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Studies on the nutrient and non-nutrient profile of lesser known seeds of tetracarpidium conophorum Mull. (Arg)

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ABSTRACTS

The nutrients and non-nutrients of Tetracarpidium conophorum were evaluated using standard laboratory methods. The results showed that moisture, crude protein and crude fat, were significantly high in percentage content (29.43;31.63;23.24% respectively) and also the energy value(1580.96Kj/100g) while carbohydrate and ash were found to be low (09.61 and 6.09%). Minerals included calcium, magnesium, potassium, sodium, iron and phosphorus were determined on a dry weight basis. T. conophorum had high potassium and phosphorus contents (02.45% and 01.16%) and all the other elements were detected in lower concentrations (0.01 to 0.65 %). Qualitative analysis of the bioactive constituents of the plant sample in its methanol and carbon tetrachloride extracts showed the presence of alkaloids, glycosides, saponins, steroids , triterpenoids,phenol and anthocyanins. Flavonoids and anthocyanins were detected in methanol extract only, while anthraquinones were detected in carbon tetrachloride extract and anthocyanins were absent. However, tannins were completely absent in both solvent extracts. Quantitative analysis of T. conophorum seeds revealed that they contained high amounts of alkaloids(12.80±0.19) and flavonoids(11.13±0.35) whereas saponins(3.65±0. 06), anthocyanins(3.33±0.74) and Phenols (1.60±0.1 6%) were found in lower concentrations. The significant role of these chemicals for the human body and the importance of these plant seeds in traditional medicine are discussed in order to assess their medicinal and nutritional contribution in body health care.

Keywords: Nutrient, non-nutrient, analysis, value, *T.conophorum*

INTRODUCTION

Tetracarpidium conophorum (Mull. Arg) Hutch & Dalz (Euphorbiaceae), commonly called the African walnut, is a perennial climbing shrub of 3-6m long. *T. conophorum* is found in the moist forest zones of Sub-Saharan Africa and India. It is cultivated principally for its nuts which are cooked and consumed as snacks [1] [2]. It serves in Congo as an edible nut eaten after cooking as a delicacy and occurs in the forest. In southern Nigerian ethno-medicine, *T. conophorum* is used as a male fertility agent and the leaves are used for the treatment of dysentery and to improve fertility in males [3].

The leaf extract is used to mitigate prolonged and constant hiccups, improvement of fertility. It can also be used to improve spermatozoa count in men and to reduce the incidence of tumor and cancer cells [4]. Solvent extracts fractions of the leaf are able to inhibit the activities of gram negative bacteria such as *Staphylococcus aureus* , *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The root extract of *T. conophorum* possess antifungal properties [3].The bark is used in tea as laxative and chewed for toothache. It helps to prevent and control high blood pressure [5].

Previous studies reported the nutritive and medicinal value of the seeds. The proximate, phytochemical , mineral compositions of the seeds [6] and the biochemical content of the nuts [7] were reported. The physico-chemical characteristics of the nut oil of *T. conophorum* [8] have been also reported. In Congo, few studies have focused on the chemistry of this wild nut. They reported the fatty acid composition of *T. conophorum* nuts[9], [10]. The aim of this work was to determine the proximate, mineral composition and phytochemical constituents of the nuts from Congo in order to establish their nutritive and medicinal values.

EXPERIMENTAL SECTION

Collection and processing of plant materials:
The experimental nuts were bought at the local Mikalou market from Talangai, North-Brazzaville area on 23rd April 2013. The plant materials were identified and authenticated by Nkouka Saminou from the national herbarium of the vegetal research centre of Brazzaville (ex-OROSTOM-Congo), where voucher specimens are conserved. The nuts were dried in an oven at 70^oC for 24 hours and milled into powder with a mechanical blender. The powder was stored at room temperature under dry conditions before analysis. Chemical analysis was carried out on the dried and powdered materials.

Chemical analysis
Proximate analysis:
The moisture content of the *T.conophorum* seeds was determined by drying them at 105°C in an oven, until a constant weight was reached. For total ash determination, the plant samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude fat content was determined by extraction with dichloromethane, using a Soxhlet apparatus. All these determinations were carried out according to AOAC[11].

The Kjeldahl method was used for crude protein determination. Carbohydrate content was determined by calculating the difference between the sum of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively[12].

Mineral analysis:
Mineral analyses of the plant sample were carried out by using the methods of Martin-Prevel [13]. Elemental analyses were carried out using an atomic absorption spectrophotometer and a flame photometer to determine calcium, sodium, potassium and magnesium content. Aluminum, iron and phosphorus were determined calorimetrically. The concentration of each element in the sample was calculated on a dry matter basis.

Preparation of solvent extracts:
The powdered sample (30g) was defatted twice in diethyl ether (150ml) for 24h before it was subjected to preliminary screening. Extraction of bioactive compounds was carried out by hot percolation in methanol. Twenty grams of the powdered sample were soaked in 150 mL of methanol and carbon tetrachloride solution for 48 h. The mixtures were then filtered and the filtrates were concentrated by evaporation using a boiling water bath. The solvent extracts thus obtained were subjected to phytochemical screening.

Preliminary phytochemical screening
Qualitative analysis of the *T.conophorum* seeds was carried out following the described methods[14-19]. Phytochemical analysis was conducted to determine the presence of alkaloids, flavonoids, glycosides, saponins, triterpenoids, steroids, tannins, phenols, anthocyanins and anthraquinones.

Quantitative phytochemical analysis
Quantitative phytochemical analysis of the *T.conophorum* seeds was performed in order to ascertain the presence of bioactive compounds, using standard methods [20-24]. The phytochemicals determined included alkaloids, flavonoids, saponins, phenols and anthocyanins.

Statistical analysis
Data were reported as means±SD of triplicate determination.

RESULTS AND DISCUSSION

Proximate composition

Table 1: Proximate composition of *T.conophorum* seeds

Parameters(DW)	Percentage composition(%)
Moisture	29.43
Crude proteins	31.63
Carbohydrates	09.61
Crude fat	23.24
Total ash	06.09
Energy(Kj/100g)	1560.96

The results showed that the *T.conophorum* seeds contained high moisture, crude protein and crude fat content of 29.43; 31.63 and 23.24% respectively and low carbohydrate and ash content values of 9.61 and 6.09% .

The crude protein value of *T.conophorum* recorded in the present study was higher than all the other nutrients recorded and was found to be in line with the 33.21% found in *M. angolensis* [25] and the 29.89% found in *L.lanceolata* [26]. However this value was found to be higher than the 21.65±0.5% [6] reported for the Nigerian species, and also very much higher than the 8.13% found in *C.procera* seeds[27].

As observed for *Moringa oleifera* the protein content in this study was very high than the range of the 16 to 65 mg/l recommended RDA for children and lactating women[28]. It suggests that the seeds could be used as potential source of plant protein in diet supplementation.

The moisture content of the sample was the second most abundant nutrient (29.43%) when compared with the others. This value was lower than the 47.92% recorded in *C.procera* [27]. It is known that high moisture content of a given food suggests a lower nutrient density [29]. This finding is in contrast with the present results due to the high amount of crude proteins and crude fat.

According to Oboh and Erema [30], moisture content has an inverse effect on carbohydrate content. This is confirmed by the results of the present study due to the low carbohydrate value (9.61%). This carbohydrate content value was lower than the 19.96 and 18.10±0.01% reported for the seeds of *T. conophorum* [5],[6].

Carbohydrates provide readily accessible fuel for physical performance, and regulate nerve tissue [31].The low carbohydrate content of the seeds recorded in the present study indicated that they cannot be considered as a good source of energy and cannot help in energy supplementation.

The crude fat content recorded in the present study was lower than the 32.73% of the almond seed lipid content [32], but it was very much higher than that for *T.conophorum* (4.28±0.01%) [6]. The high fat yield of these seeds indicates that they are considered as good source of oil, suitable for domestic purposes and could serve in oil production.

The ash content of *T. conophorum* was favorably compared to the 5.27 ±1.35% [6] recorded for this species and the 5% found in almond seeds[32]. This value was found to be higher than the 4.84 % obtained for *A. sativum* [33]. The low ash content is a reflection of the low level of minerals and high organic content of the sample.This is in accordance with the results of mineral analysis.

Mineral composition of the T.conophorum seeds

Elemental analysis showed the high potassium and phosphorus contents (2.45 and 1.16% respectively) when compared with other minerals. Calcium and magnesium were found in lower concentrations while sodium and iron were detected as trace elements. Contrarily as the major element calcium (2.10±0.01%) and potassium(1.02±0.02%) was recorded in *T.conophorum* whereas phosphorus was detected in lower quantities (0.35±0.01%)[7]. As observed in the present study potassium and phosphorus were found to be the major elements of *V. substerranea* and *C. procera* seeds [34], [27]. Potassium and phosphorus were also found in high quantities in *Garcinia Kola* [35]. High values of 27.40 and 68.31 for phosphorous were recorded in *M. scandens* and *O. gratissimum* [36] respectively. As observed for *Artocarpus altilis* the sample studied could be a moderate source of phosphorus for both adults and children[37].

Table 2: Mineral content of *T.conophorum seeds*

Element	Content(%)
Calcium	0.65
Magnesium	0.61
Potassium	2.45
Sodium	0.02
Iron	0.01
Phosphorus	1.16

A similarity when compared to the Nigerian walnut seed about the mineral content was observed for potassium as the major element whereas all others elements like sodium, calcium and magnesium were detected in high quantities [5].

The potassium level in this study was found to be higher when compared with the recommended dietary allowance value (RDA) of animals, ranging from 0.2-0.6 % (DM) [28].This value would satisfy the requirement for this element by the woman's body.

As reported early for the wild vegetables [38],[39a,b],[34] sodium was recorded as a trace element in the present study. In lower concentration it was recorded in dry sample of *T.comoporum* [7] . The high content values of sodium were recorded in *M. scandens* (26.94) and *O.gratissimum* (39.38 mg/100g) and those of calcium varied from 36.29 in (*L. guineensis*) to 51.44 mg/100g in (*O. gratissimum*) [36].

The recommended Na/K ratio is 0.6 [40] and the values obtained in the present study (0.01) (Table 2) were less than 0.6, indicating that the seeds are considered as good for consumption. The high potassium and low sodium contents of these seeds could be an advantage due to the direct relationship of sodium intake with hypertension in humans[41].

According to SCSG [42] a good menu should have a Ca:P ratio over 1. The *T.conophprum* Ca/P = 0.56 (Table 2) is less than 1 and so could be considered as a poor source of Ca and P. Consumption of the seeds may not help body with dietary deficiency in these minerals.

The magnesium content of *M. scandens* and *L. guineensis* [36] varied from 28.68 (*L. guineensis*) to 33.60 mg/100g (*M. scandens*), was found to be higher than that recorded in the present study. However this value was found to be comparable to the 0.36±0.01% recorded for this species [7].

The present work showed that the content values for most of these elements were low and varied from 0.01 to 4.39% .Comparative analysis of the present results with earlier works reported on the samples from Congo [38],[39a,b][27] showed the same trend. This indicates that vegetables and fruits from Congo could be ranked as poor mineral species, especially in sodium and iron, which were in all cases found as trace elements independently of the geographic origin of the sample.
The plant seeds contained micro and macro elements either in lower quantities or in trace amounts. Nevertheless, at these levels they may be important for women's physiology and metabolic activities. Their deficiency may lead to the risk of various diseases.

Qualitative phytochemical analysis

Table 3: Qualitative analysis of *T.conophorum seeds*

Phytochemical	Extract	
	CH_3OH	CCL_4
Alcaloides	+	+
Flavonoids	+	-
Anthraquinones	-	+
Glycosides	+	+
Saponins	+	+
Steroids	+	+
Tannins	-	-
Triterpenoids	+	+
Phenols	+	+
Anthocyanines	+	-

+ = present; - = absent

Qualitative analysis was carried out to determine the bioactive constituents of the *T.conophorum* seeds in carbon tetrachloride and methanol extracts. The results of the phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, and triterpenoids in a methanol extract of the seeds, whereas in carbon tetrachloride extract we noticed the absence of flavonoids. This result was also obtained for *V.substerranea* extracts [34] in polar solvents (methanol and ethanol) and for the aqueous extracts of some desert fruits[19].

This similarity in the results of qualitative analysis was also observed regarding the presence of alkaloids, saponins, terpenoids and steroids (except in *B. Aethiopum*) in all extracts of desert fruits [19]. However, cardiac glycosides were not found in most of these fruits.Particularly, anthraquinones were detected only in methanol and were not detected in non polar solvent (carbon tetrachloride) in contrast to previous studies which indicated their absence in polar solvents [34],[27],[43]. Tannins were absent in both solvent extracts of *C. Tetracarpidium* seeds while phenols were present. Anthocyanins were detected only in polar sovent. The *Xylopia aethiopica* extract analysis revealed that stem bark and roots contained alkaloids, saponins, flavonoids, tannins, terpenes, steroids and cardiac glycosides [43]. The phytochemical analysis of *Senna-siamea* ethanolic extract showed that the leaves contained alkaloids and saponins [44]. The presence (in polar solvent) of alkaloids, terpenoids and saponins was reported in *A.indica, H.rosasensis* and *I. Cylindrical* [45]. Steroids were not found in these plants whereas glycosides were present only in *A.indica* and *I. Cylindrica*. Fagbohun [36] reported the presence of all the screened phytochemicals in the three plant leaves. Tannins were completely absent in both solvent extracts in the present study. Similarly they were not found in aqueous extracts of *B. Aethiopum* [19].

The variation of the results of qualitative analysis of the solvent extracts may be justified by the nature of each screened compound, the specificity of the solvent (solvent effect) and a number of factors, such as genotype (species and variety), environmental conditions (sun radiation, water availability), growth rate, life stage of plant, nutrient concentration, diseases, and predators of each individual plant [46],[47].
The study showed the presence of bioactive compounds that are medicinally important in health care for their biological properties.

Quantitative phytochemical determination

Table 4: Phytochemical concentrations of *T.conophorum seeds*

Phytochemical	Amount(%)
Alkaloids	12.80 ±0.19
Flavonoids	11.13±0.35
Glycosides	0.0±00
Saponins	3.67±0.06
Phenols	1.60±0.16
Anthocyanins	3.33 ±0.74

The quantitative analysis of the bioactive compounds of the *T.conophorum* seeds showed the high concentrations of alkaloids and flavonoids however saponins, anthocyanins and phenols were recorded in lower quantities. These results are in contrast with those reported previously[5]. According to the earlier results, a small concentration of alkaloids was recorded and no traces of saponins, flavonoids and glycosides were found in the seeds of the species from Nigeria. Lower alkaloid and flavonoid contents of 0.35±0.1 and 2.70±0.1% in the dried nuts of the studied species were reported [7] and also those recorded in *A.indica, H.rosasensis* and *I.cylindrica* (0.41-0.52 and 0.32%) [45]. However the high amounts of alkaloids and flavonoids were reported in *C.aconitifolius* (12%) and *S.marianum* (21%) respectively [48],[49].

The high alkaloids content of *T.conophorum* seeds recorded in the present studies may justify the reported uses of the plant to cure various diseases[3],[5].

There are different types of flavonoids and each appears to have protective effects. A high flavonoid level may help with anti-inflammatory,stimulating properties and provide protection against oxidative induced diseases.This can justify the multiples uses of the plants [4] in ethnomedicine.

Saponins and anthocyanins were the second most abundant compounds with 3.67 and 3.33% of contents for the studied seeds. But this saponin value was found to be lower than those recorded in *T.conophorum* (5.03±0.2%)[7] and also in *cola acuminata* (7.67 and 7.30%, [50]. However, this content was higher than the range of 1.30-1.99% for tree seed plants[45].

The anthocyanin amount recorded in this study was slightly higher than that of *C.procera* seeds (2.01%) [27] but much higher than the 2.92mg/100g in *Garcinia cola* [35] and the range of 0.1g/100g to 0.08g/100g recorded for the green leafy vegetables [23].

Contrary to the content(7.45±15%). recorded for the seeds of *C.procera* from Congo phenols were determined in a lower concentration in this study(1.6±0.16 %)[27]. This phenolic content value was in line with the 1.51±0.1% found in *T.conophorum* [7] ,but higher than the range of 0.04-06% recorded for tree plant seeds [45] and the 66.33mg/100g) reported by for *G.cola* [35].

Phytochemicals are biologically active compounds, found in plants in small amounts. They are not established nutrients but nevertheless contribute significantly to protection against degenerative disease[51] .

The results of the chemical and phytochemical investigation of the seeds of *T.conophorum* showed that the nuts are rich and nutritionally and medicinally important as they contained bioactive compounds and nutrients need for body health care.

CONCLUSION

The medicinal value of the plants is determined by some chemical substances that have a definite physiological action on the human body. Phytochemicals have been reported to possess a wide range of bioactivities, which may help in treatment and protection against chronic and metabolic diseases.

The study concluded that the plant seeds investigated were nutritious as they contained nutrients in adequate concentrations. The high levels of some bioactive compounds make the seeds important in nutrition and they could be recommended for dietary supplementation.

Further research should be undertaken with the objective to isolate and to characterize these secondary metabolites, and to evaluate their biological potential in order to establish the medicinal value of the plants.

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