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Qualitative and quantitative phytochemical composition of *Paullinia pinnata* L methanolic leaf extract

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Abstract
Despite numerous bioactivities attributed to *Paullinia pinnata* L (PP) leaves such as anticancer, antioxidant, antibacterial, insecticidal, anti-inflammatory, analgesic, anthelmintic, antimalarial, anti-convulsant properties, there is little information regarding the phytochemical composition of the plant. Phytochemical analysis is a crucial process for quantifying and identifying the array of bioactive compounds in plants, such as flavonoids, alkaloids, glycosides, tannins and terpenes, which are essential for understanding their health benefits and potential applications. This analysis not only aids in development of nutraceuticals and pharmaceuticals but also enhances our knowledge of plant-based diets and their impact in human health. In this study, the qualitative and quantitative analysis of the crude methanolic extract of *Paullina pinnata* L (CMEPP) leaves was done using standard methods. The result of the qualitative phytochemical screening of the CMEPP indicates abundant presence of soluble carbohydrates, flavonoids and total phenolics; moderate presence of tannins, glycosides and reducing sugar; and mild presence of steroids, saponins and terpenoids. The result of the quantitative analysis of CMEPP reveals different amount of phytochemicals: alkaloid (207.98±11.24 mg/100g), total phenolics (8350.02±38.50 mg/100g), tannins (111.06±13.11 mg/100g), glycosides (245.63±15.09 mg/100g), saponins (19.42±3.25 mg/100g), reducing sugar (298.91±9.05 mg/100g), carbohydrates (4847.09±56.44 mg/100g), terpenoids (56.05±4.85 mg/100g), steroids (45.52±10.04 mg/100g) and flavonoids (5874.00±44.82 mg/100g). The presence of varying arrays of phytochemicals in CMEPP substantiates the numerous bioactivities of the plant.

Keywords: *Paullinia pinnata*, phytochemicals, qualitative, quantitative, analysis

Introduction
Numerous bioactivities are attributed to *Paullinia pinnata* L leaves [1, 2, 3, 4, 5]. Such bioactivities include anticancer, antibacterial, antioxidant, antibacterial, insecticidal, anti-inflammatory, analgesic, anthelmintic, antimalarial, and anti-convulsant properties [6, 7, 8]. Nevertheless, information regarding the phytochemical composition of *Paullinia pinnata* is little. The genus *Paullinia* contains five species which include *Paullinia cupana* (guarana), *Paullinia fuscescens* kunth (moldy bread and cheese), *Paullinia pinnata* L (bread and cheese), *Paullinia plumieri* Triana and planch and *Paullinia yoco* (yoco) [9]. In Nigeria, *Paullinia pinnata* is known with different names. It is popularly called *Kakansela* (Yoruba), *Yaatsa biyar* or *Goron dorina* (Hausa), *Okpanam* (Igbo), Five Finger (English), and En*u-ka kanshela* (Nupe) [10]. It is also called *Zaga Zafi*, *Furen Amarya, Hannu Biyar* or *Kana Kana,* in Hausa [11]. In many parts of Igbo in Nigeria, *Paullinia pinnata* is called edefina and aliligo It is also call it ogbe-okiye in Yoruba [12].

The unique feature of *Paullinia pinnata* makes it easily identifiable [13]. *Paullinia pinnata* is a vine mostly found as a creeping plant in semi-deciduous, moist, undergrowth forest in savannas, along creeks and in gallery forest [14]. It is an African tropical plant but also grow in abundance in South and Central America. They are naturally found and used in such countries like Nigeria, Ghana, Cameroon, Zimbabwe, Togo. Other countries where they are found include Jamaica, Brazil, Madagascar, Zambia, Senegal and Mali [14]. *Paullinia pinnata* L. (Sapindaceae) commonly known as Sweet Gum, is a woody creeper or climber with rigid stems. The leaves have pinnate form with five leaflets, the terminal leaflet being the biggest (Figure I).

Fig 1: *Paullinia pinnata* (Bread and Cheese) [9]

In traditional setting, the roots and leaves can be ground and mixed for the treatment of dysentery and to arrest a threatened abortion [13]. The leaves, roots and seeds together are mixed, ground and applied to fractures and abscesses (swollen area within body tissue, containing an accumulation of pus) [13]
Traditionally, all parts of the plant (leaves, roots, stems, and flowers) are used as a medicine for the treatment of syphilis, wounds, gonorrhoea, waist pains, eye disorders, typhoid fever, erectile dysfunction, malaria, dysentery, abdominal pains and diarrhea [1, 2, 3]
The ethanolic leaf extract of *P. pinnata* was analysed for its analgesic and anti-inflammatory and activities [14]. The result of the study showed that *Paullinia pinnata* extract exhibited analgesic and anti-inflammatory effect, suggesting that it could be used in managing pain [14]. Aqueous extract of dried leaves of *Paullinia pinnata* also showed strong analgesic and anti-inflammatory properties [15].
[16] investigated the effect of polyphenolic extract of *Paullinia* leaf against tetrachloromethane-induced oxidative stress and liver damage in female albino rats. The polyphenolic extract ameliorated tetrachoromethane-induced liver damage through the fortification of antioxidant defence mechanism [16].
Phytochemical analysis is a crucial process for quantifying and identifying the array of bioactive compounds in plants, such as flavonoids, alkaloids, glycosides, tannins and terpenes, which are essential for understanding their health benefits and potential applications. This analysis not only aids in development of nutraceuticals and pharmaceuticals but also enhances our knowledge of plant-based diets and their impact in human health. It is on this basis that this study was undertaken. In this study, the qualitative and quantitative analysis of the crude methanolic extract of *Paullina pinnata* L (CMEPP) leaves was done using standard methods.

Materials and Methods

Plant Material

Paullinia pinnata L was obtained from a farm in Okpaligbo-Ogu, Nsukka Local Government Area, Enugu State, Nigeria. The plant was identified and authenticated by Mr Felix I Nwafor, a plant taxonomist, and the Voucher Specimens (Voucher No PCG/UNN/0401) deposited at the Herbarium of the Department of Pharm. and Env. Med., Pharmaceutical Sciences Faculty, University of Nigeria, Nsukka

Preparation of Plant Material

Paullinia pinnata L leaves were harvested, washed and air-dried for two weeks and milled into fine powder using BS-350 Hammer Mill Leaf Grinding Machine (China).

Plant Extraction

Crude methanol leaf extract of *Paullinia pinnata* L was obtained by soaking dry powder of the plant leaves in methanol at the ratio of 1:5 (w/v) for 24 hrs with gentle stirring, after which the mixture was filtered. Filteration was done twice; first with a coarse sieve, then with Whatman filter paper (No 1). The filterate was collected and concentrated using water bath, producing lyophilized powder. The percentage yield was calculated and the sample stored at 4°C.

Qualitative Phytochemical Studies of Crude Methanol Extract of *Paullinia pinnata L*

Phytochemical analyses were carried out on the extract for their phytochemical constituents using standard methods [17, 18, 19].

Identification of Alkaloids

2 g of the sample was boiled with 5% sulphuric acid in 50% ethanol (20 ml). The mixture was cooled, filtered and a portion reserved. Another portion of the filtrate was put in 50 ml of sepa7rating funnel and the solution was made alkaline by addition of two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow for layer separation. The chloroform layer (lower layer) was run off into a second separating funnel while the ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml dilute sulphuric acid. The various extracts were then used for the following tests:

1. **Mayer's Test:** In one portion of the filtrate, 1 ml of Mayer's reagent (1 ml) was added drop by drop. Formation of a greenish coloured- or cream precipitate indicates the presence of alkaloids [19].
2. **Dragendoff's test:** To a portion of the filtrate, 1 ml of Dragendoff's reagent was added drop by drop. A reddish-brown precipitate formation indicates alkaloids presence [19]

Identification of Flavonoids

The sample (2 g) was completely detanned with acetone. The acetone was evaporated and then the residue was extracted with hot water. The mixture was then filtered while hot, allowed to cool and used for the following tests:

1. **Ferric Chloride Test:** To a portion of the filtrate, $Fecl_3$ solution (3 drops) was added. Production of greenish-black colour indicates the presence of flavonoids [18].
2. **Sodium Hydroxide Test:** To a portion of the filtrate, 10% NaOH solution (2 ml) was added. Formation of yellow colour indicates the presence of flavonoid. This solution turns colourless on addition of dilute hydrochloric acid [19]

Identification of Saponins

Frothing test: A quantity, 5 g sample was placed in a test tube and distilled water (10 ml) was added and shaken vigorously for 30 secs. This was allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins [18].

Identification of Tannins

The extract (2 g) was extracted with 10 ml of 50% alcohol, then filtered and the filtrate was used to carry out the following tests:

Ferric Chloride Test: To a part of the filtrate, dilute solution of $FeCl_3$(aq) (3 drops) was added and vigorously shaken. Greenish-black or blue solution which changes to olive green upon addition of more drops of ferric chloride indicates that tannins are present [19].

Identification of Carbohydrates

Molisch's test: The extract (100 mg) was dissolved in distilled water (3 ml) and mixed with Molisch's reagent (10% solution of α-naphthol in alcohol (3 drops). A Conc. H_2SO_4 (1 ml) was further added down the side of the inclined test tube such that the acid forms a layer beneath the aqueous solution without mixing it. Violet or reddish ring colour formed at the junction of the liquids shows the presence of carbohydrates [18].

Identification of Total Phenolics

The extract (500 mg) was dissolved in distilled water (5 ml). To this, neutral 5% ferric chloride solution (3 drops) were added. A dark green colour indicates the presence of phenolic compounds [20].

Identification of Steroids

Sample extract (0.2 g) was mixed with acetic acid (2 ml) and the solution was allowed to cool with the aid of ice. Then conc. H_2SO_4 was added until colour change is observed. Colour development from violet to blue or bluish green indicates the presence of a steroidal ring [19].

Identification of Terpenoids

A little quantity of the extract was dissolved in ethanol then followed by addition of acetic anhydride (1 ml). This was also followed by the carefuls addition of concentrated H_2SO_4 until colour change is observed. Formation of pink colour that changes to violet indicates that terpenoids is present [20].

Identification of Glycoside

Concentrated H_2SO_4 (1 ml) is first prepared in a test tube, and the sample solution (5 ml) is mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_3$. The above mixture is carefully added to initially prepared 1 ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. Formation of of brown ring shows that cardiac glycoside is present [18].

Identification of Reducing Sugar

The extract (100 mg) was added to dilute sulphuric acid (2.5 ml) and boiled in a water bath for 15 minutes. It was cooled and added with 20% potassium hydroxide solution for neutralization. Fehling's solution (5 ml) was added and boiled for 5 minutes. Brick red precipitate formation shows that reducing sugar is present [18].

Quantitative Phytochemical Analysis of Crude Methanol Extract of *Paullinia pinnata* L

Quantitative analysis of the organic extract was carried out according to the general method of [17] as described by [21] except otherwise stated.

Determination of Total Phenolics Contents

Modified Folin-Ciocalteu method (Kaur and Kapoor, 2002) was used to total phenol content. A quantity, 50 µl aliquots of 12.5, 25, 50, 100, 200, and 400 µg/ml methanolic gallic acid solutions were mixed with 100 µl Folin-Ciocalteu reagent (diluted ten-fold) and 100 µl (75g/l) sodium carbonate. The mixture was incubated at 25ºC for 30 minutes. Estimation of Phenol content was carried out quantitatively at 765 nm. By plotting the absorbance against concentration, the calibration curve was formed. Similar procedure was adopted for the test sample as described above. The concentration of total phenolics was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve.

Determination of Flavonoid Contents

Aluminium chloride spectrophotometric method [22] was used to determine flavonoid content with slight modification. The sample was first diluted with methanol to 100 mg/ml (w/v) solution. The calibration curve was prepared by diluting quercetin in methanol (concentration ranging from 0 to 100 mg/ml). 2 ml of Quercetin, 0.1 ml of 0.1 mM potassium acetate solution and 0.1 ml of 10% (w/v) aluminium chloride solution were mixed and incubated for 30 minutes. Then the maximum absorbance of the mixture was measured at a wavelength of 415 nm using a UV-VIC spectrophotometer. Total flavonoid content was recorded as milligram quercetin equivalent per 100 grams of the sample.

Determination of Total Tannins Contents

Tannin content of the extract was estimated by adding the extract (1 ml) to Folin-Ciocalteau's reagent (0.5 ml), followed by the addition of saturated solution of Na_2CO_3 (1 ml) and distilled water (8 ml). The mixture was kept for 30 min at room temperature. Absorbance of the supernatant was read at 725 nm using UV-Visible spectrophotometer after centrifugation. Increasing concentration of standard tannic acid was prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent per 100 grams of the sample.

Determination of Saponin Contents

The extract (1 g) was marcarated with petroleum ether (10 ml) and decanted into a beaker. Another petroleum ether (10 ml) was added into the beaker and the filterate evaporated into dryness. The residue was dissolved in ethanol (6 ml). The solution (2 ml) was transferred into a test tube and chromagen solution (2 ml) added into it. The solution was incubated for 30 minutes before the absorbance reading at 550 nm. Increasing concentration of standard oleanolic acid was prepared in a similar way and the absorbance of various oleanolic acid concentrations was plotted for a standard graph. The saponin content was recorded as mg oleanolic acid equivalent per 100 grams of the sample.

Determination of Alkaloids Contents

1 mg of extract was mixed with 20 ml of ethanol and 20% of H_2SO_4 (1:1 v/v) to marcarate. The filterate (1 ml) was added to 5 ml of 60% H_2SO_4. Then 5 mins later, 5 ml of 0.5% formaldehyde in 60% of tetraoxosulphate (vi) acid was added to the mixture and allowed to stand for 3 hours. The absorbance was read at 565 nm. Increasing concentration of standard caffeine was prepared in a similar way and the absorbance of various caffeine concentrations was plotted for a standard graph. The alkaloid content was expressed as mg caffeine equivalent per 100 grams of the sample.

Determination of Terpenoid Contents

The extract (1 g) was marcarated with 50 ml of ethanol and filtered. To a portion of the filterate (2.5 ml), 2.5 ml of 5% aqueous phosphomolybdic acid solution and 2.5 ml of conc.

Tetraoxosulphate (vi) acid were added together, allowed to stand for 30 mins before it was made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm. In a similar way, increasing concentration of linalool standard was prepared. The terpenoid content was recorded as mg linalool equivalent/100 grams of the sample.

Determination of Steroid Contents

The extract (1 g) was mixed with 20 ml of ethanol, filtered for use. To the filterate (2 ml), 2 ml of chromagen solution was added and solution left to stand for 30 min. the absorbance was read at 550 nm. Steroid content was measured using testosterone standard curve. Increasing concentration of standard testosterone was prepared in a similar way and the absorbance of various testosterone concentrations was plotted for a standard graph. The steroid content was recorded as mg testosterone equivalent/100 grams of the sample.

Determination of Glycoside Contents

The extract (1 mg) was marcarated with 50 ml of distilled water and filtered. To the filterate (1 ml), 4 ml of alkaline pirate solution was added. After gentle stirring, the mixture was heated for five minutes and cooled. The absorbance was read at 490 nm. The quantity of glycoside was determined using standard curve (digitoxin). Increasing concentration of standard digitoxin was prepared in a similar way and the absorbance of various digitoxin concentrations was plotted for a standard graph. The glycoside content was expressed as mg digitoxin equivalent per 100 grams of the sample.

Determination of Reducing Sugar Contents

The extract (1 mg) was marcarated with 20 ml of distilled water and filtered. To 1 ml of the filterate, 1 ml of alkaline copper reagent was added, boiled for five minutes and cooled. This was followed by addition of 1 ml of phosphomolybdic acid reagent and 2 ml of distilled water before the absorbance was read at 420 nm. The quantity of reducing sugar was determined using standard curve (glucose). Increasing concentration of standard glucose solution was prepared in a similar way and the absorbance of various glucose concentrations was plotted for a standard graph. The reducing sugar content was expressed as mg glucose equivalent per 100 grams of the sample.

Determination of Soluble Carbohydrate Contents

The extract (1 mg) was marcarated with 50 ml of distilled water and filtered. To one portion of the filterate (1 ml), a saturated solution of picric acid was added. Absorbance was then read at 580 nm. Increasing value of standard starch solution was prepared same way and the absorbance taken. The amount of carbohydrate was recorded as mg starch equivalent/100 grams of the sample.

Results and Discussion

Qualitative and quantitative screening result of CMEPP are as presented in Tables 1 and 2.

Table 1: Qualitative Phytochemical Analysis of Crude Methanol Extract of *Paullinia pinnata* L

S/N	Phytochemicals	Observation	Inference
1	Alkaloids (a) M		++
	Mayer's test	Cream coloured precipitate	
	Dragendoff's test	Reddish-brown precipitate	
2	Flavonoids		
	Ferric chloride test	Greenish black colour	+++
	NaOH test	Yellow colour	+++
3	Total phenolics	Dark green colour	+++
4	Tannins	Greenish precipitate	++
5	Saponins	Honey comb froth persistence	+
6	Carbohydrates	A reddish ring	+++
7	Glycosides	A brown ring	++
8	Reducing sugar	Brick-red precipitate	++
9	Steroids	Blue-green colouration	+
10	Terpenoids	From pink to violet	+

+Mild
++Moderate
+++Abundant

Table 2: Quantitative Screening of Crude Methanol Extract of *Paulinia pinnata*

S/N	Phytochemical (mg/100g)	Mean ± MD
1	Alkaloids	207.98±11.24
2	Total phenolics	8350.02±38.50
3	Tannins	111.06±13.11
4	Glycosides	245.63±15.09
5	Saponins	19.42±3.25
6	Reducing sugar	298.91±9.05
7	Carbohydrates	4847.09±56.44
8	Terpenoids	56.05±4.85
9	Steroids	45.52±10.04
10	Flavonoids	5874.00±44.82

Results are expressed as Mean ± MD (n = 3)

Qualitative phytochemical screening of the CMEPP revealed the presence of alkaloids, flavonoids, total phenolics, tannins, saponins, carbohydrates, glycosides, reducing sugar, steroids and terpenoids (Table 1). The result is comparable to that of [23] who detected the presence of tannins, flavonoids, glycoside, alkaloids and cardiac glycosides from the ethanol leaf extract of *Paullinia pinnata* L. It also corroborated the result of [24] which revealed the presence of same phytochemicals from the hot ethanol extract of the leaf of the plant. Flavonoids, alkaloids, cardiac glycosides, saponins, tannins, carbohydrates, sterols, triterpenoids, and steroidal glycosides had also been identified from *Paullinia pinnata* L [25, 26, 27, 28]. Phytochemicals, also known as secondary plant metabolites, are naturally occurring, biologically active compounds found in plants and known to provide health benefits to humans [29]. They are known as broad spectrum health-promoting agents on humans and animals [30]. They have biological properties such as antioxidant activities, stimulation of immune system, modulation of detoxification

enzymes, modulation of hormone metabolism, decrease of platelet aggregation and anticancer properties [31]. The array of phytochemicals in the CMEPP may explain why the leaf is highly regarded in Nigeria among traditional healers as a potent remedy against many diseases.

Quantifying the phytochemicals revealed them in the following order: Total phenolics (8350.02±38.50 mg/100g) > flavonoids (5874.00±44.82 mg/100g) > carbohydrate (4847.09±56.44 mg/100g) > reducing sugar (298.91±9.05 mg/100g) > alkaloids (207.98±11.24 mg/100g) > tannins (111.06±13.11 mg/100g) > terpenoids (56.05±4.85 mg/100g) > steroids (45.52±10.04 mg/100g) > saponin (19.42±3.25 mg/100g) (Table II).

The total phenolic value (8350.02±38.50 mg/100g) is less than 9020 mg/100g reported of the ethanolic extract of *Azolla microphylla* [32] but greater than 933 mg/100g reported of *Pteris confusa*; 1045 mg/100g reported of *Pteris vittata*; 805 mg/100g reported of *Pteris argyreae*; 1325 mg/100g reported of *Pteris biaurita* and 710 mg/100g reported of *Pteris multiaurita* [33]. The high amount of phenolics in this plant could be responsible for the regulation of *Paullinia pinnata* growth and its disease resistance [34].

The flavonoid content (5874.00±44.82 mg/100g) is comparable to 5850 mg/100g reported of the ethanolic extract of *Azolla microphylla* [32]. The value is greater than 1250 mg/100g reported of *Pteris confusa*; 1420 mg/100g reported of *Pteris vittata*; 1325 mg/100g reported of *Pteris argyreae*; 1755 reported of *Pteris biaurita* and 1225 mg/100g reported of *Pteris multiaurita* [33]. The high amount of flavonoid in *Paullinia pinnata* L agrees with the report that flavonoid is the most abundant phenolic compound and are ubiquitous in nature [29]. There are about 6,000 flavonoids that form the colourful pigments of vegetables, fruits, herbs and medicinal plants, tea, coffee and fruit drinks [35]. Flavonoids, which naturally occurs conjugated with sugar [36], contribute to plant's colour, flavour and aroma while protecting it from damage and diseases [37]. The presence of flavonoids in the leaf of *Paullinia pinnata* L could account for its use as anti-inflammatory agent [38], antioxidant agent [39] anti-bacterial agent [40], anti-viral agent [41], anti-cancer agent [41] It means that the plant could be used to prevent damage caused by free radicals in the body [39], and for the treatment of diarrhea [42]. It also means that *Paullinia pinnata* could be used as natural antioxidant with the potential to serve as anti-carcinogenic, cytoprotective and anti-mutagenic properties [34]. The attention of pharmaceutical industries, which attempts to design the prevention and treatment of certain disease had earlier been drawn to flavonoid because of their antimutagenic, anti-inflammatory properties coupled with their potential to control key cellular enzyme metabolic functions [30]. The best described property of majority of flavonoid classes is their ability to scavenge free radicals thereby protecting human body from free radicals and reactive oxygen species [29]. This result suggests that *Paullinia pinata* has the potential to scavenge reactive oxygen species [43], suppress generation of reactive oxygen species (ROS) through inhibition of ROS generating enzymes [44], chelate trace elements [45] and upregulate antioxidant defences. Flavonoids constitutes a wide range of compounds that help in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as DNA, carbohydrates, lipids and proteins [46]. They have low redox potential and this enables it reduce highly oxidized free radical such as peroxyl, alkoxyl, hydroxyl and superoxide radicals by proton donation [47]. Flavonoid inhibit xanthine oxidase and protein kinase which are responsible for generation of superoxide anions [44, 48]. They also inhibit other ROS generating enzymes such as cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase, lipoxygenase, NADH oxidase etc [49]. Flavonoids have important biochemical and antioxidant effect in relation to many oxidative stress-induced diseases [50]. Several flavonoids have demonstrated to reduce the production of inflammatory mediators by blocking NF-kB pathway [51]. In addition to these, they have been stated to possess many other useful properties including oestrogenic activity, anti-allergic activity, vascular activity etc [52]

The tannin content (111.06±13.11 mg/100g) is appreciably lower than 8220 mg/100g reported of the ethanolic extract of *Azolla microphylla* [32]; 306 mg/100g reported of *Pteris confusa*; 530 mg/100g reported of *Pteris vittata*; 460 mg/100g reported of *Pteris argyreae* and 526 mg/100g reported of *Pteris biaurita* [33]. Tannins, high molecular weight polyphenolic compounds, form complexes with alkaloids, minerals, proteins nucleic acid and carbohydrates [53]. The presence of tannin in this plant suggests that *Paullinia pinnata* L could be effective against diarrhoea, stomach and duodenal tumours and as diuretics [54] and also as anti-inflammatory, antiseptic and haemostatic pharmaceuticals [55]. It also suggests that the plant can elicit anti-inflammatory, anti-microbial and astringent properties [32].

The saponin content (19.42±3.25 mg/100g) is appreciably lower than 1210 mg/100g reported of the ethanolic extract of *Azolla microphylla* [32]; 622 mg/100g reported of *Pteris confusa*; 840 mg/100g reported of *Pteris vittata*; 710 mg/100g reported of *Pteris argyreae*; 1110 mg/100g reported of *Pteris biaurita* and 540 mg/100g reported of *Pteris multiaurita* [33]. The low content of saponin agrees with the report of [33]. The presence of saponin in this plant extract suggests the membrane-permeabilizing, immunostimulant, anticarcinogenic, hypocholesterolaemic potential of the plant and that the plant could have significant effect on growth, feed intake and reproduction in animals [56]. It suggests that the plant could kill protozoans and molluscs and also impair digestion of protein and the uptake of vitamins and minerals in the gut [57].

The alkaloid content (207.98±11.24 mg/100g) is comparable to 220 mg/100g reported of the ethanolic extract of *Azolla microphylla* [32] but less than 1005 mg/100g reported of *Pteris confusa*; 1210 mg/100g reported of *Pteris vittata*; 1155 mg/100g reported of *Pteris argyreae*; 1640 mg/100g reported of *Pteris biaurita* and 950 mg/100g reported of *Pteris multiaurita* [33]. The presence of alkaloid in *Paullinia pinnata* L suggest that the plant could be used as an antiarrhythmic, antimalaria, anticancer and antihypertensive agents [58]. Alkaloid is one of the first natural products to be isolated from medicinal plant [29]. When they were first obtained from the plants materials in the early years of 19th century, they were found to be nitrogen-containing bases (alkali) which formed salt with acid. That is why they were referred to as vegetable alkalis or alkaloids, and these alkaloids are used as the local anaesthetic and stimulant as cocaine [58]. They have very bitter taste [59].

Also identified from this plant is terpenoid, a class of natural products which have been derived from five-carbon isoprene units. Many of the terpenoids are commercially interesting because of their use as flavours and fragrance in foods and cosmetics [60]. The presence of terpenoids in *Paullinia pinnata* L could account for the flavour of *Paullinia pinnata* L fruits and the fragrance of its flowers [61]. It also suggests that

Paullinia pinnata L could have medicinal properties such as anticarcinogenic, anti-ulcer and antimicrobial [62].

The variation in the concentrations of the phytochemicals analysed relative to previous findings may be primarily due to variation in environmental factors such as water quality, altitude, climate, intensity of sunlight exposure, soil composition [32].

Conclusion

In this study several chemical constituents have been identified from crude methanolic extract of *Paullinia pinnata* L leaves including flavonoid, alkaloids, tannins, reducing sugar and terpenoids. Each of these compounds has its own biological importance. This explains the veracity of use of this plant part in the local setting across the world. Further study of this plant's phytochemical by insilico and invitro methods can prove its medicinal importance in future and can be an efficient and effective nutraceutical sources in cheaper rate

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