Evaluation of Phytochemical Constituents, Nutritional Composition, and *In vitro* Antioxidant Capacity of *Dennettiatripetala* Aqueous Leave Extract.

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Abstract

Dennettiatripetala (DT) leaves are commonly used as decoction in traditional medicine for the management of certain health conditions including fevers, asthma, diarrhoea, anemia, pain, diabetes and bacterial infections, due to its bioactive compounds. In this study, the aqueous extract was investigated for its phytochemical, nutritional and antioxidant potential. Fresh leaves of *Dennettiatripetala* were collected, air-dried, pulverized, macerated in distilled water and thereafter, concentrated with a rotary evaporator. Using standard methods, nutritional composition, qualitative phytochemical and *invitro* antioxidant capacity were evaluated. Results of the proximate analysis show that DT leaves contain -moisture content (11.9%), crude fibre (48.5 %), crude protein (6.21%), fat content (7.3%) and ash content (8.65%). Mineral analysis indicates phosphorus (100.5mg/100g) as the predominant mineral, followed by calcium (24.5mg/100g), potassium (24.3mg/100g), sodium (19.04mg/100g), magnesium (9.22mg/100g), iron (5.3mg/100g) and zinc (1.97mg/100g). Qualitative phytochemical analysis revealed the presence of cardiac glycosides, saponins, terpenoids and reducing sugars, while flavonoids, tannins, alkaloids, steroids and coumarins were not detected. In addition, DT effectively scavenged DPPH radical in a concentration-dependent manner. In the same vein, the extract demonstrated ferric reducing power and total antioxidant capacity. The findings of this study portray the rich contents of phytochemicals and nutrients in *Dennettiatripetala*, giving credence to its effectiveness in health management.

Keywords: Proximate analysis, antioxidant, minerals, DPPH, FRAP, bioactive compounds, traditional medicine

Introduction

Medicinal plants have served as a cornerstone of traditional healthcare systems for centuries and continue to play a vital role in the discovery and development of modern therapeutic agents. According to the World Health Organization (WHO), approximately 80% of the population in developing countries relies on traditional medicine, particularly plant-based remedies, for primary healthcare needs. The increasing prevalence of chronic diseases and growing concerns regarding the adverse effects associated with synthetic drugs have renewed interest in medicinal plants as sources of bioactive compounds with therapeutic potential. Consequently, scientific investigations aimed at validating the pharmacological properties of medicinal plants have become an important area of research in natural product chemistry, pharmacognosy and medicinal plant studies.

Dennettiatripetala Baker (syn. Uvariopsistripetala (Baker F.) G.E. Schatz) commonly called pepper fruit, is a small woody shrub 12 - 18 m tall with fibrous bark and a potent distinctive smell, belonging to the Annonaceae family (Timothy and Okere, 2008). In West Africa, it is widely cultivated in Nigeria, Ivory Coast and Cameroon. The leaves are dark green, oval shaped with prominent spine and a pointed tip. *Dennettiatripetala* fruits, bark, and leaves are primarily used as seasonings or spices for meat, sausage, stew, soup, and vegetables (Ejechi and Akpomedaye, 2005, Iseghohi, 2015).

In traditional medicine, the seeds and leaves are used for the treatment of cough, fever, asthma, catarrh, toothache, diarrhea and rheumatism (Akabueze et al., 2016). Studies have shown that the fruit possess some anti-diabetic, anti-microbial properties, anti-analgesic, anti-inflammatory and neuro-pharmacological effects (Iseghohi, 2015), due to the presence of flavonoids, polyphenols and other phytochemicals of medical significance (Dike, 2010) and are also used to enhance appetite, clear throats, check excess saliva, relieve coated tongues and stop nausea (Ejechiet al., 2005). The seeds serve as important component in the diets of women after childbirth, aiding the contraction of the uterus. The fruits have also been reported to possess several biological activities including anti-oxidant, antidiarrheal, antimicrobial, antiparasitic, anticonvulsant, antitrypanosomal, antimalarial, anti-inflammatory, anti-snake venom, and antinociceptive properties. Adedayo et al. (2010) reported that the fruits possess alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and cardiac glycosides. Although, decoctions of the leaves are used in many local homes as tea or as a vital constituent of medicinal mixtures, studies about the medicinal properties of the leaves are sparse. This study was carried out to investigate the nutritional, phytochemical constituent and antioxidant capacity of the aqueous extract of *Dennettiatripetala* leaves.

Materials and Methods

Extract Preparation

Leaves of *Dennettiatripetala* were obtained from a small farm in Benin City, Edo State. They were washed, dried at room temperature and powdered. The powdered plant sample was measured using a weighing balance. 500g was macerated in 2.5liters of water for 72hours and was stirred every 24hours. On the third day, it was filtered using a clean muslin cloth. The resultant aqueous extract was concentrated in a rotary evaporator at 40°C then dried in the oven at the same temperature.

Qualitative phytochemical screening of leaf extracts

Test for Flavonoids

One (1) ml of extract in a test tube was mixed with 5 ml of dilute ammonia and 1 ml of

concentrated H_2SO_4 was added to the mixture. A yellow color was positive for flavonoids.

Test for Tannins
1ml of extract (filtrate) in a test tube was heated for five minutes to boil. Thereafter drops of 15% ferric chloride was added, blue black colouration confirmed the presence of tannins.

Test for Cardiac glycosides
The test for cardiac glycoside was carried out using Killaini's test. 1ml of extract in a test tube was mixed with 2ml of glacial acetic acid, after which 1 drop of 15% ferric chloride and 1ml of concentrated sulphuric acid was added to the mixture. Brown colouration formed at the interface indicated the presence of cardiac glycosides.

Test for Saponins
The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins.1ml of extract was mixed with 5ml of distilled water. The mixture was mixed vigorously and observed for frothing.

Test for Steroids
Into a test tube, 1ml of the extract was added and mixed with 2ml of acetic acid and 2ml of concentrated H_2SO_4. Change of colour from violet to blue-green was positive for steroids.

Test for Terpenoids
1ml of the extract in a test tube was mixed with 2ml chloroform and 3ml concentrated H_2SO_4. Reddish brown colouration at the interface, confirmed the presence of terpenoids (Salkowski's test).

Test for Alkaloids
Hager's test**:** 1ml of extract was mixed with 3 drops of Hager's reagents. Formation of yellow coloured precipitate was indicative for alkaloids.

Coumarins
Drops of 10% NaOH (1ml) solution were added to 5ml of the plant extract in a test tube. Formation of yellow colour indicated the presence of coumarins.

Test for Reducing Sugar
Iml of extract (filtrate) was added to boiling Fehling's solution A and B in a test tube. Colour change from blue to green was positive indication for reducing sugar.

Proximate Analysis
Proximate analysis of the leaf sample was done according to the method of AOAC, (2000).

Moisture determination
Moisture content is obtained by the loss in weight that occurs when a sample is completely dried to a constant weight in an oven. With the use of a petri dish previously dried and weighed, 2 g of the sample was weighed. The sample was then completely dried in an oven for 36 hours at 65^0C, which then was allowed to completely cool in a desiccator and the weight was obtained using a weighing balance. The drying and weighing was continued until a constant weight is achieved (AOAC, 2000).

$$\%Moisture = \frac{\text{weight of sample + dish before drying} - \text{weight of sample +dish after drying}}{\text{weight of sample taken}} \times 100$$

Crude lipids or ether extract
The ether extract of a feed signifies the fat and oil in the seed. Soxhlet apparatus is the equipment used to obtain the ether extract of seed. 150 ml of anhydrous diethyl ether (petroleum ether) of boiling point of 40-60^0C was added to the flask. 2-5g of the sample was weighed into a thimble and the thimble was plugged with cotton wool. The thimble with content was placed in the extractor; the ether in the flask was heated (boiled). As the ether vapor get to the condenser through the side arm of the

extractor, it condenses to liquid form and drop back into the sample in the thimble, the ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask. The extraction continues for 4hrs at least. The thimble was removed and most of the solvent was distilled from the flask into the extractor. The flask was then disconnected from the extractor and placed in an oven for 4hrs at 65^0C before cooling in a desiccator and weighed.

$$\%\text{Ether extract} = \frac{\text{weight of flask + extract} - \text{tare weight of flask}}{\text{weight of sample}} \times 100$$

Crude fibre

The organic residue left after the extraction of seed with ether was used to determine the crude fibre (AOAC, 2003), however if a fresh sample was used, the fat contained in the sample could be extracted by adding petroleum ether, then stirred, after which allow it to settle and decant. The extraction was repeated three times. The fat-free material was transferred into a flask/beaker and 200mL of pre-heated 1.25% H_2SO_4 was added and the solution was gently boiled for 30mins, maintaining constant volume of acid by the addition of hot water. The Buckner flask funnel filtered with whatman filter paper was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under enough suction. The residue was washed numerous times with boiling water (until the residue tested neutral to litmus paper) and transferred back into the beaker. Then 200mL of pre-heated 1.25% Na_2SO_4 was added and heated for another 30 minutes. Filtration was carried out under suction and washed thoroughly with hot water and twice with ethanol. The resulting residue was dried at 65^0C for 24 hrs and the weight was obtained. The residue was transferred into a crucible and placed in muffle furnace and allowed to ash for 4hrs at 400-600^0C, then cooled in dessicator and weighed (AOAC, 2000).

$$\%\text{Crude fibre} = \frac{\text{Dry weight of residue before ashing} - \text{weight of residue after ashing}}{\text{weight of sample}} \times 100$$

Crude protein

Crude protein is obtained by quantifying the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein is obtained by Kjeldahl method. The method involves: Digestion, Distillation and Titration (AOAC, 2000).

Digestion: Two grams of the sample was weighed into Kjeldahl flask then 25 mL of concentrated sulphuric acid was added, 0.5 g of copper sulphate was also added along with 5g of sodium sulphate and a speck of selenium tablet. Heat was slowly applied using the Soxhlet apparatus in a fume cupboard at first to avoid frothing before time and digestion was continued for 45mins until the digesta turned clear pale green. The digesta was allowed to completely cool and 100 mL of distilled water was rapidly added. The flask was rinsed thoroughly about 2-3 times and added to the digest.

Distillation: Markham distillation apparatus was used for the process of distillation. It was heated up for some time and 10 ml of the digest placed into the apparatus through a funnel and allowed to heat. 10 ml of sodium hydroxide was added using a measuring cylinder so that ammonia is not lost. This was dstilled into 50 ml of 2% boric acid containing screened methyl red indicator.

Titration: The alkaline ammonium borate formed from distillation was titrated directly with 0.1*N* HCl. The titre value which was the volume of acid used was recorded for calculation.

$$\%N = \frac{(14 \times VA \times 0.1 \times W) \times 100}{1000 \times 100}$$

VA = volume of acid used
W= weight of sample
% Crude protein = % N x 6.25

Crude ash content

Ash is the inorganic residue gotten by burning up the organic matter of feedstuff using muffle furnace at 400-600^{0}C for 4 hrs (AOAC, 2000). Two grams of the sample was weighed into a pre-heated crucible. Using the muffle furnace, the crucible was heated for 4 hrs at 400-600^{0}C or until whitish-grey ash was observed. For complete cooling the crucible was then transferred into the desiccator after which it was weighed to determine the ash content.

$$\%Ash = \frac{\text{weight of crucible+ash} - \text{weight of crucible}}{\text{weight of sample}} \times 100$$

Determination of total Carbohydrate

The percentage carbohydrate content of the leaf sample was determined by summing up the percentages of moisture, ash, crude protein, fat and subtracting from 100. The difference in value was taken as the percentage total carbohydrate content of the leaf sample.

***In Vitro* Antioxidant Assay**

Determination of total antioxidant capacity

Total antioxidant capacity was determined by the phosphomolybdenum method of Prieto et al (1999). The method is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at pH 5.0. One milliliter (1 mg/mL) of each extract was added to 3 mL of molybdate reagent solution. The tubes were kept incubated at 95°C for 90 minutes. After incubation, these tubes were normalized to room temperature for 20-30 minutes and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as the standard antioxidant. The antioxidant capacities of the samples were compared to that of vitamin C and were expressed as mg ascorbic acid equivalent (AAE) / g extract.

Estimation of 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) radical scavenging ability

The free radical scavenging ability of *Dennettiatripetala*extracts against 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical was determined by the method of Brand-Williams et. al (1995). The assay is based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour was measured spectrophotometrically at 517 nm. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations (0.2 - 1.0 mg/mL) of *A. floribunda* extracts. The reaction tubes were shaken and incubated for 15 minutes at room temperature in the dark; the change in colour was measured spectrophotometrically at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank solution containing 0.5 mL of 0.3 mM DPPH and 2mL methanol was prepared and treated as the control. The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$

Where:

A_0 was the absorbance of DPPH radical + methanol
A_1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Results

Phytochemical analysis

Table 2 presents the qualitative phytochemical analyses carried out on the extracts of *Dennettiatripetala* leaves and shows the presence of phytochemical constituents. It shows that cardiac glycosides, saponins, reducing sugars and terpenoids are present in *Dennettiatripetala* leaves. From the amount of precipitate formed and degree of colour change, it was deduced that *Dennettiatripetala* moderately contained these phytochemicals. The analysis showed that flavonoids, steroids, coumarins, alkaloids and tannins were not present in *Dennettiatripetala* leave extract.

Table 1: Phytochemical screening of *Dennettiatripetala* leave extract

Phytochemicals	DT leave extract
Flavonoids	-
Cardiac glycosides	+
Saponins	+
Steroids	-
Terpenoids	+
Alkaloids	-
Coumarins	-
Reducing sugars	+
Tannins	-

Key - Present (+) not detected (-), DT - *Dennettiatripetala*

Analysis of mineral content

Table 2 shows the results of the mineral analysis of *Dennettiatripetala* leave extract.

Table 2: Mineral content of *Dennettiatripetala* aqueous leaf extract

Mineral content (mg/100g)	DT leave extract
Phosphorus	100.5 ± 0.63
Calcium	24.5 ± 0.34
Iron	5.3 ± 0.12
Sodium	19.04 ± 0.57
Potassium	24.3± 0.25
Magnesium	9.22 ± 0.08
Zinc	1.97 ± 0.04

Results are given as mean ±standard deviation, DT - *Dennettiatripetala*

Total Antioxidant Capacity

The total antioxidant capacity of *Dennettiatripetala* leaves extracts was determined and presented in Figure 1. The results demonstrated that the aqueous extract of *Dennettiatripetala* leaves possess significant antioxidant capacity *in vitro* when compared with ascorbic acid.

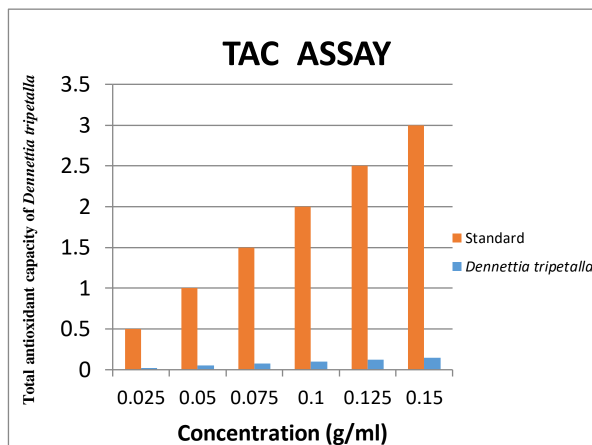

Figure 1: Total antioxidant capacity of *Dennettiatripetala* leave extract

DPPH radical scavenging activity

Figure 2 shows the results of DPPH radical scavenging activities of *Dennettiatripetala* aqueous leaf extracts. The extract sufficiently scavenged DDPH radical in a concentration dependent manner.

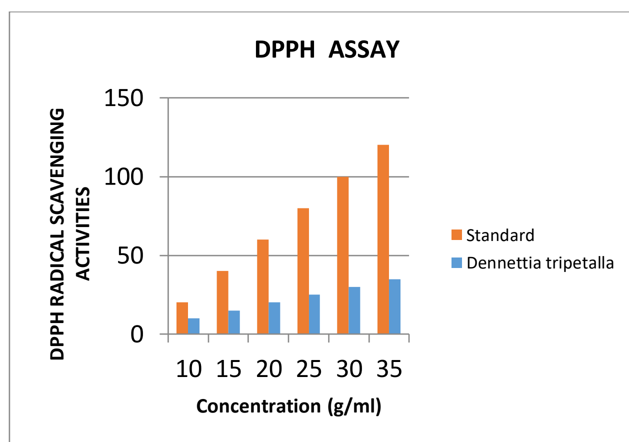

Figure 2: DPPH radical scavenging activities of *Dennettiatripetala* leave extract

Proximate Analysis

Table 3 summarizes the results of analyzing the proximate composition of *Dennettiatripetalla* leave extract. The highest proximate composition was in crude fiber (48.5±0.41). The percentage composition of the leave extract demonstrates that it contains a significant number of basic food elements including fats, carbohydrates, protein and fiber.

Table 3: Proximate analysis of *Dennettiatripetala* aqueous leaf extract

Proximate	*Dennettiatripetala* leaves (mg/100g)
Moisture content	11.9±0.08
Crude fibre	48.5±0.41
Crude protein	6.21±0.05
Fat content	7.3±0.29
Ash content	8.65±0.45

Results are given as mean ± standard deviation

Discussion

The proximate analysis of *Dennettiatripetala* aqueous leaf extract revealed its moisture content, crude fiber content, crude protein, fat content and ash content. These were present in sufficient proportion in the leave extract with crude fiber content being the highest. Crude protein content was the lowest, which could be attributed to varied environment factors that influenced plant synthesis and storage. Nonetheless, adequate nitrogen availability results in a rise in protein content, demonstrating that nitrogen is a key element in regulating plant output and nutritional composition (Mettler et al., 2023).

The result of the mineral content analysis of *Dennettiatripetala* leaf extracts confirms it contains significant minerals, which are micronutrients needed by the body in modest amounts. Phosphorus had the highest concentration, followed by calcium, potassium, sodium, magnesium, iron, and zinc. Phosphorus and calcium, in this study were higher when compared to the work done by Eze et al. (2014).

Phytochemicals are present in plants as a defense mechanism against predators while in animal cells, they play vital role in the well-being of the cell (Okungbowaet al., 2025). The therapeutic advantages of phytochemical elements in herbal medicines have been widely established. Phytochemical analysis of *Dennettiatripetala* aqueous leaf extract revealed the presence of cardiac glycosides, saponins, reducing sugars and terpenoids while flavonoids, steroids, coumarins, alkaloids and tannins were absent. The absence of steroids in *Dennettiatripetala* aqueous leaf extract, observed in our study corroborate the findings of previous authors (Mordi et al., (2021; Evuen and Kpomah, 2023, Okungbowa et al., 2025).

Alkaloids, phenols, and flavonoids are phytochemicals that serve a variety of biological roles in plant–pollinator interactions, reactive oxygen scavenging, plant defense, and metal chelation (Do Nascimento et al., 2018). Tannins have been reported to have a wide range of biological activities, such as anti-diarrheal, hemostatic, and anti-inflammatory properties, while saponins have been found to have a wide range of biological activities, such as being bioactive against diabetes (Smith and Adanlawo, 2012). The absence of flavonoids and tannins in the present study differs from some previous reports on *Dennettia tripetala*. Such variations may arise from differences in geographical location where the leaves were sourced from, climatic conditions, harvesting season, extraction methods and solvent polarity. Environmental factors have been shown to significantly influence the biosynthesis and accumulation of secondary metabolites in medicinal plants.

Antioxidant activity when increased reduces the cycle of lipid peroxidation and protects both plant and animal cells from damage (Amin et al., 2020). The reducing power assay, which is based on the hydrogen donating tendency of plant extracts, gives a significant indication of the antioxidant capacity of the plant. *Dennettiatripetala* aqueous leave extract showed reducing power ability with increasing concentration. Plant extracts with high reducing power indicates that it can operate as a vigorous electron donor in reducing free radicals and neutralizing them into stable compounds, producing a break in the chain reaction (Gulcin, 2020).

Total antioxidant capacity is a commonly used analyte for determining the antioxidant status of biological samples, and it can be used to examine the antioxidant response to free radicals produced in a disease (Rubio et al*.,* 2016). The phosphomolybdate experiment demonstrated that the *Dennettiatripetala* aqueous leaf extract had significant antioxidant capacity in the *in vitro* antioxidant assay and that the total antioxidant capacity of *Dennettiatripetala* leaves extracts slightly increased as the concentrations of the extracts increased.

The DPPH assay is the most important test for determining plant extracts' ability to absorb free radicals. Plant extracts' DPPH radical scavenging ability is mostly due to their hydrogen donating inclination. Plant extracts' phenolic content is responsible for scavenging free radicals. These free radicals have been linked to the development of a number of chronic illnesses, including cancer (Rahman et al., 2015). *Dennettiatripetala* leaf extracts had a high percentage inhibition when compared to the standard and its scavenging ability increases with increasing concentration.

The robust DPPH radical scavenging activity recorded in our study, along with the identified bioactive constituents particularly saponins, terpenoids, and cardiac glycosides, collectively substantiates the wide ethnopharmacological applications of *Dennettiatripetala* in the management of fevers, asthma, diarrhoea, anaemia, pain, diabetes, and bacterial infections. Our findings therefore provide a scientific framework that validates its continued use in traditional medicine and highlights its promise as a source of bioactive leads for future drug discovery and design.

Conclusion

The aqueous leaf extract of *Dennettiatripetala* contains important phytochemicals, essential minerals and appreciable antioxidant activity. These findings provide scientific support for the ethnomedicinal use of the plant and suggest its potential as a source of natural antioxidant compounds. Further studies involving isolation, characterization and biological evaluation of the bioactive compounds are recommended.

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