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Original Research Article

High Performance Liquid Chromatography Profiling of *Dennettia tripetala* Leaf Extract and Its Biological Activities

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ABSTRACT

There are much potential embedded in plants, ranging from air purification to ornamental and source of medicine to mankind. This study reports the biological potentials of *Dennettia tripetala* leaf extract and its flavonoid content for its possible usage in ameliorating. Flavonoid content of *D. tripetala* leaf was profiled using high performance liquid chromatography **(**HPLC), antioxidant and anticholinesterase properties were also studied using standard methods. The major flavonoids identified and quantified were catechin (56.17 mg/100g), kaemferol (49.80 mg/100g), quercetin (69.19 mg/100g), isorhammnetin (19.89 mg/100g) and naringin (19.55 mg/100g). There was evidence of antioxidant potential of *D. tripetala* leaf extract. Also, the compound, flavonoid, showed acetylcholinesterase and butrylcholinesterase inhibition of 68 and 74%, respectively. The results of the study indicated that the leaf extract of *D. tripetala* contains high concentrations of flavonoids and possesses antioxidant and anticholinesterases properties. It may be used in managing oxidative stress-related diseases and dementias.

***Keywords*:** HPLC, Anti-cholinesterases, Antioxidant, Phytochemistry, *Dennttia tripetala*.

Introduction

Dementias are complex neurological disorder characterized by impairment in the secretion and concentration of neurotransmitters metabolized by cholinesterases. These enzymes were implicated in pathogenesis of ataxia and Parkinson's disease.[1] They are characterized with low concentration of acetylcholine in the neuronal tracks and the brain. Another hypothesis attributed their pathogenesis to the effects of oxidative stress, as the free radicals attack the lipid rich membranes of the neuronal paths, thereby causing malfunctioning of the neurotransmitter flow. Management of these diseases' targets increasing acetylcholine concentration in the synaptic gaps using cholinesterase's inhibitors,[2] and the use of flavonoids rich materials that could ameliorate the effects of the free radicals released. Because of the high cost and negative side effects associated the conventional cholinesterase inhibitors; there is increase in the search for plants with these potentials.

Dennettia tripetala (pepper fruit) belongs to the annonaceae family with fruits, leaves and root that possess a characteristic pungent spicy taste consumed for leisure and used during ceremonies in the South Eastern part of Nigeria.[3, 4] The fresh leaves are used in making pepper soup as condiments,[5,6] while the older leaves are used as tea.[7] Consumption of *Dennettia tripetala* leaves provides more than just a flavour but has been proven to have a range of medicinal values when consumed alone or in combination with other medicinal plants.[8] It is a plant used in West Africa[9] for the treating diseases, such as including convulsion, typhoid, worm infestation, Gastro-intestinal diseases,[10] lowering of lipids and an alternative anti-diabetic agent of natural origin.[11] Essential oil of *D. tripetala* seed possesses antibacterial, antiradical properties,[12] antifungal and insecticidal activities,[13] also the seed extract was reported to possess grain protectant property.[14] However, there is dearth of information on the characterization of flavonoid content, *in-vitro* cholinesterase and antioxidant properties of *D. tripetala* leaf extract. In this study, flavonoid concentration was studied in the leaf principle of *D. tripetala*, together with its *in-vitro* cholinesterase and antioxidant properties.

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Materials and Methods

Sample collection

Fresh leaf of *D. tripetala* was collected from its natural habitat, Edem-ani village, Nsukka Local Government Area on 22nd November, 2019 and identified by Mr. Felix Nwafor of Pharmacognosy Department, University of Nigeria, Nsukka as *Dennettia tripetala* Bak. f. and deposited in their herbarium with voucher number PCG/UNN/0362.

Sample preparation

The leaf sample was dried at 25ºC for 7 days, milled into fine particles using electric blender and stored in a refrigerator (4ºC). 200g *D. tripetala* leave powder was soaked in 500 mL of 70% ethanol for 48 h with occasional shaking in an air tight container. The mixture was filtered and concentrated using rotary evaporator.

Qualitative phytochemical analysis

The following phytochemicals (alkaloids, glycosides, tannins, saponin, flavonoids, resins, steroids, terpenoids) were analyzed using A. O. A. C method,[15] with little modification.

Quantitative phytochemical analyses of D. tripetala leaf extract

The total phenolic content was evaluated using the folin ciocalteau reagent as described by Nwidu *et al*.[16] The total flavonoid, alkaloid and tannin contents of *D. tripetela* leaf extract were determined according to Senguttuvan*et al*. [17]Quercetin was used as a reference

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compound for flavonoids. Total flavonoid (TFC) content was determined as µg quercetin equivalent/gram of extract.

Acetylcholinesterase and Butrylcholinesterase inhibitory activity assay

Acetylcholinesterase and butrylcholinesterase inhibition were assayed using Ellman*et al*.[18] methods. Acetylthiocholine and butrylcholine iodides were used as substrates. Each sample concentration was mixed with 500 µL enzyme solution and incubated at 37°C for 30 min. The absorbance was read at 412 nm after adding3.5 mL; 0.5 mM acetylcholine and butyrylthiocholine, 1 mM DTNB, in 50 mM sodium phosphate buffer (pH 8.0) using JENWAY 6404 spectrophotometer. Assay reactions with plant sample were all performed in duplicate.

The percentage of enzyme inhibition was calculated using the following formula:

$$AcetylCholinesterase\ activity\ \% = \frac{Ao - A1}{Ao} \times 100$$

$$Butyrylylcholinesterase\ activity\ \% = \frac{Ao - A1}{Ao} \times 100$$

Where A_o is control absorbance and A_1 is sample absorbance.

In-vitro 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging potential

In vitro antioxidant property was carried out using DPPH assay as described by Shen *et al*.[19] 1 mL of different concentrations of the sample was added to1 mL of DPPH (0.1 mM) was added in the test tube and incubated for 30 min at room temperature. The absorbance was read at 517 nm. The percent DPPH radical scavenging was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{Control\ absorbance\ -\ Sample\ absorbance}{Control\ absorbance} \times 100$$

Statistical analysis

Data were analyzed using the statistical program GLM model (Statistical Analysis Systems, SASInstitute, Cary, NC, USA, 2001). The means of values were compared using independent t test of significance ($p < 0.05$).

Results and Discussion

The qualitative and quantitative phytochemical results of the leaf extract of *D. tripetala* are presented in Tables 1 and 2, respectively. Alkaloid, tannin, saponin, flavonoids and steroids were detected, while glycoside and terpernoids were not detected in the leaf extract. Table 2 shows the quantitative analysis of total flavonoids and phenolic contents of the leaf extract of 0.718 mg/GAEq and 1.683 mg/QEq respectively, while alkaloid and tannin contents were 0.508% and 0.129 mg TEq/g, respectively.

Plant materials such as the leaves, roots and stems have been in use as medicine as long as mankind. *D. tripetala* leaf is rich in phytochemicals as previous studies have reported alkaloids and saponins in *D. tripetala* seed extract.[11] Also, alkaloids, tannins, saponins and flavonoids were detected in the root extract of *D. tripetala*.[20] The pharmacological or medicinal properties of *D. tripetala* leaf could be attributed to the rich presence and diverse nature of phytochemicals they possess. Another study on the fruit extract of *D.* tripetala,[9] reported that the extract contained tannins, flavonoid, saponins, phenol and alkaloid.

This result is indicative that the leaf extract could possess antioxidant activity due to the ability of these polyphenols to mop up free radicals when generated in the system. The total phenolic content of *D. tripetala* leaf is lower when compared to 212.5 mg/g obtained by Okolie*et al*.[20] from their study on the root extract of *D. tripetala*. Many pharmacological properties such as hypocholesterolemic, hypoglycaemic and antifungal activities have been attributed to saponins.[21] Flavonoids are potent antioxidant agent[22]and anticarcinogenic.[23]Phenolics inhibit the proliferation of pathogenic microbes.[24] Alkaloid is another essential phytochemical with medicinal properties such as antimalarial, anesthetics, anti-cancer and anti-hypertensive agents.[25]These suggest the rationale behind the use of *D. tripetala* leaf in many folk medicines.

DPPH radical scavenging potentials of the leaf extract of *D. tripetala* at varying concentrations (10-200 mg/ml) are presented in Figure 1. At 100 mg/mL, the highest *in vitro* scavenging activity against DPPH was observed. Also, ferric cyanide reducing power of *D. tripetala* was also studied. A corresponding increase of the extract's absorbance was observed (Figure 2). This suggests that the plant extract possesses the capacity to reduce ferric ions at the concentrations studied.

Omage*et al*.[9] reported the ability of *D. tripetala* fruit extract to have scavenged free radical produced by DPPH. This ability could be due to the presence of polyphenols in the leaf extract, which possess different antioxidant potentials. Antioxidant property of leaf extract could be attributed to its flavonoids content, which has been reported to scavenge free radical generated by DPPH.[26] This suggests that the plant extract has the capacity to mop up ferric ions at the concentrations studied, which is in agreement with the report of Okolie *et al*,[20] they observed concentration dependent ferric ion reducing power by *D. tripetala* root extract.

Table 1: Qualitative phytochemical analysis Of *D. tripetala* leaf extract

Phytochemicals	Inference
Alkaloid	+
Tannin	+
Saponin	+
Glycoside	-
Flavonoid	+
Resin	+
Steroid	+

Table 2: Quantitative phytochemical composition of ethanol leaf extract *D. tripetala*

Phytochemicals	Composition
Alkaloid	0.508%
Tannin	0.129 mgTEq/g
Phenolic	0.718 mgGAEq/g
Flavonoid	1.683 mg QEq/g

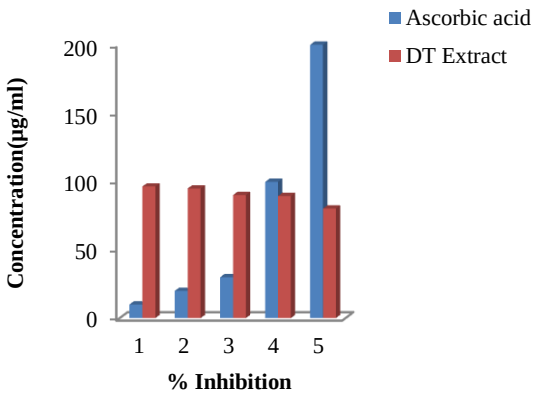

Figure 1:*in vitro* DPPH scavenging activity of the ethanolic extract of *D. tripetala* leaf.

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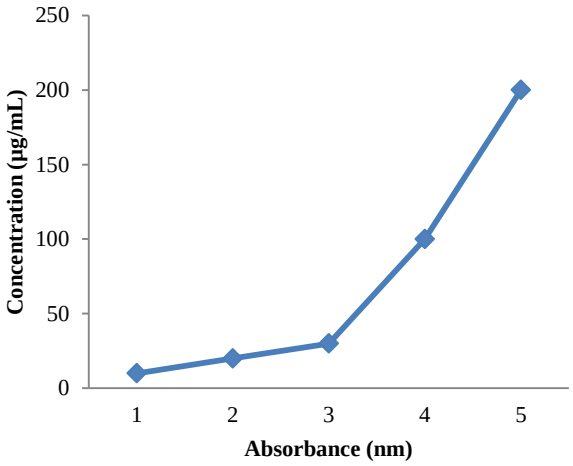

Figure 2: Reducing power of of*D. tripetala* leaf extract

The quantification of flavonoids in the leaf extract was studied using HPLC/MS. The compounds identified and quantified (mg/100 g) were catechin (56.17), apigenin (7.91), resveratrol (6.72), genistein (2.18), daidzein (2.00), butein (9.76), narigenin (1.55), biochanin (1.55), luteolin (18.82), kaemferol (49.80), (-)-epicatechin (11.38), (-)-epigallocatechin (3.25), gallocatechin (2.71), quercetin (69.19), epicatechin-3-gallate (6.98), epigallocatechin-3-gallate (4.82), isorhammnetin (19.89), robinetin (9.85), myricetin (1.22), baicalein (8.55), nobiletin (5.72), baicalin (3.65), tageretin (4.04), artemetin (2.42), silymarin (2.33), naringin (19.55) and hesperidin (3.98) respectively as shown in Table 3 and Figure 3. Among all the flavonoids identified, catechin, kaemferol, quercetin, isorhamnetin and naringin had highest concentrations of 56.17, 49.80, 69.19, 19.89 and 19.55 mg/100 g, respectively.

Previous studies have shown that quercetin plays some pharmacological roles including neuroprotective and cardioprotective effects,[27]thus these functions could be achieved by the leaf extract. Catechin has been reported to possess antioxidant properties[28] and protects the skin against UV radiation.[29]Medicinally, isorhmannetin serves anti-inflammation and antioxidative functions,[30]while naringin possess many properties such as anti-inflammation, tumor-inhibiting and bone regeneration.[31]

Table 3: Concentrations of flavonoids identified in *D. tripetala*leaf extract

S/N	Retention Time (min)	Amount (mg/100g)	Name of compound
1.	13.75	56.17	(+)-catechin
2.	14.49	7.91	Apigenin
3.	15.16	6.72	Resveratrol
4.	15.62	2.18	Genistein
5.	15.90	2.00	Daidzein
6.	16.30	1.07	Daidzein
7.	16.61	9.76	Butein
8.	16.98	1.55	Naringenin
9.	17.16	1.55	Biochanin
10.	17.78	18.82	Luteolin
11.	18.06	49.80	Kaemferol
12.	19.53	11.38	(-)-Epicatechin
13.	20.60	3.25	(-)-Epigallocatechin
14.	21.68	2.71	Gallocatechin
15.	22.61	69.19	Quercetin
16.	22.86	6.98	(-)-Epicatechin-3-gallate
17.	23.47	4.82	(-)-Epigallocatechin-3-gallate
18.	23.97	19.89	Isorhamnetin
19.	24.19	9.85	Robinetin
20.	24.79	1.22	Myriceetin
21.	25.62	8.55	Baicaletin
22.	26.14	5.72	Nobiletin
23.	26.30	3.65	Baicalin
24.	26.50	4.04	Targeretin
25.	26.83	2.42	Artemetin
26.	27.00	2.33	Silymarin
27.	27.37	19.55	Naringin
28.	28.31	3.98	Hesperidin

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Figure 4: HPLC/MS Chromatogram of *D. tripetala* leaf extract

Similarly, the inhibitory potentials of the leaf extract on cholinesterases (AchE and BchE) were studied. The extract showed 68 and 74% inhibition of the cholinesterases, respectively. Inhibitions of acetylcholinesterases lead to increase in the neural acetylcholine levels. The high inhibition percentage of cholinesterase could be attributed to the appreciable content of flavonoid content of the extract. Previous researchers have reported anti cholinesterase activity of flavonoids.[32]Similarly, 39.33 and 61.90% inhibition of acetylcholinesterase activity by essential oil from fresh leaf and fruit of *D. tripetala* was reported by Okolie *et al*.,[20] which is lower than 68% inhibition obtained in this study. This could be attributed to the effect of oil extraction process that may have denatured or removed some phytochemicals responsible for the inhibition in the cholinesterases.

Conclusion

The experimental study identified and quantified flavonoids content of *D. tripetala* leaf using HPLC, its antioxidant and anticholinesterases potentials. The medicinal potentials (antioxidant properties and anti-cholinesterases inhibition) could be attributed to the rich content of flavonoids in the leaf extract.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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