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Proximate Analysis and Phytochemical Screening of *Triclisia subcordata* Oliv Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Proximate analysis and phytochemical screening of *Triclisia subcordata* Oliv leaf were investigated using standard analytical methods. Results of the proximate analysis showed contents for moisture (79.28±2.32%), ash (1.91±0.21%), crude fibre (1.83±0.13%), lipid (1.81±0.16%), crude protein (8.32±1.20%) and carbohydrate (6.91±0.74%). Phytochemical screening showed the presence of all six metabolites studied but at different concentrations. Saponin and flavonoids > alkaloids > phenol, tannin and cyanogenic glycoside. Tannins, saponins, alkaloids, flavonoids, cyanogenic glycosides and phenol gave 0.01±0.00%, 5.81±0.23%, 2.32±0.13%, 6.01±0.36%, 0.20±0.00% and 0.03±0.00%. Given observed high contents of moisture, flavonoids and saponins, moderate amounts of alkaloids and low contents of lipid, cyanogenic glycosides, tannins and phenols in *Triclisia subcordata* Oliv leaf, this study rationalises the medicinal use of the plant, and unveils its potential as a source of micronutrients.

Keywords: Triclisia subcordata oliv; proximate analysis; phytochemical screening; analytical methods; traditional medicine.

1. INTRODUCTION

Phytochemicals are secondary metabolites produced by plants. These products are biologically active, naturally occurring chemicals in various parts of a plant, providing health benefits for humans further than those attributed to macronutrients and micronutrients. These

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compounds have been linked to human health by contributing to protection against degenerative diseases [1].

Triclisia subcordata Oliv (Menispermaceae) is a medicinal plant traditionally used for the treatment of various diseases in West Africa [2]. *T. subcordata* Oliv is commonly called “Alugboran” in Yoruba language, “ogwu-aju” in Nsukka (which means antidizziness), “Ezize” in Ohafia (which means benign skin growth), “ike mbekwu” in Umuoji in Eastern part of Nigeria (which is gotten from the shape of the leaf) [3]. The Menispermaceae is a temperate to tropical family of around 70 genera (including Triclisia) and 450 species of dicotyledenous tropical flowering vines that are short in pedunculate clusters with twining stems, an appressed puberscent fruits and a few herbs, shrubs and trees. Leaves are reticulate, alternate and simple, but may be palmately veined and often lobed [2]. The stem is used as a rough fibre (tietie) [4].

It is a nature plant of west tropical Africa including Nigeria, Ghana, Ivory Coast, Sierra Leone, Senegal and Togo [5]. The plant frequently serves as rope used for tying purposes; the importance of the species however rest with their medicinal application including the use of root extract for the treatment of snake bite, ulcer, diarrhea, malaria pyorrhea, swelling of extremities, anemia joint pains, cancer, rheumatic pains and hypertension in Nigeria [3,4].

Evidenced-based studies revealed that *Triclisia subcordata* has antiulcer, antihistamine, antimicrobial, anticancer, antioxidant and antidiabetic activities [6,7,8,9]. The cytotoxicity Effects and Apoptosis Induction in ovarian and cancer cells by Bisbenzylisoquinoline and Isochondodendrine and 2′-norcocsuline alkaloids from *Triclisia subcordata* was assayed by Uche et al. [10,11].

Plate 1. Photograph showing *Triclisia subcordata* Oliv leaf

Medicinally, several hypotheses suggest that a decoction of the root is taken to treat fever and malaria. Also, root decoction is also taken as an emmenagogue and abortifacient (drugs or chemicals that induce abortion). The root pulp is rubbed in scarifications as a treatment for rheumatism, arthritis, anaemia and sleeping sickness. The leaf or a root juice is mixed with salt in palm wine and taken as treatment for coughs and bronchial disorders and as well function as sedative on the heart and to help wash palpitations. The leaf sap has a soothing effect on coughs. A decoction of the leaves and twigs is drunk, or leaf pulp is rubbed in, to treat oedema of the legs. Furthermore, it was hypothesized that a decoction of the leaves and stem are used as a nasal or ocular instillation and as a purgative or bathe against epilepsy and as treatment for stomach ache respectively. The stem bark is powdered and applied topically to syphilitic sores and leprosy while the bark pulp is used as a purgative. A methanolic leaf extract has shown significant anti-ulcer effects [12]. Due to it's acclaimed pharmacological importance by African natives, this paper is aimed at analyzing the proximate content and phytochemical screening of *Triclisia subcordata* Oliv leaf.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh *Triclisia subcordata* Oliv leaf with identification number UPHMO472 was obtained from University of Port Harcourt campus environment, Choba, Rivers State, Nigeria. The plant was identified by a taxonomist (Dr. Suleiman M.) of the department of pharmacognosy and phytotherapy, facaulty of pharmaceutical sciences, University of Port Harcourt. The leaves were pulverized using a mechanical grinder to obtain a smooth mixture.

2.2 Experimental Design

After grinding of the leaf, approximately 50 g was weighed using electronic weighing balance and used for proximate and phytochemical screening using standard analytical methods.

2.3 Proximate Analysis

2.3.1 Determination of moisture content [13]

A dry clean petridish was placed in an oven at 80°C for about 30 minutes, cooled in a desiccator and weighed as (w). 5 g of the samples was added to the petridish and weighed as (b). The petridish and its content were placed in an oven adjusted to 70°C. After 5 hours, the petridish containing the sample was removed and quickly transferred to a desiccator for cooling. The petridish was put back into the oven and adjusted to 105°C for another 5 hours after which it was removed, put in desiccators for cooling. This process was repeated and weighed until a constant weight (c) was obtained.

The % moisture content was determined as follows;

$$\% \text{ moisture content} = \frac{b-c}{b-w} \times 100$$

Where W = weight of moisture can, b = weight of petridish + sample, c= weight of petridish + sample after drying.

2.3.2 Determination of ash content [13]

An empty crucible was first ignited in a muffle furnace for 1 minute and allowed to cool in desiccators containing silica gel. 5 g of the sample was accurately weighed into the preheated dish. The weight of the porcelain dish and the samples were noted. Afterwards, the dish was heated with a Bunsen burner in a fume cupboard until smoking ceases and later transferred into a muffle furnace at 550-570°C for about 18-24 hours to burn off all organic matter. After ashing, the crucible was removed from the furnace and placed in desiccator to cool at room temperature and weighed. The percentage ash content of the sample was calculated thus;

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$
$$= \frac{W3-W1}{W2-W1} \times 100$$

Where; W_1 = weight of empty crucible,
W_2 = weight of crucible + sample before ashing
W_3 = weight of crucible + sample after ashing

2.3.3 Determination of lipid [13]

A 5 g of the sample was weighed into a thimble and was extracted with petroleum ether until it siphons using the Soxhlet extraction method. The lipid was exhaustively extracted using petroleum ether at 40 – 60°C for 6 hours. The sample in the thimble was removed and dried in air at 50°C for 5 minutes, cooled in a desiccator and weighed. The % lipid content was calculated as follows;

$$\% \text{ Lipid} = \frac{\text{weight of sample (extracted fat)}}{\text{weight of sample}} \times 100$$

$$= \frac{W2-W1}{W} \times 100$$

Where; W_1 = weight of empty thimble, W_2 = weight of thimble + sample W = weight of sample

2.3.4 Determination of crude fiber [13]

A 2 g of the defatted sample was weighed into conical flask and 200 mLs of 1.25% of boiling sulphuric acid was added within a minute. The content of the flask was filtered through a buchner funnel prepared with wet 12.5 cm filter paper. The sample was washed back into the original flask with 200 mLs of 1.25% NaOH, and boiled for 30 minutes. All insoluble matter was transferred to the crucible and treated till the sample was free from acid. The sample was again washed in a muffle furnace at 550°C/hour. The crucible was then cooled in desiccator and reweighed.

$$\% \text{ Crude fiber} = \frac{W2-W1}{W} \times 100$$

Where; W = weight of sample, W_1 = weight of crucible + sample
W_2 = weight of crucible+ filter paper after ashing.

2.3.5 Determination of Crude protein [13]

A 1 g of the sample was weighed and transferred into Kjeldahl flask. Few chips of antibumping granules, 4 g of digestion catalyst and 20 mLs of concentrated sulphuric acid were added at a 40°C angle with a retort stand on an electro thermal heater. The flask was gently heated for frothing to occur and subside, and then heat was increased to about 250°C. The digestion was carried out within 2-6 hours by which time the entire sample was digested completely. The digest was cooled to room temperature and diluted to 100 mLs with distilled water. For distillation, 20 mLs aliquot of the digest was transferred into a round bottomed flask. This flask was connected to a Liebig condenser through a monoarm steel head (Adaptor). The liebig condenser was connected to a receiver flask through a receiver adapter. 10 mLs of 2% boric acid and two drops of double indicator were pipetted into the distillation flask. 30 mLs of 40% sodium hydroxide was injected into the distillation flask through a cork with the aid of a syringe. The flask was heated for 10 minutes to digest the content. The distillate was collected in the boric acid and then titrated with 0.1M HCL. The volume of HCl added was recorded as the titre value.

The % Crude protein was calculated thus;

$$\% \text{ Crude protein} = \% \text{ Nitrogen x } 6.25$$

$$\% \text{ Nitrogen} = \frac{\text{titre value} \times 1.4 \times 100 \times 10}{1000 \times \text{wt of sample} \times \text{aliquot digest}}$$

Where, 1.4 = N_2 equivalent to 0.1NHCl used in titration
100 = Total volume of digest

2.3.6 Determination of total carbohydrate [13]

The total carbohydrate content of the sample was estimated as the Nitrogen free extract (NFE). The arithmetic different methods involve adding the total percentage value of crude volume.

$$\text{Total CHO} = 100 - (\% \text{ fibre} + \% \text{ protein} + \% \text{ Moisture} + \% \text{ ash} + \% \text{ fats})$$

2.4 Phytochemical Screening

2.4.1 Preparation of the leaf extracts

Ten grams (10 g) of the leaf sample was soaked in 100 mL of water in a beaker and left for about 8 hours. The solution obtained was filtered using filter paper, and the filtrate used for phytochemical screening.

2.4.2 Qualitative determination of phytochemicals

Screening for presence of secondary metabolites (qualitative) were performed following standard methods by AOAC [13].

2.5 Quantitative Determination of Phytochemicals

2.5.1 Determination of Saponins [13]

A 10 g of the ground sample was measured into a conical flash and 100 mL of 20% ethanol added to it. The suspension was heated over hot bath at 55°C for 12 hours with continuous stirring using a magnetic stirrer. The mixture was filtered and the residue re-extracted with another 200 mL of 2% aqueous ethanol. The combined extract was reduced to 40 mL of the original size over a water bath at about 55°C. The purification process was repeated two more times. A 4 g of Sodium chloride (NaCl) was added to adjust the pH meter. The solution was shaken with 60 mL and 30 mL portions of n-butanol extract and later washed twice with 10 mL of aqueous NaCl. The

remaining solution was evaporated to dryness in water bath. After evaporation, the sample was dried in the oven to a constant weight.

The saponin content was calculated in g/100g as;

$$\% \text{ saponins} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

$$\% \text{ saponins} = \frac{W_2 - W_1}{W} \times 100$$

Where; W = weight of sample, W_1 = weight of evaporating dish

W_2 = weight of evaporating dish + saponin content after drying

2.5.2 Determination of Alkaloids [13]

A 2 g of freshly crushed sample was weighed and dispensed into 100 mL of 10% acetic acid. The mixture was shaken and allowed to stand for 4 hours before filtration. The mixture was filtered to remove all debris and evaporated to ¼ of the original volume. A 1% concentrated ammonium hydroxide (NH_4OH concentration) was added drop wise to precipitate the alkaloids. It was filtered with well weighed precipitate in the filter paper, oven dried at 60°C for 30 minutes and then reweighed.

The alkaloid content was calculated as;

$$\% \text{ Alkaloid} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

Where; W = weight of sample, W_1 = weight of empty filter paper

W_2 = weight of filter paper of precipitate

2.5.3 Determination of Flavonoids [14]

The method used for determination of flavonoids was that of Bohn and Kocipai (1994). 5 g of the blended sample was extracted repeatedly and separated with 50 mL of 40 % aqueous methanol at room temperature. The solution was shaken for homogeneity and left to stand for about 4 hours and later filtered into a weighed beaker. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath, then dried in an electric oven to a constant weight.

The flavonoid content was expressed in percentage as follows;

$$\% \text{ flavonoid} = \frac{\text{weight of residue}}{\text{weigh tof sample}} \times 100$$

2.5.4 Determination of cyanogenic glycoside [13]

One gram (1 g) of sample was weighed into a 250 mL round bottomed flask. 200 mL distilled water was added and allowed to stand for 2 hours (for autolysis to occur). Full distillation was then carried out and 150-170 mLs of distillate was collected in a 250 mL conical flask containing 2 mLs of 2.5% NaOH. Anti-foaming agent (tannic acid) was added before distillation. To 100 mL of the distillate containing cyanogenic glycoside, 8 mL of 6N NH4OH and 2 mLs of 5% potassium iodide (KI) was added, mixed and titrated with 0.02 M silver nitrate ($AgNO_3$) using a micro burette against a black background. Permanent turbidity indicated end point.

Cyanogenic glycoside in the sample was calculated thus;

$$\text{cyanogenic glycosides (mg/kg)} = \frac{\text{Titre value(mL)} \times 1.0 \text{ g} \times \text{extract volume (mL)} \times 100}{\text{Aliquot volume (mL)} \times \text{weight of sample (g)}}$$

2.5.5 Determination of tannin [13]

A 0.5 g of the sample was weighed into 100 mL plastic bottle. 50 mLs of distilled water was added and shaken for 1 hour in a mechanical shaker. Then 5 mLs of the filtrate was pipette out into a tube and mixed with 3 mLs of 0.1 M Iron (III) chloride ($FeCl_3$) in 0.1N hydrochloric acid (HCl) and 0.008 M potassium ferrocyanide $K_4[Fe(CN)_6]$. The absorbance was measured in spectrophotometer at 720 nm wavelength within 10 minutes. A blank sample was prepared, and the colour also developed and read at same wavelength. A standard was prepared using tannic acid to get 100 pp m and measured using the formula below.

$$\text{Tannin} = \frac{An}{As} \text{ x C x } \frac{100}{W} \text{ x } \frac{vf}{vg}$$

Where; An = Absorbance of test sample, As = Absorbance of standard solution, C = Concentration of standard solution, W = Weight of sample used, Vf = Total volume of extract, Vg = volume of extract analyzed.

2.5.6 Determination of phenol [13]

A 0.1 g of sample was weighed into a 250 mL round bottomed flask. 100 ml distilled water was added, boiled for 30 minutes and the volume made up to 100 mL. A 2.5 mL aliquot is measured into a conical flask and 5 mL of 0.1M NaOH added into it. The solution was heated to 50°C and allowed to cool, then 2.5 mL of 0.005 Iodine added. The flask was cork with a foil and allowed to stand at room temperature for 2 hourrs. 0.5 ml of concentrated HCl was added and titrated to pale yellow with 0.1 M $Na_2S_2O_3$ with the addition of 3 drops of starch indicator. The solution is titrated again with 0.1 M $Na_2S_2O_3$. A blank is formed using 2.5 mL distilled water and absorbances read off.

2.6 Method of Data Analysis

All data collected were subjected to Students t-test for statistical analysis. All data were represented in mean ± standard deviation (M ± S.D). Level of significance was determined at a confidence level of determination ($p \leq 0.05$).

3. RESULTS

The result of the proximate analysis, phytochemical screening (qualitative and quantitative analysis) of *Triclisia subcordata* oliv leaf are presented in Tables 1, 2 and 3. The proximate analysis revealed that the leaf comprises more of moisture and less of lipids. Saponins and flavonoids had the highest percentage of phytochemicals while tannins had the lowest.

4. DISCUSSION

The proximate content of *Triclisia subcordata* oliv leaf showed appreciable amount of the parameters as expressed in Table 1. From this study, the moisture content was very high (79.28%) when compared with findings of [15] on fluted pumpkin pod which imply that this leaf may not be stored for long and can be prone to microbial attack.

Ash contents express the mineral index contents in biological mass. This study revealed relatively lower ash in *Triclisia subcordata* oliv leaf (1.91%) than in fluted pumpkin leaf (9.68%), bitter leaf, (15.86%) and *Moringa oleifera* (15.09%) leaves [16]. This implies that *Triclisia subcordata* oliv leaves are not good source of mineral elements (Table 1).

Lipid in food absorbs and retains the flavour thereby enhancing the palatability [17]. The lipid content recorded in this study (1.81%) is far lower than values obtained by [15] which implies consumption of *T. subcordata* will not enhance pallatability.

Proteins are necessary for the build-up of tissues and substances like hormones and enzymes. The values of protein obtained in this study (8.32%) are low when compared with [15] 24% in *Amaranthus vividis* [18], 20.72% in *Moringa oleifera* [19], 21.0% in *Lasianthera africana* and 15.0% in *Heinsia crinata* [16,20] which makes it insufficient source of protein.

The crude fibre contents of *Triclisia subcordata* oliv leaf (1.83%) is very low when compared with those of *Lasianthera africana* (15.3 – 18.1% dry mass) [21] and *Heinsia crinata* (13 – 15% dry mass) [20]. Although intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, diabetes, obesity, constipation and cardiovascular diseases, colon and breast cancer [22,23]. The major problem associated with nutrition of vegetables by human is the high fibre content which can cause intestinal irritation and lower nutrient bioavailability [16,24]. Intake of fibre can stimulate peristaltic movement, weakening of hunger, increase stool bulk and reduce serum levels of cholesterol [25,26].

Table 1. Proximate analysis of *Triclisia subcordata* Oliv leaf

Proximate content	*Triclisia subcordata* (%)
Moisture	79.28 ±2.32
Ash	1.91 ±0.21
Lipid	1.81 ±0.16
Crude protein	8.32 ±1.20
Crude fibre	1.83 ±0.13
Carbohydrate	6.91 ±0.74

Values are Mean ± SD of triplicate determinations. n=3

Table 2. Qualitative phytochemical screening of *Triclisia subcordata* Oliv leaf

Phytochemical content	*Triclisia subcordata* Oliv leaf
Tannins (%)	+
Saponins (%)	+++
Alkaloids (%)	++
Flavonoids (%)	+++
HCN (mg/kg)	+
Phenol (%)	+

Key: + = slightly present; ++ = moderately present; +++ = highly present

Table 3. Quantitative phytochemical screening of *Triclisia subcordata* Oliv leaf

Phytochemical content	*Triclisia subcordata* Oliv leaf
Tannins (%)	0.01±0.00
Saponins (%)	5.81±0.23
Alkaloids (%)	2.32±0.13
Flavonoids (%)	6.01±0.36
HCN (mg/kg)	0.20±0.00
Phenol (%)	0.03±0.00

Values are Mean ± SD of triplicate determinations. n=3

The carbohydrate content recorded was 6.91%. Carbohydrates are pivotal nutrients needed for a balanced diet [27]. Generally, the low contents of protein, lipid and carbohydrate in this leaf imply that it is not a good source of energy.

Table 2 revealed that saponin, flavonoids were highly present, alkaloids were moderately present while cyanogenic glycosides, tannins and phenols were slightly present in *Triclisia subcordata* oliv leaf.

Table 3 revealed the phytochemical screening of *Triclisia subcordata* oliv leaf quantitatively. The study showed substantial amounts of flavonoids, saponins and alkaloids and small amounts of phenol, tannins and cyanogenic glycosides. When compared with the study of [28], tannins, saponins, alkaloids and phenols were lower while flavonoid content was high.

Tannins are complex moiety with wide pharmacological activities and are produced by majority of plants as protective substance. Tannin has astringent property, hastens the healing of wounds and inflamed mucous membrane and has been used since past as tanning agents. Tannin has received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties [29,30].

Saponins protect against hyperglycaemia, hypercholesterolaemia, hypertension [31], have antibiotic properties and antiinflammatory property and aid healing [32].

Alkaloids have been reported to be powerful pain relievers, exert an anti-pyretic, antihypertensive, antifungal, anti-inflammatory, anti-fibrogenic effect [33], stimulating, anaesthetic action [34] and inhibiting activity against most bacteria [35].

The flavonoids show antioxidant activity and have strong anti-cancer activity [36]. Flavonoids exhibit their antioxidative properties through several mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, inhibition of hydrolytic and oxidative enzymes inhibit antibiotics resistant microbes and also act as anti-inflammatory agent [37].

5. CONCLUSION

It can be concluded that *Triclisia subcordata* oliv leaf has high moisture (which show their low shelf-life), alkaloids, flavonoids and saponins (pharmacological relevance) with low lipid, cyanogenic glycoside, tannins and phenols. The low cyanogenic glycoside, tannin and phenol implies that the plant is non-poisonous and may inhibit inflammatory enzymes. Generally, the proximate analysis does not support their nutritional relevance as macronutrient supplement (as they are seen in smaller quantity when compared to other plants (such as *Telfairia occidentalis*) but provide the micronutrients required for proper body functioning. The low concentration of lipids obtained makes it suitable and healthy for people on low fat diet. However,

the phytochemical screening backs their usage in traditional medicine research and would be helpful in copping with different diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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