react with various biomolecules in the body and lead to diseases such as stroke, hypertension, asthma and diabetes [11] [39]. Therefore, phenols and antioxidants would counter the effects of free radicals because they are stable even if an electron has been taken from them.

4. Conclusion

This study on the extraction and characterization of *Cucumeropsis mannii* seed oil have confirmed that the oil is edible and also the oil is of good nutritional value due to the presence of the phytochemicals hence the oil pose no significant health risk to humans. Since the oil is edible it is suggested that Batswana should be encouraged to cultivate and commercialize the white melon seeds for oil production, as this will boost and diversify the economy. The low acid and peroxide results also show that the oil is of high quality with long shelf life and is suitable for human consumption. The presence of phytochemicals is important due to the fact that the phytochemicals contain medicinal compounds such as antioxidants and compounds that may inhibit carcinogenesis and other lifestyle diseases.

Acknowledgements

O. Tibe is the main author, GD Haki and M. Batlhophi are responsible for correcting the manuscript, T. Gaba and A. Muzila, S. Thatitswe and KM Letswee were responsible for analysis of the samples.

Conflicts of Interest

The authors declared no conflict of interest regarding the publication of this paper.

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