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Original Article

Extraction, Physicochemical, Phytosterols and Fatty Acid of *Celosia spicata* Leaves

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ABSTRACT

Objective: This study assessed the minerals, phytosterols, fatty acid, physical and chemical characteristics of oil extracted from *Celosia spicata* leaves.
Methods: The Leaves of *Celosia spicata* were washed, dried and dry-milled into flour. Oil from the flour was extracted with petroleum ether using Soxhlet extractor. The extracted oil was evaluated for physicochemical properties and phytosterols using standard classical methods of analysis. The fatty acid methyl esters were determined using a chromatographic technique and the mineral elements were analyzed by dry-ashing the sample in a muffle furnace at 550^{O}C and dissolving the ash with 3mL of 3M HCl in 100mL standard flask. Sodium and potassium were determined by flame photometer and other elements were determined by Atomic Absorption Spectrophotometer.
Results: The results showed that potassium was the highest mineral with the value of 659±0.04mg/100g followed by magnesium (463±0.01mg/100g) while copper was the lowest with the value of 0.40±0.002mg/100g). The results of the physico chemical properties studied were: specific gravity (0.86), saponification value (191mgKOH/g), iodine value (112mgI_2/g), acid value (3.90mgKOH/g), flash point (258^{0}C) and fire point (274^{0}C). The phytosterol analysis of *Celosia spicata* revealed the presence of sitosterol (102.76mg/100g). Palmitic acid ($C_{16:0}$) (29.84%) and linoleic acid ($C_{18:2}$) (23.29%) were the highest fatty acids while arachidic acid ($C_{20:0}$) (0.505%) was the lowest.
Conclusions: It can be concluded that the oil contained high amount of unsaturated fatty acid and also exhibited good physical and chemical properties, which makes it edible and useful industrially. Therefore, its cultivation is encouraged.

Keywords: Minerals, Physicochemical, Phytosterols, Fatty acid, *Celosia spicata*, Leaves.

INTRODUCTION

Fruits and vegetables contain a number of essential nutrients that cannot be found in other types of food. They contain anti oxidants, high amount of fiber and low

fat that our body needs to: reduce cholesterol, cleanse, rid off waste/toxins and regular bowel movements thereby preventing constipation as well as intestinal cancer[1]. *Celosia spicata* is an edible, ornamental plant in the amaranth family '*Amaranthaceae*'. It originated from Africa but well known food in Indonesia and India. *Celosia spicata* is an annual leafy vegetable that grows up to 2m in height. Its flowers yield large numbers of seeds that are 1mm in diameter. It grows well in humid areas with moist soil[2]. When cooked, it is slightly bitter in taste and the bitterness is usually removed by washing, squeezing severally, then grinded melon and other condiments may be added in order to prepare a palatable recipe[2]. *Celosia spicata* is of high economic value in Nigeria, particularly during the dry season[3]. They are very rich in vitamin C and E, which are both very powerful antioxidants. Daily intake of vegetables would help to protect the body from developing cancerous cells and heart diseases[1]. Some works like the vitamins, amino acid and proximate composition had been studied on the other specie of *Celosia* called *Celosia argentea* but little or no work has been done on the present sample specie. Therefore, the present work is aimed at determining the minerals, physicochemical properties, phytosterols and fatty acid composition of *Celosia spicata* leaves.

MATERIALS

Celosia spicata vegetable was obtained from Alagbayun-Oremeji, Ibadan, Oyo state, South west Nigeria in Africa**.** The vegetable leaves were removed, air dried and then milled into flour, using a Kenwood blender and sieved to obtain fine flour, packaged in rubber container and kept in freezer prior analyses.

METHODS

Extraction of oil

The Soxhlet extractor was used for the extraction of oil according to method described by AOAC[4]. Fifty gram grinded leaves of the sample was folded in a filter paper and inserted into the thimble of the extractor. The reflux was done for 7 hours using petroleum ether of boiling point range of 60-80^{o}C followed by cooling. The mixture of the oil and solvent was transferred into a Rotavapor apparatus for solvent recovery. The oil obtained was drained and stored in the freezer (-4^{o}C) for physicochemical, phytosterols and fatty acid analyses.

Determination of minerals

The minerals were analyzed by dry ashing the sample at 550ºC to constant weight and dissolving the ash in 100 mL standard flask using distilled deionized water with 3mL of 3M HCl. Sodium and potassium were determined by using a flame photometer (model 405, corning, U.K). All other minerals were determined by Atomic Absorption Spectrophotometer (Perkin & Elmer model 403, USA)[4].

Determination of physicochemical properties

Saponification value

A 2.0mL of the oil sample was added to the 20mL of ethanolic potassium hydroxide in 500mL round bottom flask. The flask with its content was refluxed for 30 minutes. 2mL of phenolphthalein indicator was added and the hot solution was allowed to cool and later titrated against the 0.5M hydrochloric acid. A blank titration was carried out using the same procedure[5].

$$Saponification\ value = \frac{56.1M(V_1-V_2)}{W} \quad (1)$$

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Where:

M = molarity of hydrochloric acid.
V_1 = volume of HCl used in the test.
V_2 = volume of HCl used in the blank.
W = weight of sample oil.

Peroxide value

A 2.0g of the oil sample was weighed into the 200mL conical flask containing 20mL of petroleum ether and heated for 30 seconds in a water bath. 20ml of 50% aqueous solution of potassium iodide and 25mL of distilled water were added. The resulting mixture was titrated with 0.002M sodium thiosulphate solution. During the titration a milky white precipitate was observed and the total disappearance of the precipitate indicated the end point of the titration. The peroxide value of the sample oil was estimated on the basis of the equation below. The same procedure was repeated for the blank[6].

$$Peroxide\ value = \frac{100\ (T_B - T_S)\ mgEqiv.O_2/kg}{Weight\ of\ sample\ oil} \quad (2)$$

Where:

M = molarity of thiosulphate.
T_S = volume of thosulphate used in the sample test.
T_B = volume of thiosulphate used in the blank.

Acid value

A 5g of the sample oil was weighed into a 250 mL conical flask. 50 mL of hot neutralized alcohol was measured into the flask. The content in the flask was boiled on a water bath, after which 5 drops of phenolphthalein indicator was added into the content of the flask. The mixture was then titrated with 0.1M sodium hydroxide using a burette until a pink colour was observed, indicating the end point[5].

$$Acid\ value = \frac{M \times T_B - T_S}{\text{Weight of sample oil}} \quad (3)$$

Where:

M = molarity of sodium hydroxide.
T_S = Titre value of the sample.
T_B = Titre value of the blank

Iodine value

A 0.2g of the sample oil was transferred into a flask containing 10mL carbon tetrachloride. 25mL of Wijs solution was added into the flask containing the sample (Wijs solution consists of iodine monochloride in glacial acetic acid). Blank was prepared. The mixture was stored in a dark place for 30 minutes at temperature of 25°C after which 15mL potassium iodine solution was added along with 100ml of distilled water. The resulting mixture was titrated with 0.1M sodium thiosulphate solution using 2mL of 1% starch indicator. The titration was continued until the blue colour just disappeared, indicating the end point[6].

The iodine value was calculated on the basis of the following equation:

$$Iodine\ value = \frac{12.692\ (T_B - T_S) \times M}{Weight\ of\ the\ sample\ oil} \quad (4)$$

Where:

M = molarity of the solution.
T_S = Titre value of the sample.
T_B = Titre value of the blank.

Unsaponifiable matter

After saponification, 300mL of the mixed solvent of ethanol (70%), toluene (25%) and 5mL oil was added to the packed glass column. It was allowed to run through the column at the rate of 12mL / minute. The glass column was washed with 150mL of the solvent mixture at the same rate. It was concentrated to 25mL using rotary evaporator and then transferred to the tarred dish for evaporation in oven at 105°C for 15 minutes. The dried sample was weighed and titrated for the remaining acids; the weight was corrected for the unsaponifiable matter[4].

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Specific gravity

The sample (40mL) was homogenized and poured into a 500mL measuring cylinder gently to avoid air bubbles. The temperature was controlled to avoid drifting in the temperature value. Hydrometer was dipped into the oil carefully to avoid resting on the wall of the cylinde33r and the reading was then taken[6].

Refractive index

The oil was dried to make it free of moisture. Two drops of the oil was put on the lower prism of the equipment and the prism was closed up. The water was passed through the jacket at 45^{o}C and the jacket was adjusted until the equipment read temperature of 40^{o}C. The light was adjusted and the compensator was moved until a dark border line was observed on the cross wire. The reading on the equipment was recorded[6]

Kinematic viscosity

The capillary viscometer was used for kinematic viscosity determination. The sample was filtered to remove impurities and then introduced into the viscometer and was allowed to stay in a regulated water bath long enough to reach the desired temperature. The head level of the test sample was adjusted to a position in the capillary arm of the equipment to about 5mm ahead of the first timing work. As the sample was flowing freely, the time required for the meniscus to pass from the first time mark to the second was read[7].

The equation used was:

$$V = C\,T \qquad (5)$$

V - Kinematic viscosity
C - Calibration constant
T - Flow time in seconds

Flash and Fire points

The dried sample was poured into the cup of the tester to the mark and then placed the cup and the cup cover with the left hand pointing toward the left front corner of the test compartment. Stirrer was fixed into the tester properly and the resistance thermometer probe connected. Flame and the pilot light were carried out by lighting and the drought screen was closed. The tester was put on and the heater temperature was regulated and the stirrer switch was on simultaneously with the tester for homogeneity. A flash occurred when large flame was observed on the cup and the temperature at which this occurred was recorded as the flash point for the oil sample. The fire point was the temperature observed when the oil combustion was sustained after the flash point of the sample oil was recorded[8].

Pour point

The sample was homogenized and poured into the test jar to mark level. The jar was closed tightly with the cork carrying the high pour thermometer that was placed 3mm below the surface of the oil. The disc was placed in the bottom of the jacket and the ring gasket was placed around the jar at the 25mm from the bottom. The test jar was then placed in the jacket. The oil was allowed to cool without disturbance to avoid error. The test jar from the jacket was removed carefully and tilted to ascertain whether there is a movement of the oil. The procedure continued in this manner until a point was reached at which the oil in the test jar showed no movement when the test jar held in a horizontal position for 5 minutes[8].

Cloud point

The determination of cloud point was done using a high precision cloud meter (wave guide sensor total - reflection type), the wave guide sensor have an incidence channel, emergence channel and a detector surface that intersect along the detection surface. The incidence optical fibre

connected to the exit of the emergence channel, and a cooling / heating of the waveguide sensor was done within a desired temperature range. The sample oil was placed on the detection surface and light introduced into the incidence optical fibre. The emergence light from the optical fibre was detected. The wave guide sensor was cooled / heated thereby cooling / heating the sample and the temperature wherein the total reflection of light in the emergence optical fibre was the cloud point of the sample oil[8].

Determination of phytosterols

The phytosterols extraction and analysis were carried out by following the modified method of AOAC[4]. **A** 50.0g of the sample flour was weighed and transferred into corked flask and treated with petroleum ether until the flour was fully soaked. The flask was shaken at every one hour for the first 6hours and then kept and agitated after 24hours. This process was repeated for 3days and then the extract was filtered. The extraction was collected and evaporated to dryness by using nitrogen steam. The extract (0.5g) was added to the screw-capped test tube and saponified at 95^{0}C for 30minutes using 3mL of 10% KOH in ethanol which 0.20mL of benzene was added to ensure homogeneity. The deionized water (3mL) with 2mL n-hexane was added to extract the non-saponifiable materials e.g. sterols. Three sequential extractions of 2ml each with n-hexane were performed for 1hour, 30minutes and 15minutes respectively to achieve complete extraction of sterols. The mixture was concentrated to 2mL for chromatography analysis.

Determination of fatty acid

The fatty acid profile was determined using a method described[9]. The fatty esters analyzed using a PYE Unicam 304 gas chromatography fitted with a flame ionization detector and PYE Unicam computing integrator. Helium was used as carrier gas. The column initial temperature was 150^{o}C rising at 5^{o}C min^{-1} to a final temperature of 200^{o}C respectively. The peaks were identified by comparison with those of standard fatty acid methyl esters.

RESULTS

See Tables 1, 2, 3, 4 and 5.

DISCUSSIONS

The results of nutritionally important minerals of *Celosia spicata* leaves are shown on Table 1. Minerals are important in human nutrition. It is an established fact that enzymatic activities as well as electrolytic balance of blood fluid are related to the adequacy of Na, K, Mg and Zn. Potassium is very important for balancing body pH fluid and osmotic equilibrium. Metal deficiency problems like rickets and calcification of bones is caused by calcium deficiency. The potassium, magnesium and calcium levels were found to be higher than those of *Parinari curatellifolia* seeds with K (459mg/100g) and Mg (428mg/100g) [10] and *Bridela Ferruginea benth* seeds with K (29.8mg/100g), Mg (21.3mg/100g) and Ca (24.2mg/100g)[11]. Sodium content in the sample was higher than those values for the leaves of *Cucurbita maxima* (19.5mg/100g), *Amaranthus viridis* (21.3mg/100g) and *Basella alba* (20.4mg/100g)[12], while the Phosphorus value was higher than those reported for *F. asperifolia* and *F. sycomorus*[13]. The iron value was higher than that of African nutmeg (3.0mg/100g)[14] but comparable with that of cowpea (4.9mg/100g)[15]. The value of zinc in *Celosia spicata* leaves was found to be higher than those of *Luffa cylindrica* (1.0mg/100g)[16], guinea corn (1.8mg/100g), maize (1.48mg/100g) and cocoyam (2.4mg/100g)[17]. Zinc is an important mineral in the body as its deficiency may result to dwarfism and hypogonadism

among adolescents[18]. Calculated minerals ratios were also shown on Table 1. The Na/K and Ca/P ratios are important nutritionally, modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine[19]. This necessitated the ideal of the Ca/P ratio. If the Ca/P is low (low calcium, high phosphorus) more than the normal amount of calcium may be lost in the urine thereby decreasing the calcium level in bones[19]. Food is considered 'good' if the ratio is above one and 'poor' if the ratio is less than 0.5[20]. This indicates that Ca/P ratio was higher than 0.5 recommended value[20] which is the minimum ratio required for maximum calcium absorption in the intestine for bone formation[19,20]. Na/K ratio was lower than 0.6, indicating that the sample will not promote high blood pressure when utilized by the body. For normal retention of protein during growth and for balancing metabolic fluid, a Na/K ratio of 0.6 is recommended[21]. The Ca/Mg ratio obtained was lower than the recommended value is 1.0mg/100g. Both calcium and magnesium would need adjustment for good health.

The results obtained for the physicochemical properties of *Celosia spicata oil* are shown on Table 2. The specific gravity of *Celosia spicata* oil was close to *Cassia tora* oil (0.88)[22] but higher than that of the value for velvet tamarind oil (0.79)[23]. The flash and fire points were comparably higher than those for *Irvingia gabonensis* oil flash point (120^{o}C) and fire point (168^{o}C)[24]. The acid value was lower than those of castor oil (15.00mgKOH/g) and palm kernel oil (16.60mgKOH/g)[25]. The low acid value of *Celosia spicata* oil indicates that the oil was acidic and may be recommended for soap making and other cosmetic applications. The saponification value was lower than that value obtained for coconut oil (338.20mgKOH/g)[26] but higher than those of soybean oil (13.47mgKOH/g) and sesame oil (3.29mgKOH/g)[27]. The iodine value was higher than that of *Citrullus vulgaris* oil 38.50mgI_2/g[26] and some vegetable oils [28]. The peroxide value was higher than that for castor oil (2.270mgEquiv O_2/kg)[25]. The high peroxide value indicates that the oil is saturated. The unsaponifiable matter was comparably higher than that of *Irvingia gabonensis* oil (0.44%)[24], and that of free fatty acid was higher than that of groundnut seed oil (0.250%)[25]. It has been observed that, it is desirable to ensure that free fatty acid value of cooking oil lies within the limits of 0.0 – 3.0%[29]. Therefore, the low value of free fatty acid in *Celosia spicata* oil is an indicator that the oil is edible and would not spoil easily via oxidative rancidity. The refractive index is the measure of the extent of purity or clarity of oil. The refractive index value of the sample oil was in agreement with those of sesame oil (1.47)[27] and *Irvingia gabonensis* oil (1.48)[24]. The kinetic viscosity obtained at 40^{o}C was 54.40Cst which indicated that at higher temperature, there is restriction in the fluidity of the oil.

The phytosterols levels of the sample oil are shown on Table 3. The results phytosterols of *Celosia spicata* revealed the presence of sitosterol which value was lower than those reported on raw (111mg/100g), cooked (110mg/100g) groundnut oil[30]. Sitosterol took the first position in *Celosia spicata*. Sitosterol is one of several phytosterols with chemical structures similar to that of cholesterol. It is widely distributed in the plant kingdom. Phytosterol reduces blood levels of cholesterol and is sometimes used in treating hypercholesterolemia. β-sitosterol plays a major role in treatment of herbal therapy of prostatic hypertrophy[31]. Sitosterol had the highest value while cholesterol had the lowest value. *Celosia spicata* oil also contained some amounts of campesterol which was lower than those of

the processed groundnut oil[30]. The cholesterol level was lower than those reported on raw groundnut oil (8.93mg/100g)[30]. Ergosterol is a precursor of vitamin E that is useful for human sterility. The value of ergosterol in the sample oil was lower than that of roasted groundnut seed oil (30.2mg/100g)[30], while stig-masterol value was higher than that reported for cooked groundnut seed oil (8.95mg/100g)[30]. Plant sterols have been suggested to have dietary significance and to protect vegetable oils from oxidative polymerization during heating at frying temperatures[32]. The fatty acid composition of the oil *Celosia spicata* is shown on Table 4. Palmitic acid had the highest concentration, which was higher than those of *Irvingia gabonensis* oil (7.95%)[24] and velvet tamarind oil (0.76%)[23]. The linolenic acid ($C_{18:3}$) and stearic acid ($C_{18:0}$) values were higher than those for coconut oil; linolenic acid (5.35%) and stearic acid (4.16%)[27]. Linoleic acid ($C_{18:2}$) value was higher than of *Parinari curatellifolia* (0.1%)[10]. It has been shown that Linoleic acid was the most concentrated fatty acid in pigeon pea (54.8%)[33], corn oil (55.7%) and safflower oil[34] (72.6%), but this differs in the case of the sample studied. The oleic acid ($C_{18:1}$) was higher than that of velvet tamarind oil (1.02%)[23] while palmitoleic acid ($C_{16:1}$) value was higher than that of walnut oil (0.36%)[35]. Table 5 shows fatty acids distribution *Celosia spicata* oil. The total saturated fatty acid (TSFA) was in close agreement with total saturated fatty acid (TSFA) of winged termite oil (39.9%)[36], but higher than those of variegated grasshopper oil (19.2%) and roasted cashew kernel oil (17.5%)[36]. The total saturated fatty acids (TSFA) sample oil was also compared favourably with that of soybean oil (15.10%)[37]. The Oleic acid/Linoleic acid (O/L) ratio of (0.36%) was in good agreement with that of calabash seed oil (0.35%) but higher than that of *Citrullus linatus* oil (0.27%)[38]. The (O/L) ratio helps to know the detrimental effects of dietary fats. The higher the ratio, the more nutritionally useful is the oil[36]. The total essential fatty acid (TEFA) of the sample oil was higher than that of African yam bean oil (38.2%)[39] but lower than those of kidney bean oil (50.3%) and soybean oil (53.2%)[40]. It was observed that total unsaturated fatty acid (TUFA) was relatively greater than that of total saturated fatty acid (TSFA). It has been discovered that saturated fatty acid (SFA) increases serum cholesterol while unsaturated fatty acid (UFA) lowers serum cholesterol[41].

CONCLUSIONS

It can be concluded that the sample is rich in nutritionally valuable minerals and exhibits good physical and chemical properties. The high amount of total unsaturated fatty acids in *Celosia spicata* oil makes it edible and good for industrial utilization. The cultivation and consumption are highly recommended.

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Table 1. Minerals composition of *Celosia spicata* leaves

Mineral	mg/100g
Iron (Fe)	4.90± 0.04
Copper (Cu)	0.40 ±0.002
Manganese (Mn)	1.70 ± 0.03
Zinc (Zn)	4.20 ±0.09
Calcium (Ca)	242±0.01
Magnesium (Mg)	463±0.01
Potassium (K)	659± 0.04
Sodium (Na)	25.3±0.05
Phosphorus (P)	102±0.02
Na/K	0.04
Ca/P	2.37
Ca/Mg	0.52

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Table 2. Physicochemical properties of *Celosia spicata* oil

Parameter	Value
Free fatty acid (%)	1.42
Saponification (mgKOH/g)	191
Peroxide value (mgEquiv O_2/kg)	3.96
Unsaponifiable matter (%)	1.39
Iodine value (mgI_2/g)	112
Specific gravity	0.86
Refractive index @ 40^0C	1.47
Acid value (mgKOH/g)	3.90
Kinetic viscosity @ 40^0C, Cst	54.4
Flash point (^{0}C)	258
Cloud point (^{0}C)	11.0
Fire point (^{0}C)	274
Pour point (^{0}C)	4.00

Table 3. Phytosterol Analysis of *Celosia spicata* oil

Component	mg/100g
Cholesterol	1.65×10^{-5}
Cholestanol	4.58×10^{-4}
Ergosterol	8.07×10^{6}
Campesterol	27.54
Stig-masterol	10.24
5-avensterol	4.81
Sitosterol	102.76

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Table 4. Fatty acid composition of *Celosia spicata* oil

Fatty acid	Concentration (%)
Caprylic ($C_{8:0}$)	ND
Capric ($C_{10:0}$)	ND
Lauric ($C_{12:0}$)	ND
Myristic ($C_{14:0}$)	ND
Palmitic ($C_{16:0}$)	29.84
Palmitoleic ($C_{16:1}$)	2.396
Margaric ($C_{17:0}$)	ND
Stearic ($C_{18:0}$)	6.923
Oleic ($C_{18:1}$)	8.289
Linoleic ($C_{18:2}$)	23.29
Linolenic ($C_{18:3}$)	26.368
Arachidic ($C_{20:0}$)	0.505
Arachidonic ($C_{20:4}$)	ND
Behenic ($C_{22:0}$)	0.533
Erucic ($C_{22:1}$)	1.004
Lignoceric ($C_{24:0}$)	0.852

ND= Not Detected

Table 5. Total saturated, unsaturated, mono-unsaturated and poly-unsaturated fatty acid

Fatty Acid	Concentration (%)
Total Saturated Fatty Acid (TSFA)	38.65
Total Unsaturated Fatty Acid (TUFA)	61.35
Monounsaturated Fatty Acid (MUFA)	11.69
Poly-Unsaturated Fatty Acid (PUFA)	49.66
Total Essential Fatty Acid (TEFA)	49.66
Oleic/Linoleic ratio (O/L)	0.36