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Biochemical Characterisation of Fruits for Establishing Phylogenetic Relationship among Members of Arecaceae (Palmae) in North-Central Nigeria

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ABSTRACT

Background and Objective: The Arecaceae (Palmae) is a family of perennial flowering plants in the monocot order Arecales. The very slow rate of convergent evolution among some morphological characters has a limited phylogenetic resolution for the palm family. This study was aimed at determining the biochemical composition of selected members (*Phoenix dactylifera, Hyphaene thebaica, Cocos nucifera* and *Elaeis guineensis*) of Arecaceae in North-Central Nigeria and using the traits to establish phylogenetic relationships among the members. **Materials and Methods:** Before the comparative nutritional study of the selected members, ash content, moisture content, crude fibre, crude protein, crude fat, carbohydrates and mineral compositions were assessed using standard methods. All data was assumed significant at $p<0.05$. Similarly, all the data obtained was used to draw a dendrogram based on Unweighted Pair Group Method Average (UPGMA) to establish the phylogenetic relationship among the palms. **Results:** Results showed that the highest percentages of moisture content (11.47%) and carbohydrates (66.33%) were recorded in Date palm (*Phoenix dactylifera*). Doum palm (*Hyphaene thebaica*) had significantly the highest ash content, calcium, sodium and potassium (7.30%, 303.14, 195.22 and 949.90 mg/100 g, respectively). The highest percentages of crude protein (10.63%), crude fibre (27.53%) and magnesium (343.89 mg/100 g) were recorded in a coconut fruit. Palm fruit had significantly the highest crude fat content (51.29%). **Conclusion:** Phylogenetic relationship showed that coconut and oil palm was the closest among the four palm groups while date palm is closer to them than the Doum palm. The results obtained have enhanced our knowledge of establishing phylogenetic relationships among palm trees in North-Central Nigeria using their nutritional composition.

KEYWORDS

Arecaceae, palms, biochemical composition, phylogenetic relationship, dendrogram, nutritional composition

INTRODUCTION

The Arecaceae is a family of perennial flowering plants in the monocot order Arecales. Their growth form can be climbers, shrubs, tree-like dichotomously branched and stem-less plants generally known as

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palms[1]. Those having a tree-like form are called palm trees. The family contains several commercially important species such as coconuts, areca nuts, doum palms and date palms etc[2]. Christenhus and Byng[3] mentioned that currently 181 genera with about 2,600 species are known within Arecaceae, most of them are said to be restricted to tropical and subtropical climates. Most palms are distinguished by their large, compound, evergreen leaves, known as fronds, arranged at the top of an un-branched stem. However, palms exhibit an enormous diversity in physical characteristics and inhabit nearly every type of habitat within their range, from rainforests to deserts. Palms are among the best-known and most extensively cultivated plant families. Because palms provide so many common products and foods, they have been important to humans for a long time[4]. Palms are one of the most economically important plants nowadays since they are commonly employed in landscaping. Palms have always been cited as important materials for construction, food, handcrafts and therapeutics. Quantitative methods have been used in ethnobotanical studies to analyze the knowledge and use of palms by local people Martins *et al*.[5]. Several inhabitants of the tropical and sub-tropical regions of the world use the members of the Arecaceae family as food resources[6,7]. Arecaceae plants provide carbohydrates, fats, proteins and minerals as well as a significant amount of molecules that help to confer antioxidant properties that are important to human life[8]. Some of the most important economic palms fall within the Arecoideae, such as oil palm (*Elaeis guineensis*), coconut (*Cocos nucifera*) and betel nut palm (*Areca catechu*) and many important species in the global horticultural trade[4]. Some of the edible wild plants are regularly consumed by people in the form of staple food. Many wild species may constitute an interesting genetic resource for the development of new food sources which could be used as alternative food and select promising types for domestication[9]. It is based on this background that this research intends to compare the biochemical composition among selected members of the family Arecaceae in Minna, Nigeria.

MATERIALS AND METHODS

Study area and sample collection: The samples, which were the fruits of the different palms (coconut, date palm, oil palm and doum palm), were collected during the growing season of the year 2021. They were collected between June and September, 2021 from different locations in Minna (North-Central Nigeria) metropolis as presented as follows (Table 1).

The date palm (*Phoenix dactylifera*) sample was collected from a border area between Tunga and Chanchaga, Minna. Doum palm (*Hyphaene thebaica*) sample was collected at the front of the Man 'O' War command unit at Bosso, Minna. Coconut (*Cocos nucifera*) was collected around the Maikunkele area, an outskirt of Minna. The oil palm (*Elaeis guuineensis*) was collected at Dama village, Bosso, Minna (Table 1), Fig. 1a-h.

The flesh of the fruits was separated from the seed manually and thoroughly rinsed so that all traces of impurity was cleared and dried in an oven at 50°C. After the drying, the seeds were ground into powder. Then, the powder of each seed sample was sieved and stored in air-tight sample containers and kept in the refrigerator until required for appropriate analysis.

Determination of moisture and ash contents: The moisture content of the samples (*Phoenix dactylifera, Hyphaene thebaica, Cocos nucifera* and *Elaeis guineensis*) was determined using the method described by the Abdulrahman *et al*.[10]. A 2.0 g of each sample was placed into the Petri dish and dried in an oven for three hours at 105±2°C. The dried samples were cooled in a desiccator for 30 min and weighed to a constant weight. The loss in weight is equivalent to the moisture content of the sample. The moisture content was then calculated as below:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight after drying}}{\text{Initial sample weight}} \times 100$$

Fig. 1(a-h): Whole fresh fruits and ground fruits of palms, (a) Palm kernel fruit with fleshy epicarp and stony mesocarp removed, (b) Palm kernel fruit (whole fruit), (c) Doum palm fruit (whole fruit), (d) Ground doum palm fruit, (e) Coconut fruit (whole fruit), (f) Ground coconut fruit, (g) Fresh fruit of date palm and (h) Ground fruit of date palm

Table 1: Locations of the samples collected

Palm species	Common name	Longitude	Latitude
Phoenix dactylifera	Date palm	9°36'54.86"N	6°32'51.94"E
Hyphaene thebaica	Doum palm	9°39'52.45"N	6°31'19.44"E
Cocos nucifera	Coconut palm	9°39'52.46"N	6°14'84.43"E
Elaeis guuineensis	Oil palm	9°35'37.00"N	6°32'10.75"E

Ash content of the samples was determined according to the method of Abdulrahman *et al.*[10]. A 2.0 g of each sample was weighed into a pre-weighed porcelain crucible and burned slightly over a small flame and transferred into a muffle furnace at 550°C for 6 hrs. The crucible was cooled in a desiccator and weighed immediately. The procedure of heating, cooling and weighing was repeated to achieve a constant weight. The residue in the crucible is the ash contained in the sample and hence, the total ash was calculated as follows:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{1\,\text{g}\,(\text{oven - dried weight})} \times 100$$

Crude fat determination: The concentration of crude fat was determined using the method of AOAC 2019[11]. Two grams were weighed from the oven-dried sample from moisture content determination into a thimble (W_1) and the weight of an empty thimble (W_0). It was further dried in the oven for 5 hrs at 100°C. Beakers to be used for fat determination were dried for about 1 hr at 100°C and cooled in a desiccator. The weights were taken and recorded (W_2). The thimble containing the sample was placed in a soxhlet unit connected to a condenser and a heating flask. About 400 mL of petroleum ether was poured into the flask in the extraction unit. The heating mantle was set to 60°C. The crude fat content will then be determined using:

$$\text{Fat (\%)} = \frac{(W_1 - W_2) \times VC}{VA \times SW} \times 100$$

where, W_2 is the weight of glass tube and dried extract (g), W_1 is the weight of empty dried glass tube (g), VC is the total volume of chloroform (mL), VA is the volume of extract dried (mL) and SW is the weight of the sample in grams.

Crude fiber determination: The concentration of fibre was determined according to the method highlighted by AOAC, 2019[11]. A 5.0 g of each sample of *Phoenix dactylifera, Hyphaene thebaica, Cocos nucifera* and *Elaeis guineensis* was weighed into a one-litre conical flask. Then, 200 mL of boiling 1.25% (v/v) sulphuric acid was added and boiled for 30 min over a burner. The solution was allowed to cool and filtered under suction on a piece of coarse texture linen. The residue will then be transferred back to the conical flask and then 200 mL 1.25% sodium hydroxide (NaOH) solution was added and then refluxed for 30 min. It was cooled and filtered again through a piece of coarse-textured linen. Lastly, the samples were dried in an oven at 100°C for 1 hr, then cooled in a desiccator, filtered through a piece of coarse-textured linen and weighed (W_1). The sample was packed into a crucible in a furnace at 55°C for 3-4 hrs and was allowed to cool in a desiccator and weighed again (W_2). Fibre content was calculated thus:

$$\text{Fiber (\%)} = \frac{W_1 - W_2}{\text{Weight of the sample}} \times 100$$

Crude protein determination: The crude protein content of the samples was determined according to the method of Abdulrahman *et al.*[10]. Some 2.5 g of dried and ground samples were weighed into the digestion tubes. Fifteen grams (15 g) Na_2SO_4, 1 g $CuSO_4$, one or two solemnized boiling granules and 25 mL of concentrated H_2SO_4 was added to the tube. It was digested at about 400°C until a solution was almost colourless (2 hrs for inorganic material) and then at least a further 30 min. It was cooled down, 200 mL of water was cautiously added. About 100 mL 0.1 N HCl was pipette into a 500 mL conical flask, 1 mL Conway's indicator was added and the flask was placed under the condenser of the distillation apparatus ensuring that the condenser tip is immersed in the acid solution. To the Kjeldahl tube containing the digested samples, 100 mL of 50% NaOH solution was slowly added down the side of the Kjeldahl tube so that it formed a layer underneath the digestion mixture. The digestion mixture was immediately transferred to the distilling bulb of the distillation apparatus and corked. It was heated until all ammonia passed over into the standard acid. Approximately 150 mL was collected. Excess standard HCl in distillate was titrated with NaOH standard solution until the colour changed from purple to green indicating endpoint. Percentage nitrogen was calculated (wet weight basis) as follows:

$$\text{Nitrogen (wet) (\%)} = \frac{(\text{A-B}) \times 1.4007}{\text{Weight (g) of sample}} \times 100$$

Where:

A = Vol. (mL) std., HCl×normality of std., HCl

B = Vol. (mL) std., NaOH×normality of std., NaOH

Nitrogen content on a dry basis was calculated (when the moisture content is known) as follows:

$$\text{Nitrogen (dry) (\%)} = \frac{\text{Nitrogen (wet)}}{\text{100-moisture}} \times 100$$

Calculate the percentage protein (wet or dry basis) as follows:

$$\text{Protein (\%)} = \text{Nitrogen} \times 6.25$$

where, 6.25 is the protein-nitrogen conversion factor.

Carbohydrate content determination: To determine the concentration of carbohydrates, the percentage of crude fibre and nitrogen-free extract represent the total carbohydrate in the sample i.e., the sum of the crude fibre and nitrogen-free extract. It was calculated thus:

$$\text{Carbohydrate (\%)} = \text{Crude fiber} + \text{Nitrogen-free extract}$$

Mineral content determination: The determination of mineral content was done according to the method of Abdulrahman *et al.*[10] Some 1.0 g of the pulverized samples were placed in a crucible and ignited in a muffle furnace at 550°C for 6 hrs. The resulting ash was dissolved in 10 mL of 10% HNO_3 and heated slowly for 20 min. After heating, the dissolved ash was filtered and the filtrate was used for the determination of mineral content. Atomic Absorption Spectrophotometer (AAS) was used to determine Calcium and Magnesium, while, a flame photometer was used for the determination of Sodium and Potassium in the filtrate.

Data analysis: Quantitative data were subjected to Analysis of Variance (ANOVA) to compare the means using SPSS version 22. Duncan Multiple Range Test was used to separate the means. Differences between means were considered statistically significant at $p<0.05$. Each value for nutritional parameters was expressed as Mean±SEM for the samples from *Phoenix dactylifera, Hyphaene thebaica, Cocos nucifera* and *Elaeis guineensis*. Dice dissimilarity index was used to calculate dissimilarity values between the germ lines. A cluster diagram was drawn using Unweighted Pair Group Method Average (UPGMA).

RESULTS

The results of the proximate analysis of the selected members of Arecaceae revealed distinct variations among coconut, doum palm, date palm and oil palm fruits. The highest percentage of moisture content (11.47%) was found in the date palm fruits and this value was significantly different ($p<0.05$) from those obtained from coconut fruit (3.12%) and palm fruit (2.57%) but significantly the same with that of Doum palm fruit (9.87%). For the crude fat, the highest value was recorded in the palm fruit (51.29%), this value was significantly different from all other values, however, the percentage of crude fat in coconut fruit (44.09%) was quite higher than those of date palm (6.55%) and Doum palm (15.32%). The highest percentage of Ash content of 7.03% was found in Doum palm fruit while the lowest was recorded in palm fruit (2.29%), however, there was no significant difference ($p>0.05$) between the highest and the lowest (Table 2).

The highest percentage of Crude protein was found in coconut (10.63%), this was followed by palm fruit (10.05), whereas the lowest value was recorded in Doum palm fruit (3.60%) and the value was significantly different from those of coconut and oil palm fruit but significantly the same ($p>0.05$) with date fruit (3.74%). The highest percentage of Crude fibre was recorded in coconut fruit (27.53%) with date fruit having the lowest percentage of crude fibre (8.73%). The crude fibre in oil palm fruit and Doum palm fruit were 14.83 and 20.10%, respectively. The highest percentage of carbohydrates was found in date fruit (66.37%) with coconut fruit having the lowest percentage (11.31%), these values were significantly different from each other, Doum palm fruit and palm fruit contained a percentage carbohydrate of 44.06 and 18.98%, respectively.

The highest percentage of calcium (303.14 mg/100 g) was found in Doum palm and palm fruit contained the lowest percentage of calcium constituent (37.53 mg/100 g), these values were significantly different from each other and date fruit and coconut (165.21 and 171.36 mg/100 g), respectively. Coconut fruit had the highest magnesium constituent (343.89 mg/100 g) and the lowest percentage was found in oil palm fruit (52.95 mg/100 g), these values were significantly different from each other and those produced by date fruit and Doum palm fruit (85.66 and 136.58 mg/100 g), respectively. The highest percentage of

Table 2: Proximate composition of selected members of arecaceae

Proximate composition	Moisture content (%)	Crude fat (%)	Ash (%)	Crude protein (%)	Crude fibre (%)	Carbohydrates (%)
Date fruit	11.47±1.36^b	6.55±0.57^a	3.15±0.32^a	3.74±0.27^a	8.73±0.91^a	66.37±0.18^d
Dum palm fruit	9.87±0.23^b	15.32±0.43^b	7.03±3.07^a	3.60±0.20^a	20.10±1.24^c	44.087±1.44^c
Coconut fruit	3.12±0.35^a	44.09±1.20^c	3.05±0.49^a	10.63±0.34^b	27.53±0.16^d	11.31±1.10^a
Palm fruit	2.57±0.41^a	51.29±0.58^d	2.29±0.24^a	10.05±0.12^b	14.83±0.22^b	18.98±0.89^b

Values are Means±Standard errors, values followed by different superscripts along the same column are significantly different at $p<0.05$

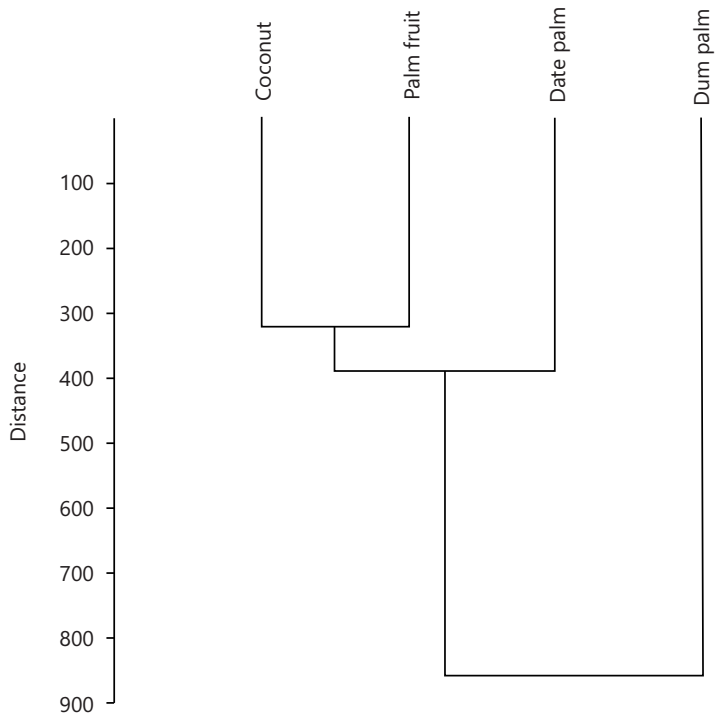

Fig. 2: UPGMA hierarchical cluster diagram based on biochemical composition analyses of the fruits of selected members of Arecaceae

Table 3: Mineral composition of selected members of Arecaceae

Proximate composition	Ca (mg/100 g)	Mg (mg/100 g)	Na (mg/100 g)	K (mg/100 g)
Date fruit	165.20±5.18^{d}	85.66±1.78^{b}	8.15±0.74^{a}	359.27±19.68^{b}
Dum palm fruit	303.14±15.88^{c}	136.58±4.40^{c}	195.22±5.18^{c}	949.90±41.71^{c}
Coconut fruit	171.36±5.22^{a}	343.89±7.11^{b}	32.07±0.77^{b}	39.94±0.74^{a}
Palm fruit	37.53±0.44^{b}	52.95±0.83^{a}	31.31±1.06^{b}	33.64±0.84^{a}

Values are Means±Standard errors, values followed by different superscripts along the same column are significantly different at $p<0.05$

sodium was found in Doum palm fruit (195.22 mg/100 g) while date palm fruit had the lowest percentage of sodium constituent (8.15 mg/100 g), the values were different significantly from each other and those produced by other fruits (coconut fruit 32.07 mg/100 g and palm fruit 31.31 mg/100 g). The highest percentage of potassium was found in Doum palm fruit (949.90 mg/100 g), while the lowest percentage was recorded in coconut fruit (39.94 mg/100 g), palm fruit contained 33.64 mg/100 g of potassium while, date fruit contained 359.27 mg/100 g, there was significantly different ($p<0.05$) among the values (Table 3).

The phylogenetic relationship among the selected members of Arecaceae was presented in Fig. 2. At a genetic distance of 350, the plants were separated into three different clusters. Cluster 1 comprises coconut and palm fruit, while clusters 2 and 3 comprise date palm and Doum palm respectively. This indicates that coconut and oil palm are closer while date palm is closer to them than the Doum palm. Doum palm appeared to be distinctly separated from all the other palm groups.

DISCUSSION

Proximate and nutritional analysis play an essential role to assess the human-derived health benefits of various food products. The importance of fruits as a source of nutrients has attracted the attention of various researchers throughout the world, especially in Nigeria by Umar *et al.*[12]. The present study has identified variations in the nutrient composition of the selected members of the Arecaceae family. In this study, the moisture content, ash content, crude fibre, crude protein and minerals of doum

palm, oil palm, coconut and date palm were assessed. The result showed a reasonable difference in these parameters to the plant samples.

In the present study, the highest percentage of moisture content (11.47%) was consistent with the study of Ogungbenle[13], who reported a moisture content of 11% in date palms. The high moisture content in date palms indicated the fruit is more inclined to decay since nourishments with high dampness substance are more inclined to perishability if not well preserved as reported by Omoregie and Osagie[14]. The lowest moisture content (2.75%) that was found in oil palm fruit is an indication that palm fruit may not be inclined to decay as obtainable in other fruits.

This study revealed that the highest value of crude protein was recorded in the palm fruit (51.29%), thus indicating that palm fruit contains essential molecules that could mediate cell responses as reported by Whitney and Rolfes[15], who opined that crude protein serves as an enzymatic catalyst, mediate cell responses, control growth and cell differentiation. The low crude protein (3.74±0.27) that was recorded in date palms is quite higher than those reported by Al-Harrasi *et al.*[16] those who reported low crude protein content from 2.637-3.783 g/100 g in date palms from Oman. However, this value falls within the range of value obtained for date palms collected in some parts of Nigeria[10]. This indicated that date palm is not an excellent protein source compared to the selected fruits as has been previously reported by Assirey[17].

In this study, the highest percentage Ash content of (7.03%) that was found in Doum palm fruit indicates that doum palm may have appreciable amounts of mineral elements. This is consistent with the work of Datti *et al.*[18] in their study on the biochemical properties of *Hyphaene thebaica* in Kano, Nigeria. They reported high ash content (6.24%) of doum palm. The slight difference in the values might be due to variation in soil minerals which could influence the overall mineral compositions of the plants.

The present study revealed that the highest percentage of Crude fibre was recorded in coconut fruit (27.53%) with date fruit having the lowest percentage (8.73%). Dietary fibre is known to influence digestion and absorption processes in the small intestine, Habib and Ibrahim[19]. The high crude fibre content of coconut fruit compared with other fruits indicates that coconut fruit may aid digestion. This is consistent with the study of Al-Farsi and Lee[20] that reported that high crude fibre content aid in digestion, absorption of water from the body and bulk stool and also prevents constipation. Low fibre content (8.73%) was recorded in date palms. This slightly corroborates the study of El-Sharnouby *et al.*[21], who reported a 9.4% crude fibre content of date palm.

The present study has identified that the highest percentage of carbohydrates was found in date fruit (66.37%) while, coconut fruit had the lowest percentage. Carbohydrate contributes the greatest amount of energy required by man and animals Oni *et al.*[22]. This probably explains why this fruit is considered a staple fruit in semi-arid regions of the world[22]. Generally, the carbohydrate content of date palms has been reported to be largely composed of fructose and glucose Sadiq *et al.*[23], which are readily metabolized in cell energy production pathways. The present study observed that date palm is rich in carbohydrates having 66.37% is dissimilar to the study of Assirey[17], who reported 74.3% of the carbohydrate content in date palm and it is also slightly lower than 80.70% reported by Ogungbenle[13]. The variation could be due to the different genetic bases of the samples used. The results showed that the date palm is a good source of carbohydrates which may be considered a good source of energy for the human system.

Minerals are important components in the human diet and are considered essential in human systems including growth, cell differentiation and tissue and bone formation[22]. Minerals such as calcium, potassium, magnesium and sodium have all been identified in varying quantities in this study. This could

be attributed to the difference in their distribution among plant species which may be attributed to several environmental factors. The study revealed doum palm has the highest calcium percentage (313.14%), which indicated that the doum palm is an excellent source of calcium and can be included in the diet as a bone strengthener as calcium has been reported in several studies to strengthen bone and teeth including the study of Lima *et al*.[24]. The present study revealed that coconut fruit has the highest magnesium constituent (343.89 mg/100 g) and the lowest percentage was found in palm fruit (52.95 mg/100 g). This result confirmed previous studies on the high magnesium content of coconut fruit including the study of El-Beltagi *et al*.[25]. This study also revealed that the highest percentage of sodium and potassium was found in Doum palm fruit 195.22 and 949.90 mg/100 g, respectively. This indicated that the doum palm contains a considerable amount of minerals. This is consistent with the study of El-Beltagi *et al*.[25], who reported high sodium content (238.09 mg/100 g) in doum palm.

The phylogenetic relationship as revealed by the biochemical constituents of the selected palm trees in Minna, north-central Nigeria is in line with those reported by both morphological and molecular characterisations by Hahn[26]. In various studies, the genus *Hyphaene* has been placed in separate groups from *Phoenix*, *Cocos* and *Elaeis*. In the study of Hahn[26] and Xiao *et al*.[27], both *Cocos* and *Elaeis* have been grouped in the Arecoid line of Palmae, whereas *Phoenix* and *Hypahene* have been grouped in a separate group, i.e. Coryphoideae and Caryoteae respectively. In a more specific study by Baker *et al*.[4], both coconut palm and oil palm trees were grouped into a large Arecoideae group. Both species were further separated into sub-group Attaleinae (*Cocos nucifera*) and Elaeidinae (*Elaeis guineensis*). This further buttressed the suitability of using biochemical constituents in palm fruits to further understand the phylogenetic relationship among the different palm groups. The reason why both Coconut and African oil palm are closely related could be attributed to the fact that both store oil in their endosperms, while date palm fruits contain very little oil Xiao *et al*.[27]. Thus, the biochemical composition of fruits of members of Arecaceae could serve as an important taxonomic key for establishing phylogenetic relationships among members of the plant family in the study area.

CONCLUSION

This study revealed the nutrient composition of the selected members of the Arecaceae family. The study revealed there is variation in the nutrient composition among the selected members. This indicated Date palm is inclined to spoilage but is a very good source of energy. Doum plant has the highest Ash content, Calcium, Sodium and potassium. This indicated that the Doum plant contains a considerable amount of minerals and can be used as a bone strengthener. The highest percentages of Crude protein and Crude fibre as well as Magnesium, were recorded in Coconut fruit. This indicated that Coconut can be included in the diet of people because of its high fibre content. Lastly, African oil palm fruit has the highest Crude fat content, no wonder it serves as an excellent source of natural oil for consumption. The phylogenetic relationship showed that coconut and oil palm are closer to each other than date palm and doum palm. Date palm however is closer to both of them than the Doum palm. The results obtained have enhanced our knowledge of some biochemical compositions of the plants and their phylogenetic relationship.

SIGNIFICANCE STATEMENT

This study established the possibility of using biochemical constituents of fruits of members of the palm family to establish a phylogenetic relationship. It further revealed that among coconut, oil palm, date palm and doum palm trees, coconut and oil palm are the most closely related among the palms while date palm is somehow next to them in terms of closeness. However, the doum palm was distinctly separated from the group, indicating that it is distantly related to the other members. Synergistic effect of vitamin E, calcium and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination and possibly other combinations, may be arrived at.

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