

ORIGINAL RESEARCH ARTICLE

Comparative Analysis of Antioxidant Properties in Green and Reddish-Brown Leaves of *Terminalia catappa* Using DPPH and Nitric Oxide Assays

Ndu Chukwukaie Favour¹, Victoria Moltong Yilwa¹, Onwumere G. Brian¹, Joseph Appah¹, Sunday Gandu

Audu^{1,2}, Enoch Emmanuel^{1,2}, Nwankwo Cornelius Tochukwu^{2,3} , Duru Daniel⁴ and Pai Yunusa Yusuf^{2,3}.

¹Department of Biological Sciences, Faculty of Science, Nigerian Defence Academy, Kaduna, P.M.B. 2109, Nigeria

²Department of Biotechnology, Faculty of Science, Nigerian Defence Academy, Kaduna, P.M.B. 2109, Nigeria

³Department of Biotechnology, Faculty of Science, Mewar International University, Masaka, Nasarawa, Nigeria

⁴Department of Psychology, University of Ibadan, P.M.B. 5017, Ibadan, Nigeria

ABSTRACT

This study evaluated and compared the antioxidant properties of the reddish-brown and green leaves of *Terminalia catappa*. The reddish-brown and green leaves of *T. catappa* were collected from Agwan-kadara Television, Chikun LGA of Kaduna State in Nigeria. These were collected and processed separately; 270g of each of the ground samples was used to prepare the methanol and aqueous extracts, respectively. Phytochemical analysis was conducted to determine the quantities of polyphenols, flavonoids, tannins, saponins, and alkaloids in the leaf samples. The antioxidative potential of the plant was determined by analyzing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and nitric oxide reducing activity. Methanol and aqueous extracts of the reddish-brown leaves of *T. catappa* yielded 31.6 g and 52.2 g of extract, respectively. The phytochemical constituents found in the methanol and aqueous extracts of reddish-brown leaves of *Terminalia catappa* showed a significant difference in flavonoids and polyphenols, but there was no significant difference in alkaloids ($13.9 \pm 0.02 \text{ mg/g}$), tannins ($90.4 \pm 0.07 \text{ mg/g}$), and saponins ($152.2 \pm 0.85 \text{ mg/g}$). The methanol and aqueous extracts of the green leaves of *Terminalia catappa* showed no significant difference in flavonoids ($96.9 \pm 7.02 \text{ mg/g}$), polyphenols ($110.0 \pm 14.1 \text{ mg/g}$), and alkaloids, but there was a significant difference in tannins ($123.2 \pm 0.38 \text{ mg/g}$) and saponins ($143.6 \pm 1.03 \text{ mg/g}$). The high presence of phytochemicals and antioxidant potential of *Terminalia catappa* justifies its use in traditional medicine because of its beneficial medicinal activity. This study also suggests both the reddish-brown and green leaves of *T. catappa* as effective antioxidants.

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INTRODUCTION

Terminalia catappa L. known as the Tropical almond/Indian almond, is often regarded as one of the most significant medicinal plants that can be found everywhere in the globe. Many regions inside and outside of India employ it as a traditional form of medical treatment for anti-inflammation, antimicrobial, hepatoprotective, vermifuge, antioxidant, anticancer, and antidiabetic agent (Mwangi *et al.*, 2024; Chinaka *et al.*, 2017). It is a member of the Combretaceae family (Combretum family); it is cultivated for its decorative value and can also grow wild because it does so well in tropical environments, has a single stem that can grow to a height of around 10 meters, after which it branches out horizontally and forms rosette-like clusters of leaves at the tips of each branch. Before they fall off, the leaves go

through a transformation in which they transition from green to red, yellow, or gold and a reddish-brown copper tint.

Terminalia catappa can be beneficial in the treatment of inflammatory disorders, the healing of wounds, allergies, skin issues, asthma, ulcers, cardiovascular disease, diarrhoea, and many other conditions. It is also believed to be beneficial for reviving one's sense of vigour and vitality. It is believed that the chemical constituents of *T. catappa* leaves include essential chemicals such as chebulagic acid, chorilage, gentisic acid, Grenadine-B, and kaempferol; on the other hand, the seeds are abundant in arachidic acid, ascorbic acid, fibre, fat, linoleic acid, palmitic acid, and other similar substances. It has a high concentration of minerals, including calcium, potassium, phosphorus, and sodium (Chang *et al.*, 2018).

Correspondence: Nwankwo Cornelius Tochukwu. Department of Biotechnology, Faculty of Science, Nigerian Defence Academy, Kaduna, P.M.B. 2109, Nigeria. ✉ corneliusnwankwo@nda.edu.ng.

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Phytochemicals such as phenolic compounds found in medicinal plants can have biological effects and are thought to be helpful to human health because they lower the chance of developing degenerative diseases by scavenging oxidative agents, preventing macromolecular oxidation and boosting the immune system (Olaoye *et al.*, 2021).

It has been determined that the LD₅₀ value of *T. catappa* is larger than 5000mg/kg bwt, which suggests that it is practically non-toxic in accordance with the classification provided by the Organization for Economic Cooperation and Development Guideline for Chemical Testing (OECD) (Ugwah-Oguejiofor *et al.*, 2019). Previous findings record that numerous species of *Terminalia*, when extracted using a variety of solvents, provide LD₅₀ values that are so low as to be considered virtually non-toxic (less than 2,000 to 5,000 mg per kg of bwt) (Arjariya *et al.*, 2013).

A subacute study of 28-day repeat-dose treatment of *T. catappa* in experimental animals demonstrated that it did not elicit any clinical signs of morbidity, toxicity, or mortality in any of the treatment groups. (Dongmo *et al.*, 2019). Alterations in organs and changes in body weight are crucial indications for access to the tested compound, the unfavourable effect of the medicinal plant, and the overall health conditions of experimental animals (Dongmo *et al.*, 2019).

Although the antioxidant properties of *T. catappa* leaves have been studied, there is limited information on the comparative effects of green versus reddish-brown leaves. This study explores the potential of both leaf types as natural antioxidants, focusing on their chemical composition and medicinal value. Therefore, this research aimed to determine and compare the phytochemical and antioxidant properties of the reddish-brown and green leaves of *T. catappa* responsible for its medicinal properties.

MATERIALS AND METHODS

Collection and Extract Preparation of *Terminalia catappa* Leaves

Terminalia catappa leaves were collected in Agwan-kadara Television, Chikun LGA Kaduna state, Nigeria. The reddish-brownish and green leaves were collected separately in a clean bag.

Extraction with Methanol

Terminalia catappa reddish-brown and green leaves were rinsed, air dried, and crushed. Separately, 270g of reddish-brown leaf powder and 270g of green leaves were weighed and soaked for 48 hours at room temperature in 1.2 litres of 100 percent methanol. The mixture was filtered using Whatman filter paper. The filtrate was concentrated dry using a rotary evaporator at 40°C and the extract yield was weighed and labelled accordingly.

Extraction with Distilled Water

Terminalia catappa's reddish-brown and green leaves were washed, air dried, and crushed. 270 g of reddish-brown leaf powder and 270 g of green leaves were weighed and soaked for 48 hours at room temperature in 3.2 liters of separately distilled water. The mixture was filtered using number 1 Whatman filter paper. The filtrate was concentrated dry in a water bath at 40°C, and the yield of the extract was weighed correspondingly.

Screening for Phytochemicals

Procedure for Estimating Polyphenols

- In a clean, dry beaker, 20 mg of gallic acid was weighed, 10 mL of methanol was used to dissolve it, and the volume was increased to 20 mL using the same solvent to get the solution concentration to 1 mg/mL.
- The polyphenol content of *T. catappa* leaf extract was determined using a modified Folin-Ciocalteu colorimetric technique. The standard was gallic acid, and the results were given as Gallic Acid Equivalent (GAE) per gram of material.
- Gallic acid concentrations ranging from 0.2 to 0.7 mg/mL were produced in methanol. In a 96-well plate, aliquots of 20 µL each of the sample and standard will be mixed with 150 µL of Folin–Ciocalteu and left to stand for 3-5 minutes at room temperature. Following that, 80 l of a saturated sodium carbonate solution (7.5 percent w/v) was added to each reaction well and incubated at room temperature for one hour with intermittent shaking. At 765 nm, the absorbance was measured, and a calibration curve for the gallic acid standard was created (Kim *et al.*, 2024)

Calculation

$$\text{Polyphenol Content} = \frac{V \times C}{M}$$

Where; C = concentration of gallic acid from calibration curve in mg/ml

V = volume of extract in mL

M = weight of extract in grammes

Total Flavonoids Estimation

A stock solution of 1 mg/mL quercetin was prepared prior to the commencement of the experiment. Total flavonoid content (TFC) of *T. catappa* leaf extract was determined using a colorimetric assay of aluminum chloride with slight modifications. 20 µL of extract and standard solution (1 to 0.5 mg/mL quercetin) were mixed with 15 µL of sodium nitrite solution (5 percent NaNO₂ w/v) in a 96-well plate and incubated for 6 minutes at room temperature. Following that, 15µL of aluminum chloride solution (10% AlCl₃, w/v) was added to each reaction well and left for 6 minutes before adding 80µL of sodium hydroxide (4 percent NaOH, w/v) to each well. The absorbance at 510 nm was measured after an additional 15 minutes of incubation. The total flavonoid content (TFC) was estimated as mg of quercetin equivalent per gramme of sample using a quercetin calibration curve. (Jang *et al.*, 2019).

Calculation

$$\text{Total flavonoid Content} = \frac{V \times C}{M}$$

Where; C = concentration of quercetin from calibration curve in mg/mL

V = volume of extract in ml

M = weight of extract in grammes

Determination of Alkaloids

Terminalia catappa leaf extract (20mg) was diluted in 4 ml of NHCl and then filtered. One milliliter of this solution was placed to a separatory funnel and rinsed with ten milliliters of chloroform. Then 0.1N NaOH was used to modify the PH of the phosphate buffer solution to neutral. In a separating funnel, 1 milliliter of this solution was transferred, and 5 milliliters of bromcresol solution and 5 milliliters of phosphate butter were added (7.0). The complex formed was fractionated using chloroform after the mixture was violently agitated. The fractions were collected in a 10 mL graduated flask and diluted to volume with chloroform. At 470 nm, the complex's absorbance in chloroform was determined. As a control, atropine was used. (Jang *et al.*, 2019)

Tannins Determination:

To evaluate the tannins, 0.2mL of *T. catappa* extract was put to a volumetric flask (2 mL) containing 1.5 mL of distilled water and 0.1 mL of Folin-Ciocalteu phenol reagent, 0.2 mL of 30% Na₂CO₃ solution, and 2 mL of distilled water. The mixture was properly combined and set aside for 30 minutes at room temperature. A series of reference standard solutions of gallic acid (20, 40, 60, 80, and 100µg/mL) were prepared in the same manner as stated previously. At 725 nm, the absorbance of the test and reference solutions was measured using a UV/VIS spectrophotometer against a blank. The tannin content was determined in milligrams of GAE per gram of extract. (Olaoye *et al.*, 2021).

Determination of Saponins Content

(5mg/mL) of *T. catappa* leaf extract was taken after diluting 5ml in 50% aqueous methanol and suitable aliquot. Following the vanilin reagent, 2.5mL of sulfuric acid (72 percent v/v) was added (0.25mL; 8 percent). It will be properly mixed and incubated for 10 minutes in a 60°C Water bath. The absorbance at 544 nm (UV visible spectrophotometer) was measured after incubation against a blank containing no extract. The standard calibration curve was made with aliquots of diosgenin (0.5mg/mL in 50% aqueous methanol). The total saponin concentration was given in milligrams of diosgenin equivalent (DE) per gram of dry weight (DW) (Olaoye *et al.*, 2021).

Determination of Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl)

The antioxidant activity of *T. catappa* leaf extract was evaluated using the free radical scavenging method DPPH

(2,2-diphenyl-1-picrylhydrazyl) according to the protocol with minor changes. The 180µL of 0.2 mm DPPH methanolic solution was prepared and mixed with 12µL of samples, ascorbic acid, and methanol (blank) for 30 minutes at 37°C in the dark on a microwell plate. The absorbance values for each combination were measured at 517nm after incubation. The proportion of radical scavenging activity (percent inhibitions) was calculated from the absorbance data using the formula below. (Hoque *et al.*, 2023)

$$\% \text{ of Inhibition} = \frac{A \text{ of Blank} - A \text{ of Sample}}{A \text{ of Blank}} \times 100$$

Where: A of Blank is the Absorbance of the control reaction (containing all reagents except the test sample). A of Sample is the Absorbance of the extracts/standard.

Nitric Oxide

Terminalia catappa and ascorbic acid extracts were prepared using 50% methanol at a concentration of 200mg/mL. The samples are then serially diluted with the same methanol to achieve 10, 20, 30, and 40 mg/mL concentrations.

Griess' reagent was made by combining equal parts of 2% sulfonamide, 5% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride right before use.

A volume of 50µL of 10mmol/L sodium nitroprusside in 0.1mmol/L phosphate buffer pH 7.4 was added to 50µL of the extracts made in 96-well plates, which were then incubated at 25°C for 180 minutes. After that, 100µL of Griess' reagent was added. Similarly, a control sample was made with an equal volume of methanol but no extract.

The absorbance values at 542nm were obtained, and the percentage of inhibition of the extract and ascorbic acid at various concentrations was determined using the same formula as the DPPH. The falling optical density readings indicate that nitric oxide radicals have a significant scavenging activity. (Akinyede *et al.*, 2022).

Statistical Examination

Data obtained from the study were summarized and expressed as mean ± standard error of mean. Data analysis was performed using Statistical Package for Social Science (SPSS), version 23.0. To compare the results obtained from different groups, one-way ANOVA followed by Duncan Multiple Range Test comparison tests were performed to determine statistical significance. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Extraction Yield of Green and Reddish-Brown Leaves of *Terminalia catappa*

Figure 1 shows Percentage yields of methanol and aqueous extracts of the Reddish-brown and green leaves of *Terminalia catappa*

Phytochemical Constituents of Green and Reddish-Brown Leaves of *Terminalia catappa*

The results of the phytochemical screening indicated the presence of flavonoids, polyphenols, alkaloids, and tannins (Table 1). Quantitatively, no significant

differences ($p > 0.05$) emerged in the flavonoids and polyphenols in methanol and in the aqueous extracts of the reddish-brown or green leaves of *T. catappa*; however, significant differences ($p < 0.05$) in alkaloids, tannins, and saponins were observed in the extracts of *T. catappa* leaf samples (Table 1).

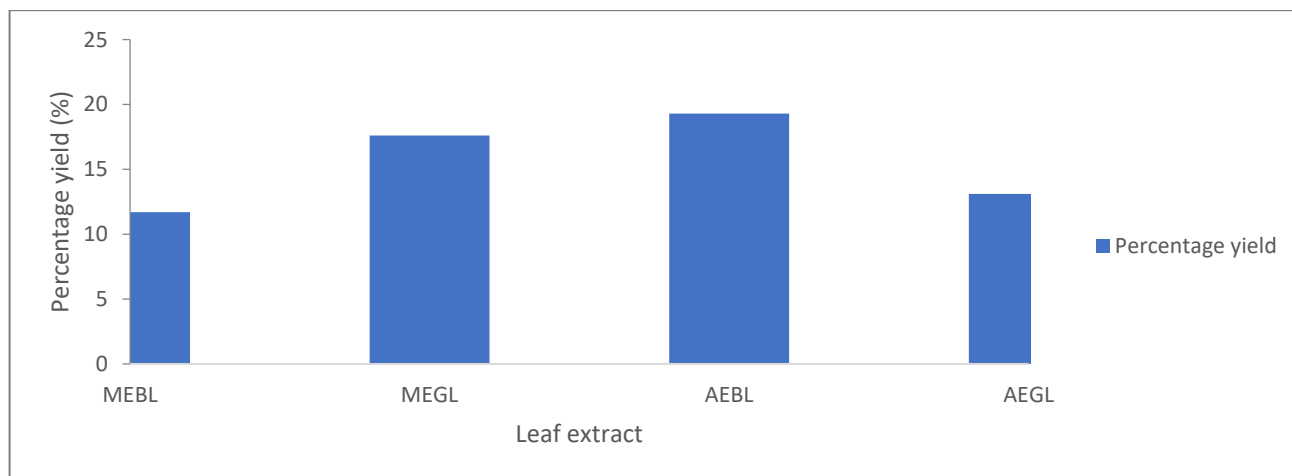


Figure 1: Percentage yields of methanol and aqueous extracts of the Reddish-brown and green leaves of *Terminalia catappa*. (MEBL: Methanol Extract of the Reddish-brown leaves of *T. catappa*; MEGL: Methanol extract of the Green leaves of *T. catappa*; AEBL: Aqueous extract of the Reddish-brown leaves of *T. catappa*; AEGL: Aqueous extract of the green leaves of *T. catappa*).

Table 1: Quantitative phytochemical constituents of the methanol and aqueous extracts of the Reddish-brown and green leaves of *Terminalia catappa*

Samples	Phyto-constituents (mg/g of extract)				
	Flavonoids	Polyphenols	Alkaloids	Tannins	Saponins
MEBL	96.4±9.07 ^a	100.0±0.00 ^a	14.3±0.02 ^a	90.4±0.07 ^b	152.2±1.85 ^a
MEGL	96.9±7.02 ^a	110.0±14.1 ^a	14.2±0.03 ^a	123.2±0.38 ^a	143.6±1.03 ^b
AEBL	102.4±11.8 ^a	105.0±7.07 ^a	13.9±0.02 ^b	74.9±0.73 ^c	110.2±1.06 ^c
AEGL	97.5±7.62 ^a	110.0±14.1 ^a	14.0±0.02 ^a	56.9±0.07 ^d	66.6±0.20 ^d

Values are given as mean±standard deviation; in each column, values with different superscripts have statistical significant difference ($p < 0.05$)

KEY: **MEBL**: Methanol Extract of the Reddish-brown leaves of *T. catappa*; **MEGL**: Methanol extract of the Green leaves of *T. catappa*; **AEBL**: Aqueous extract of the Reddish-brown leaves of *T. catappa*; **AEGL**: Aqueous extract of the green leaves of *T. catappa*.

Antioxidant Effect of Methanolic and Aqueous Extracts of Reddish-Brown and Green Leaves of *Terminalia catappa*

Radical Scavenging Action of DPPH: Radical scavenging activity standards were much higher than that of *T. catappa* leaf extracts, with a mean percentage inhibition of 2.68 ± 0.36 . No significant difference was seen among all the extracts' radical scavenging activity (Table 2). The methanol extract of *T. catappa*'s reddish-brown leaves had the highest DPPH radical scavenging activity, with a mean value of 0.55 ± 0.31 percent, and the aqueous extract of *T. catappa*'s green leaves had the lowest, with a mean inhibition of 0.24 ± 0.26 percent. *T. catappa*'s methanol extract of green leaves and aqueous extract of reddish-brown leaves had mean values of 0.48 ± 0.45 and 0.43 ± 0.46 percent, respectively (Table 2).

Assay for Nitric Oxide Reduction: Except for the aqueous Reddish-brown leaf extract, all three solvent extracts of *T. catappa* displayed concentration-dependent nitric oxide lowering action, with increasing activity linked

with graded extract concentrations (Table 3). At the highest concentration (40mg/mL) the methanol and aqueous green leaf extract value was 99.74 and 98.28 percent, respectively, while methanol and aqueous reddish-brown leaf extract were 36.34 and 52.28 percent, respectively. The results also showed that methanol and aqueous green leaf extracts had considerably higher ($p < 0.05$) nitric oxide lowering activities than methanol and aqueous reddish-brown leaf extracts at all doses examined (Table 3).

The plant leaves subjected to different extractants gave different yields, which could be attributable to differences in compound composition and polarity of the extraction solvent (Kuppusamy *et al.*, 2015). The presence of phytochemical constituents such as flavonoids, polyphenols, tannins, saponins and alkaloids in the leaves of *T. catappa* have also been documented by Kankia, (2014). High content of these bioactive compounds, except for alkaloids, were recorded. Past research has strongly encouraged long-term consumption of diets rich

in plant polyphenols because it could help in the prevention of several degenerative diseases.

Many studies have recorded that tannins are polyphenolic compounds that exhibit antioxidant, tissue repair, and antimicrobial properties (Vasconcelos *et al.*, 2010). Flavonoids are made up of 4000 compounds out of 8000 known polyphenolic compounds that are water soluble and anti-allergen, anti-oxidative, anti-microbial, and anti-inflammatory. Alkaloids from *Lotus plumules* have been recorded to have hepato-protective efficiency (Liu *et al.*, 2019). Saponins have been seen from past research to control dietary hypercholesterolemia, suppress colon cancer cell proliferation, prevent peroxidation of lipids, and protect the liver by accelerating the secretion of thyroid hormones (Sayama *et al.*, 2012).

Table 2: DPPH radical scavenging activities of the methanol and aqueous extracts of the Reddish-brown and green leaves of *T. catappa*

Samples	% inhibition
DPPH	2.68±0.36 ^a
MEBL	0.55±0.31 ^b
MEGL	0.48±0.45 ^b
AEBL	0.43±0.46 ^b
AEGL	0.24±0.26 ^b

Values are given as mean±standard deviation; in each column, values with different superscripts have statistical significant difference ($p < 0.05$)

KEYS: **DPPH**: 2, 2-diphenyl-1-picrylhydrazyl; **MEBL**: Methanol Extract of the Reddish-brown leaves of *T. catappa*; **MEGL**: Methanol extract of the Green leaves of *T. catappa*; **AEBL**: Aqueous extract of the Reddish-brown leaves of *T. catappa*; **AEGL**: Aqueous extract of the green leaves of *T. catappa*;

Table 3: Nitric oxide reducing activity of the methanol and aqueous extracts of the Reddish-brown and green leaves of *T. catappa*

Conc. (mg/mL)	MEBL	MEGL	AEBL	AEGL
10	0.106 ^d	43.23 ^b	12.43 ^c	40.74 ^b
20	16.22 ^c	98.25 ^a	10.19 ^d	98.33 ^a
30	23.59 ^b	98.36 ^a	38.02 ^b	98.07 ^a
40	36.34 ^a	99.74 ^a	52.28 ^a	98.28 ^a

Values are given as percentage inhibition. Values with different superscripts in each column have statistically significant differences ($p < 0.05$).

Key: **MEBL**: Methanol Extract of the Reddish-brown leaves of *T. catappa*; **MEGL**: Methanol extract of the Green leaves of *T. catappa*; **AEBL**: Aqueous extract of the Reddish-brown leaves of *T. catappa*; **AEGL**: Aqueous extract of the green leaves of *T. catappa*

Free radical scavenging activity of the plant is similar to the reducing ability which is attributable to different quantities of the plant's phytochemicals constituents (Akinyede *et al.*, 2022). Generally, the antioxidant activities of plant extracts are attributed to the constituents of total phenolic, total flavonoid, and total antioxidant capacity. The analysis of the antioxidant activity showed that the DPPH scavenging activities of the methanol extract of *T. catappa* leaves were higher than that of the

aqueous extract, and this agrees with earlier publication reported by Ebrahimzadeh *et al.* (2009) for the free radical scavenging activity of *Hydroxyannmus squareous*, and by Omenna, (2015) on the antioxidant activity of almond leaves of *T. catappa* prove the efficacy of the solvent. Muhie and Endalew (2023), reported similar results on *in vitro* antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. In their study, the methanolic extract of the plant had the highest scavenging activities among other extracts used.

Sodium nitroprusside reacts with oxygen to produce nitric oxide, and nitrite scavenges free radicals through diazotisation with a sulphanilamide acid coupled reaction, giving rise to a pink colour (Akinyede *et al.*, 2022). The Nitric Oxide assays' antioxidant activities involved the donation of protons to the nitrite radicals that resulted decrease in absorbance. In this study, the Nitric Scavenging activity of the green leaves of *T. catappa* was higher than that of the reddish-brown leaves, and this agrees with Olaoye *et al.* (2021) in their study analyses of antioxidants of *Moringa oleifera* leaf extracts from southwestern states in Nigeria. Their results revealed a concentration-dependent free radical scavenging activity of the plant extracts. Following the decrease in absorbance observed, points out the extent of nitrite radical scavenging potentials, which could be attributed to components such as flavonoids, as reported in Akinyede *et al.* (2022).

CONCLUSION

The green and reddish-brown leaf extracts of *T. catappa* contain high Polyphenols, Flavonoids, Tannins, Saponins and low Alkaloids. The DPPH radical scavenging activity ability of the Green and Reddish-brown leaves extract of *T. catappa* are not significantly different, and the Green leaves of *T. catappa* demonstrated higher Nitric oxide scavenging activity than the Reddish- brown leaves. This suggests that both the Green and Reddish-brown leaf extracts of *T. catappa* are both effective oxidants since the scavenging activity and Nitric Oxide assay of *T. catappa* leaf extracts (Aqueous and Methanol) revealed no significant difference.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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