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Physicochemical Properties, Fatty Acid Composition and Antimicrobial Potential of Turk's Cap (*Croton penduliflorus*) Seed Oil

Ojo, Ayomipo Martins[1*], Amoo, Isiaka Adekunle[2], Ayoade, Gbolahan Wahab[2] and Ojo, Bukola Caroline[1]

[1]Department of Industrial Chemistry, Federal University Oye-Ekiti, P.M.B. 373, Ekiti State, Nigeria.
[2]Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AIA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OAM and AGW managed the analyses of the study. Author OBC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Physicochemical properties, fatty acid composition and antimicrobial potential of *Croton penduliflorus* seed oils were investigated using standard methods. Physicochemical properties of whole and dehulled seed oils were; colour (dull cinnamon- brown colour and cinnamon- brown colour), specific gravity (0.79 and 0.80), refractive index (1.46 and 1.46), acid value (14.5 and 23.6) mgKOH/g, free fatty acid (7.3 and 11.7) as oleic acid, peroxide value (1.14 and 1.60) meq/kg, iodine value (96.20 and 88.32) mg/g, saponification value (162.69 and 169.70) mg/g, and unsaponifiable matter (0.97 and 1.43) % respectively. The fatty acid compositions of whole and dehulled seed oils respectively were; stearic (2.9 and 4.8) %, myristic (0.3 and 0.7) %, palmitic (25.2 and 26.1) %, oleic (36.4 and 37.2) %, linoleic (25.4 and 27.5) %, linolenic (0.2 and 0.3) % and arachidic acid (0.1 and 0.2) %. The fatty acid of whole and dehulled seeds contain high amount of nutritionally valuable unsaturated fatty acids especially in dehulled seed oil. The oils exhibited high antimicrobial properties which suggest their application in controlling or treatment of various bacterial and fungal infections.

**Corresponding author: E-mail: ayomipo.ojo@fuoye.edu.ng;*
E-mail: ayoadegbolahan@yahoo.com;

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1. INTRODUCTION

Croton penduliflorus (family Euphorbiaceae) is an important medicinal plant in southern Nigeria. It is commonly known as Turk's cap (Yoruba Aworo Oso, Igbo Ogwuaki and Aki Ozara) and thought to originate from Malaysia as a tropical evergreen plant. It is widely distributed in the southern part of Nigeria. *Croton* seed and its oil have been used in the treatment of wide range of disorders in the past, [1]. *Croton* oil is extensively used in herbal medicine in Nigeria. *Croton penduliflorus* oil has been used in traditional Chinese medicine to treat severe constipation since the seed of the plant can cause diarrhea. It is a source of organic compound phorbol and its tumor promoting esters. Its seeds are commonly used as a purging nut and possess anti-inflammatory, vesicant and contraceptive properties in Nigeria [2]. It is particularly used for the treatment of stomach complaints [3]. Its oil is used as liniment for acute rheumatism, arthritis, neuralgia and diseases of the joints [4]. *Croton* seeds and oils have been used in the treatment of a wide range of disorders in pregnant and non-pregnant individual. In Cote d'Ivoire a leaf infusion is taken to treat menstrual disorders. In Ghana its leaf infusion is externally applied to treat fever. In Nigeria, its seed extract is taken to treat stomach complaints and uterine tumour or as an abortifacient. The seeds mashed with cassava are eaten as a purgative. It is credited with antimicrobial, anti venom, antiparalytical, rubefacient, and anti-tumor potencies. It forms a major component of herbal contraceptives, abortifacent and anti fibroid concoctions used in local treatment of fibroids [5,6]. This study aimed at investigating the acclaimed medicinal importance of *Croton penduliflorus* to establish the scientific proof of its application in treatment of various diseases.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample collection

Croton penduliflorus (aworo oso) seeds were purchased from Oja Oba market in Ado-Ekiti, Ekiti State and identified by a curator at Crop, Soil and Pest Management Department, School of Agriculture and Agricultural Technology of the Federal University of Technology, Akure, Ondo State, Nigeria. All the reagents used in this study were of analytical grade and all glass distilled water was used throughout the research.

Plate 1. Whole seeds of Croton penduliflorus

Plate 2. Dehulled seeds of *Croton penduliflorus*

2.1.2 Sample preparation

The seeds were sorted to remove debris and sun dried until constant mass was obtained. It was later divided into two parts. The seed coat of one part was removed by dehulling, while the second part was left with seed coat intact. Both samples were ground into powder using Marlex Excella mixer/grinding machine, packed in air tight containers and kept in the refrigerator at 4°C for further processing.

2.1.3 Extraction of oil from the seed

About 300 mL of n-hexane was poured into the round bottom flask of the extractor. Two hundred gram of the each sample seed pulp was wrapped in a white muslin cloth and placed in the thimble of the soxhlet extractor. The oil sample was extracted from the seed flours by Soxhlet

extraction using n-hexane of Analar grade, boiling range 40-60°C. The soxhlet was coupled with condenser and flask already filled with n-hexane. The set up was heated on heating mantle at 65°C to allow solvent boiling, in the process the solvent vapour travels up a distillation arm and flowed into the chamber housing the sample material. The extract seeps through the pores of the thimble and fills the siphon tube where it flows back down into the round bottom flask. The process was allowed to continue for 3 h until a clear solvent was obtained in the thimble chamber. At the end of the extraction, the resulting mixture containing the oil was heated to recover the solvent from the oil. The recovered oil was stored at room temperature prior analysis.

2.2 Methods

2.2.1 Determination of the physicochemical properties of the oil

The acid, ester, saponification, iodine and peroxide values were determined using the methods described by [7] while the methods described by [8] were adopted for the determination of the specific gravity, refractive index and density of the oil.

2.2.2 Determination of fatty acid constituents of the oil

The oil extracted was converted to the fatty acid methyl ester using the method of [9]. The fatty acid methyl ester were analyzed using a PYE Unicam 304 gas chromatograph (PYE Unicam, Cambridge, UK) fitted with a flame ionization detector and a PYE Unicam PU4810 computing integrator. Helium was used as the carrier gas. The column initial temperature was 150°C rising at 5°C/min to a final temperature of 220°C. The injection port and the detector were maintained at 220°C and 250°C respectively. A polar (25QC3/BP1-0.5) capillary column (25.00 m × 0.33 mm; SGE Scientific Glass Engineering Co., UK) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Sigma Chemical Co. (St Louis, MO, USA).

2.2.3 Antimicrobial assay

The extracted oils of *Croton penduliflorus* were tested for antimicrobial activities using agar disc diffusion technique to determine the diameter of growth inhibition zones using the method of [10] with slight modification. Briefly, 0.1 ml of suspension of the test microorganisms containing 1.5×10^6 cfu/ml of bacteria or 1.5×10^6 spores/ml of yeast was spread on Mueller Hinton agar or Sabouraud dextrose agar medium respectively. Filter paper discs (Whattmann n°3, 6 mm in diameter) were placed on the seeded plates. Gentamicin and penicillin (10 µg/disc) were used as positive reference drugs for bacteria and fungi respectively. The plates were incubated at 37°C (24 h) for bacterial strains, and 35°C (48 h) for yeast. Antimicrobial activity was evaluated by measuring the diameters of inhibition zones. The experiment was repeated three times in each case.

2.3 Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA), and differences between samples at $P \leq 0.05$ were determined by Duncan multiple range test using the Statistical Package for the Social Sciences (SPSS 17) program. Experimental results were expressed (where appropriate) as mean ± SDV (standard deviation).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties

Physicochemical properties of extracted oil of the seeds are presented in Table 1. There was no significant difference in values of density, specific density and refractive index for whole and dehulled seeds oil. Acid value is an important index of physicochemical property of oil which is used to indicate the quality, age, edibility and suitability of oil for use in industries such as paint [11]. According to [12] acid values are used to measure the extent to which glycerides in the oil have been decomposed by lipase and other physical factors such as light and heat. Thus, the high acid value of both samples suggests that the oil was highly susceptible to lipase action. This value (14.5 mgKOH/g) and (23.5 mgKOH/g) for whole and dehulled seeds oil respectively were higher than 0.6 mgKOH/g proposed by [13] for edible vegetable oil. The acid values were also higher than 11.04 mgKOH/g reported for *Annona cherimoya* by [14], 5.64 mgKOH/g reported for *Baua racemosa* seed oil by Amoo and Moza, 1999 and lower than 68.71 and 34.79 mgKOH/g reported for L. *aegyptica* and *castenae* sp respectively by [15]. Peroxide value of dehulled seeds (1.60) meq/kg was higher than whole seeds (1.14) meq/kg. Oils with peroxide

values ranging from 20.00 to 40.00 meq/kg are considered rancid [16]. The peroxide value contrast the value 0.06 meq/kg reported for Soybean oil [17]. Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage. The iodine value is a measure of the unsaturation of fats and oils [18]. The iodine value of whole seed (96.20 mg/g) was higher than dehulled seed (88.32 mg/g) categorized them in semi drying oil group. High iodine value of *Croton* oil is caused by high content of unsaturated fatty acid such as oleic acid and linoleic acid. The results are very high compared to the value for *Trichosanthes cucumerina* seed oil reported by [19]. Saponification value is an index of average molecular mass of fatty acids in oil sample. The saponification value of dehulled seeds oil (169.70) mg/g was higher than whole seeds (162.69) mg/g, this was higher than 148.3 and 126.8 reported for crude and refined oils of Canerium fruit by [20], 108.23 and 28.28 reported for L. *aegyptica* and *castenae* sp respectively by [15]. High saponification value makes the oil useful in the production of liquid soaps and shampoos [21]. The unsaponifiable matter in the seeds oil was low, an indication of low values of natural products like steroids, alkaloid and others which can limit the domestic consumption of the oil by toxicity increase [22]. The result showed that whole and dehulled seed oils had FFA content of 7.3 and 11.8 respectively. The FFA and moisture contents have significant effect on the transesterification of glycerides with alcohol using catalyst [23]. The density of a material is defined as the measure of its mass per unit volume in g/dm^3. Generally the density of oil decreases with molecular weight, yet increase with unsaturation level [24]. The densities of both whole and dehulled oil samples were 0.74 and 0.75 g/dm^3 respectively.

3.2 Fatty Acid Compositions

Fatty acid compositions of *Croton penduliflorus* seeds are presented in Table 2. There are three main types of fatty acids that may be present in a triglyceride which are saturated (Cn:0), monounsaturated (Cn:1) and polyunsaturated with two or three double bonds (Cn:2,3). The most prominent of fatty acids in whole and dehulled seeds were Oleic acid (C18:1) (36.4 and 37.2) % and Linoleic acid (18:2) (25.4 and 27.5) % respectively. Dehulled seeds have a higher composition of saturated fatty acid (31.8%) compared to whole seeds (28.5%). The ratio of saturated to unsaturated fatty acid was (0.459) in whole seed and (0.489) in dehulled seeds. The various fatty acids are higher in dehulled seed oil .The Palmitic acid content of both samples (25.2%) whole seed and (26.1%) dehulled seeds was lower than 40.1% of *Periploca sepium* reported by [25]. The oleic acid percentage concentration of whole seed (36.4%) and dehulled seed (37.2%) are higher than coconut oil (7.2%) as reported by [26]. This implies that *C. penduliflorus*is a better source of oleic acid compared to coconut oil. Various vegetable oil is a potential feedstock for the production of a fatty acid methyl ester or biodiesel but the quality of the fuel will be affected by the oil composition. Ideally the oil should have low saturation and low polyunsaturation, and high in monounsaturated fatty acid [24]. The stability of oil depends on the oleic acid/ linoleic acid (O/L) ratio and iodine value; high (O/L) ratio signifies longer shelf life, stability and potentiality of the oil for deep frying. The O/L level of the oil samples (1.43) and (1.35) for whole and dehulled respectively in the present study was closed to peanut oil of 1.48 reported by [27] hence *Croton penduliflorus* seed oil is as stable as peanut oil.

Table 1. Physicochemical properties of the extracted oil of whole and dehulled seeds of *Croton penduliflorus*

Parameters	Whole seed	Dehulled seed
Refractive index (32°C)	1.46± 0.00	1.46± 0.00
Specific gravity	0.79± 0.01	0.80± 0.01
Density (g/dm^3)	0.74 ± 0.00	0.75 ± 0.00
Acid value (mgKOH/g)	14.5 ± 0.00	23.5 ± 0.01
Free fatty acid (mgKOH/g as oleic acid)	7.3 ± 0.00	11.8± 0.01
Saponification value (mg/g)	162.69± 0.03	169.70± 0.02
Unsaponifiable matter (%)	0.97 ± 0.01	1.43± 0.01
Iodine value (mg/g)	96.20± 0.01	88.32± 0.00
Peroxide value (meq/kg)	1.14± 0.05	1.60± 0.01

Mean ± standard deviation.

Table 2. Fatty acid composition (%) of both whole and dehulled seed oils of *Croton penduliflorus*

Fatty acids	Shorthand	Formula	Whole seed	Dehulled seed
Myristic acid	C14:0	$C_{14}H_{28}O_2$	0.3	0.7
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	25.2	26.1
Stearic acid	C18:0	$C_{18}H_{34}O_2$	2.9	4.8
Oleic acid	C18:1	$C_{18}H_{34}O_2$	20.4	27.2
Linoleic acid	C18:2	$C_{18}H_{32}O_2$	25.4	27.5
Linolenic acid	C18:3	$C_{18}H_{30}O_2$	0.2	0.3
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	0.1	0.2
Saturated			28.5	31.8
Monounsaturated			36.4	37.2
Polyunsaturated			25.6	27.8
Total			90.5	96.8

Table 3. Antimicrobial properties of the whole and dehulled seed oils of C. *penduliflorus*

Microorganisms	Whole seed oil	Dehulled seed oil	Reference
Bacteria			**Gentamycin**
Escherichia coli	23.12^{c}±0.08	31.16^{b}±0.07	43.18^{a}±0.11
Klebsiella pneumonia	30.17^{c}±0.02	37.26^{b}±0.09	51.24^{a}±0.02
Pseudomonas aeruginosa	26.18^{b}±0.03	18.11^{c}±0.03	37.21^{a}±0.06
Salmonella typhi	11.13^{c}±0.01	14.25^{b}±0.02	26.54^{a}±0.02
Staphylococcus aureus	46.57^{c}±0.11	51.23^{b}±1.05	58.16^{a}±0.05
Enterococcus faecalis	20.43^{c}±0.16	24.38^{b}±0.17	40.03^{a}±0.13
Fungi			**Penicillin**
Candida albican	16.24^{c}±0.05	19.83^{b}±0.06	28.43^{a}±0.03
Candida tropicalis	9.16^{c}±0.02	10.52^{b}±0.02	17.32^{a}±0.02
Candida krusei	12.36^{c}±0.03	15.47^{b}±0.05	33.61^{a}±0.05
Candida glabrata	8.45^{c}±0.02	11.33^{b}±0.03	24.35^{a}±0.02

Mean ± standard deviation of triplicate determinations. Means follows the same superscript are not significant at ($p < 0.05$)

3.3 Antimicrobial Activities

The extracted oil of C. *penduliflorus* showed antimicrobial activities against all the bacteria and fungi tested. The highest activity was observed on bacteria especially staphylococcus aureus (51.23±1.05). The diameters (mm) of inhibition zones for bacterial ranged from 11.13 – 46.57 whole seed oil, 14.25 – 51.23 in dehulled and 26.54 – 58.16 in control (gentamicin) and fungal strains were in the ranges 8.45 – 16.24 in the whole seed oil, 10.52 – 19.83 in dehulled and 17.32 – 33.61 in control (penicillin) respectively as shown in Table 3 above. The results of the antimicrobial activities showed that both oils of C. *penduliflorus* had moderate antimicrobial activities against all the microorganisms compared to reference. These oils showed greater antibacterial and antifungal activities with the standard method of disc diffusion. This established that the extracted oils were capable of controlling bacterial and fungal diseases especially when it was dehulled.

4. CONCLUSION

The results of physicochemical properties of whole and dehulled seed oil revealed that there was significant increase in acid value, free fatty acid, saponification value and unsaponifiable matter in the dehulled seed oil. There was little or no significant difference in refractive index, specific gravity and density while there was reduction in iodine value due to dehulling. The low peroxide value of both oils makes them ideal for long time storage without going rancid. Fatty acid composition of whole and dehulled seed oils showed the presence of saturated fatty acid at low concentration with high amount of unsaturated fatty acid especially in dehulled seed oil which makes it ideal in the food industry to reduce the incidence of heart attack caused by high level of cholesterol. Both oils possessed high antimicrobial activities by inhibit the growth of bacteria and fungi thereby makes them a good alternative medicine in treatment of bacterial and fungal infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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