Evaluation of the Nutritional Composition, Antioxidant Properties, and α-Amylase Inhibitory Activity of Methanol Leaf Extracts of *Terminalia catappa* and *Ocimum gratissimum*

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Abstract
This study comparatively evaluated the nutritional composition, phytochemical constituents, in vitro antioxidant properties, and α-amylase inhibitory activities of methanol leaf extracts of Terminalia catappa and Ocimum gratissimum. Standard procedures were used for proximate and phytochemical analyses, while spectrophotometric methods assessed total flavonoid and phenolic contents, antioxidant capacity (via DPPH, TAC, FRAP, and reducing power assays), and α-amylase inhibitory activity. Proximate analysis showed no significant difference ($p > 0.05$) in crude fiber and total carbohydrate contents between the two extracts. T. catappa exhibited significantly higher ($p < 0.05$) total phenolic content and total antioxidant capacity, while total flavonoid content showed no significant variation between the extracts. The DPPH radical scavenging activity was concentration-dependent, with T. catappa demonstrating a lower IC_{50} value (1.35 ± 0.16 mg/mL), indicating stronger antioxidant potency compared to O. gratissimum (2.65 ± 0.17 mg/mL). However, O. gratissimum displayed superior α-amylase inhibitory activity in a dose-dependent manner, suggesting potential for glycemic control. These findings highlight the therapeutic potential of both plants: T. catappa as a promising antioxidant source and O. gratissimum as a candidate for antidiabetic intervention.

Keywords: *Terminalia catappa, Ocimum gratissimum, Antioxidant activity, α-Amylase inhibition*

INTRODUCTION

For millenia, plants have been used for their therapeutic properties to treat various diseases (Ateş and Yalçın, 2022). According to a report, there are between 35,000 to 70,000 plant species that have been used for medicinal purposes in the world (Amjad *et al.*, 2020). The basis of all extractions in medicinal plant research is to isolate the dissolvable plant metabolites, excluding the insoluble cellular marc known as the residue (Fotsing *et al.*, 2022). The basic crude extract obtained contains a complex blend of numerous plant metabolites, for example, alkaloids, glycosides, phenolics, terpenoids and flavonoids, and has a potential for utilization in oral or external applications for disease treatment (Abubakar and Haque, 2020).

Diabetes mellitus has become a major global health concern due to its rapidly increasing prevalence. It is a metabolic disorder that causes chronic hyperglycemia and glucose intolerance because of either endogenous insulin shortage, a reduced ability of insulin to act, or both (Antar *et al.*, 2023). Since the body can not manage the level of sugar in the blood,

individuals with diabetes suffer from side effects, such as frequent urination (polyuria), unquenchable thirst (polydipsia), weight reduction, tiredness, increased hunger, extremely dry skin and slow healing of wounds (Chang, 2022). The International Diabetes Federation (IDF) projects that the number of adults aged 20-79 living with diabetes worldwide will surge from 589 million (11.1% of this age group) in 2025 to 852.5 million by 2050, with low- and middle-income countries expected to bear 95% of this increase due to rapid population growth (International Diabetes Federation, 2025).

In Nigeria, type 2 diabetes mellitus has become very common.While the International Diabetes Federation (IDF) noted a prevalence rate of 3.7% in 2019, a recent national meta-analysis found a considerably higher overall prevalence of 7.0% (95% CI: 5.0–9.0%) among adults in Nigeria. Having more than 120,000 participants, this study revealed regional variations; the North-Central zone had the lowest at 2.0%, while the South-South zone had the highest frequency at 11.4%. Among the noted primary risk factors include obesity (BMI > 25 kg/m²), urbanization, physical inactivity, and dietary habits typified by low vegetable intake (Olamoyegun *et al*., 2024).
Numerous hypoglycemic medicines exist for diabetes treatment,although many of these synthetic agents have negative consequences like low efficacy, high cost, and unpleasant side effects (Karau *et al*., 2012; Dhyani *et al*., 2025). Investigating reasonably priced, readily available, safer plant-based medicines is thus,becoming more crucial.

Terminalia catappa and *Ocimum gratissimum* have been used to treat diabetes and associated diseases in many cultures.Studies have shown that the aqueous leaf extracts of *Terminalia catappa* and *Ocimum gratissimum* possess antioxidant and hypoglycemic activities (Sharma *et al.*, 2022; Ramanan *et al.*, 2024). *Ocimum gratissium*, locally known as “Scent leaf” belongs to the family *Lamiaceae*. It is also called clove basil and is a local of tropical Africa, Asia and South America (Ugbogu *et al.*, 2021). Its different names include *Nchuanwu* in Igbo, *Daidoya* in Hausa and *Efinrin* in Yoruba (Onwudiwe *et al.*, 2025). In Nigeria, *O. gratissimum* has been used traditionally to treat various diseases such as epilepsy, diarrhea, fever, headache, cough, kidney infection, gastric ulcer and conjunctivitis (Edo *et al.*, 2023). It is rich in phytochemicals such as alkaloids, tannins, flavonoids, saponins, terpenoids and reducing sugars and steroid (Offor *et al.*, 2025).

Terminalia catappa also known as Indian/tropical almond or *ebelebo* (Nigeria) is one of the species of catappa belonging to the *Combretaceae* family. It is native to Southeast Asia and grows well in subtropical and tropical climate regions including Nigeria (Iyekowa *et al*., 2023). The leaves of *T. catappa* are rich in bioactive compounds, including antioxidants, flavonoids such as kaempferol, as well as tannins, phenols, polyphenols, alkaloids, steroids, cardiac glycosides, saponins, and coumarins (Mwangi *et al.*, 2024; Ramanan *et al.*, 2024). The leaves are used traditionally in the treatment of several diseases because they possess antimicrobial, antiparasitic, antibacterial, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer properties (Sirat and Senjaya, 2025).

Given the increasing burden of diabetes in Nigeria and the potential of plant-based remedies to offer alternative management strategies, this study aims to compare the nutritional composition, phytochemical contents, *in vitro* antioxidant activities, and α-amylase inhibitory properties of methanol leaf extracts of *Terminalia catappa* and *Ocimum gratissimum*.

MATERIALS AND METHODS.

Collection of plant materials.

T. catappa leaves and *O. gratissimum* were collected from Benson Idahosa University campus, Ugbor, Edo State. Taxonomists at the University of Benin, *Faculty of Life Sciences*, identified the plants, with voucher numbers USH-T258 for *T. catappa* and UBH-O341 for *O. gratissimum.*Voucher specimens of the plants were deposited at the Herbarium for future reference.

Preparation of extracts.

Fresh leaves of *Terminalia catappa* and *Ocimum gratissimum* were air-dried in the laboratory. The dried leaves were then crushed into crumbs by hand. One hundred grams (100g) of the dried form of the plant materials were extracted by soaking in 1000 mL of methanol for 72 hours (3 days). Stirring was done twice daily, and the supernatant was obtained by filtering with a muslin cloth at the end of the third day. The extracts were concentrated in a rotatory evaporator, stored in a well-closed container and kept in the refrigerator at 4^{o}C to protect against sunlight and moisture for subsequent use (Sutharson *et al*., 2007).

PROXIMATE ANALYSIS.

Determination of % crude fibre content.

Five grams (5g) of dried leaf sample was weighed and set in a 1-litre conical flask. A 150 mL pre-heated 0.128M H_2SO_4 was included, and the substance was boiled for 30 minutes. The substance was separated through the fluted funnel, and the residue was washed multiple times with heated water. To the digest, 150 mL pre-heated 0.15M KOH was included, and heated to a boil. A few drops of antifoaming agent (n-octanol) were included. The substance was boiled for 30 minutes, filtered, and the residue washed thrice with boiling water, followed by washing 3 times with acetone. The resulting residue was dried in an oven at 130^{o}C for 1 hour, cooled in a desiccator and weighed, and after that, ashed at 500^{o}C for 30 minutes, cooled in a desiccator and later weighed using a weighing balance (Lemus-Mondaca *et al*., 2016). The percentage of crude fibre was calculated as follows:

% crude fibre = $\frac{W2-W3 \times 100}{W1}$

Where W_1 = Sample weight before drying, W_2 = Weight of residue after drying, and W3 = residue after ashing.

Total Carbohydrate Content

Total carbohydrate content was determined using the anthrone method. Carbohydrates are dehydrated with concentrated sulfuric acid to form Furfural, which condenses with anthrone to form a green color complex, which can be measured using a spectrophotometer at 620nm. Then, 0.5 mL of the sample was measured in a test tube, and 2 mL of distilled water was added. Immediately, 4 mL of Anthrone reagent (0.2% in concentrated sulfuric acid) was added. The test tubes were covered with foil paper, boiled for 10 minutes at 100^{o}C in a water bath, then cooled at room temperature, and the absorbance was read at 620nm. Glucose (1mg/mL) was used as standard. Blank was prepared using only 2.5 mL of distilled water and 4 mL of Anthrone reagent (Ohemeng-Ntiamoah and Datta, 2018). Total carbohydrate was calculated as:

$$\frac{\text{Abs of sample}}{\text{conc. of sample}} = \frac{\text{Abs of standard}}{\text{conc. of standard}}$$

$$C_{\text{sample}} = \frac{A\,(\text{sample}) \times C(standard)}{A(standard)}$$

QUANTIFICATION OF PHYTOCHEMICALS

Determination of Total Phenol Content.

One milliliter (1mL) of aliquots and standard (gallic) acid (100, 200, 400, 600, 800, 1000µg/ mL) was measured into test tubes and 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent were added. After 5 minutes, 1.5 mL of 20% sodium carbonate was added. It was made up to 10 mL with distilled water. It was incubated for 2 hours at room temperature. A dark blue colouration was noticed. After incubation, absorbance was read at 750nm with a spectrophotometer. The standard was performed in triplicate. Gallic acid was used as a standard (Patel *et al*., 2010; Bhalodia *et al*., 2011).

Determination of Total Flavonoid Content.

One milliliter (1 mL) of an aliquot and 1 mL of standard quercetin solution (100, 200, 400, 600, 800, 1000µg/ mL) were measured into test tubes, and 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution were also added to each. After 5 minutes, 0.3 mL of 10% aluminum chloride was added. At the 6^{th} minute, 2 mL of 1M sodium hydroxide was added. The volume was created up to 10 mL with distilled water and mixed well. An orange-yellowish colour was noticed. The absorbance was read at 510nm by using a spectrophotometer. A blank experiment was carried out with distilled water. Quercetin was used as a standard. The experiments were carried out in triplicate (Satish-Kumar *et al*., 2008; Patel *et al*., 2010; Pallab *et al*., 2013).

ANTIOXIDANT ANALYSIS

DPPH (1, 1-Diphenyl-2-Picryl Hydroxyl) Radical Scavenging Capacity.

The free radical scavenging activities of the plant extracts were determined by the Brand Williams *et al* (1995) method. The absorbance was measured at 517nm. Briefly, 0.5 mL of 0.3 mM DPPH was added to different concentrations of leaf extract (0.4, 0.8, 1.0, 1.6, 2.0 mL) and was made up to 2 mL with methanol. The test tubes were then shaken and incubated at room temperature for 15 minutes. All tests were performed in triplicate, and ascorbic acid was used as a standard. Blank solution was made using 0.5 mL of 0.3 mM DPPH and 2 mL of methanol. The radical scavenging activity was calculated using the formula:

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

Where A_0 = the absorbance of DPPH radical + methanol, A_1 = the absorbance of DPPH radical + sample extract.

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.

Determination of Reducing Power

Different (0.2-1.0 mL) concentration of extracts in methanol solvent were mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 mL of 10% tri-chloroacetic acid was added and centrifuged at 3000rpm for 10minutes whenever necessary. The supernatant (2.5 mL) of the solution was mixed with 2.5 mL distilled water and 0.5 mL freshly prepared ferric chloride solution. The absorbance was measured at 700nm. Control was prepared in a similar manner excluding samples. Ascorbic acid at same concentrations of the sample was used as standard. Increased absorbance of the reaction mixture indicates an increase in reducing power (Jayanthi and Lalitha, 2011).

Ferric Reducing Antioxidant Power Assay.

The ferric reducing antioxidant power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). To 1.5 mL of freshly prepared FRAP solution [25 mL of 30mM acetate buffer pH 3.6, 2.5 mL of 10mM 2, 4, 6-tripyridyls- triazine (TPTZ) in 400mM HCL, and 2.5 mL of 20mM ferric chloride ($FeCl_3.6H_2O$) solution] was added to 1 mL of the leaf extracts (1mg/mL) and standard at concentrations of 100-600µM. The reaction mixtures were incubated at 37°C for 30 minutes and the increase in absorbance at 593nm was measured. $FeSO_4$ was used for standardization curve and ascorbic acid was used for control. FRAP values (expressed as mg Fe (II)/g of the extracts) for the extracts were then calculated from the standard curve.

Determination of Total Antioxidant Capacity.

Total antioxidant capacity (TAC) was estimated by the phospho- molybdenum assay (Prieto *et al*., 1999). One milliliter of the extracts (1mg/ mL) was added to 3 mL of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 minutes. After incubation, these tubes were normalized to room temperature for 20-30minutes, and the absorbance of the reaction mixture was measured at 695nm. Ascorbic acid was used as the standard.

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Anti-diabetic assay

***In vitro* Inhibitory α-amylase Assay.**

In vitro α-Amylase inhibition was determined using the method described by Sangeetha and Vedasree (2012). Test extract (0.1 mL) was allowed to react with 0.2 mL of alpha amylase enzyme and 0.1 mL of 2 mM phosphate buffer (pH 6.9). After 20 minutes of incubation, 0.1 mL of 1% starch solution was added. The same was performed for the control, where 0.2 mL of the enzyme was replaced with buffer. After incubation for 5 minutes, 0.5 mL of DNSA (3, 5 5-dinitrosalicylic acid) reagent was added to the control and test, and they were kept in the boiling water bath for 5 minutes. The absorbance was recorded at 540nm using a spectrophotometer, and the percentage inhibition of α-Amylase was calculated as:

$$\text{Inhibition (\%)} = 100 \left(\frac{\text{control}-\text{test}}{\text{control}}\right)$$

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Fisher's pairwise comparison. The probability of $p < 0.05$ was considered significant.

RESULTS

Proximate Composition

The proximate composition results (Figure 1.0) showed no significant difference ($p > 0.05$) in crude fiber and total carbohydrate content between *Terminalia catappa* and *Ocimum gratissimum* leaves. However, *O. gratissimum* had a relatively higher carbohydrate content, while *T. catappa* had a slightly higher crude fibre content.

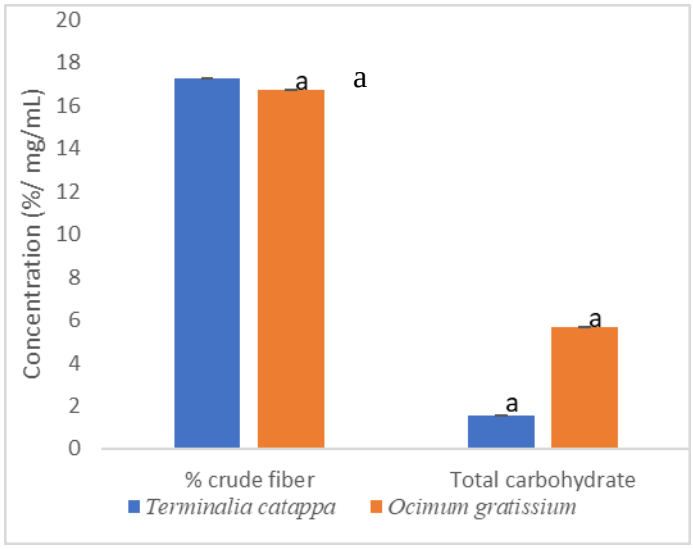

Figure 1.0: Crude fibre and Total Carbohydrate Content of *Terminalia catappa* and *Ocimum gratissimum* leaves.

Phytochemical Quantification

Figure 2.0 presents the total phenolic and flavonoid contents of the extracts. *T. catappa* had significantly higher ($p < 0.05$) total phenol content (0.606 ± 0.060 mg GAE/g) than *O. gratissimum* (0.292 ± 0.010 mg GAE/g). No significant difference ($p > 0.05$) was observed in the total flavonoid content between the extracts.

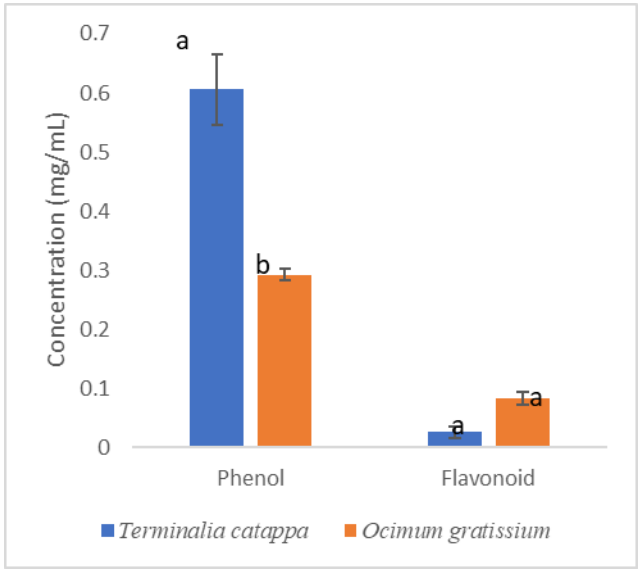

Figure 2.0: Total phenol and total flavonoid content of *Terminalia catappa* and *Ocimum gratissimum* leaf extracts.

Phenol is expressed as mg gallic acid Equivalent (GAE) /g extract, and flavonoid is expressed as mg quercetin Equivalent (QE) /g extract.

Antioxidant activity

Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP)

The TAC and FRAP results (Figure 3.0) showed that *T. catappa* exhibited significantly ($p < 0.05$) higher antioxidant capacity than *O. gratissimum.*

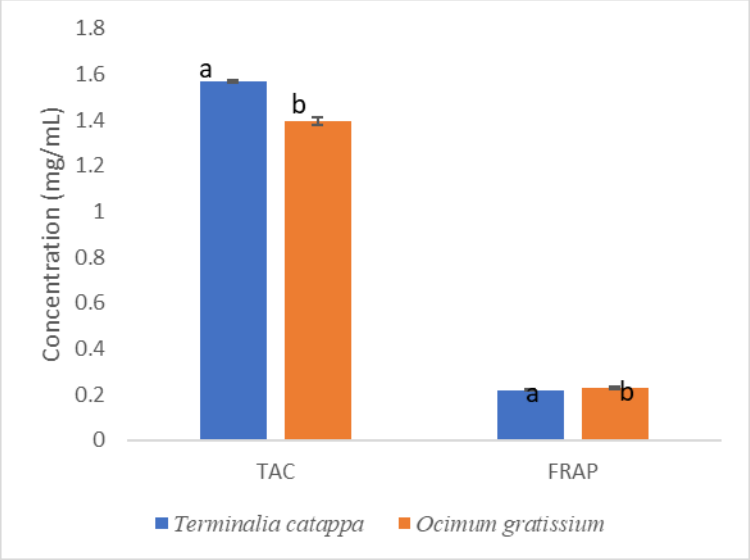

Figure 3.0: TAC and FRAP Analysis of Methanol Extracts of Terminalia catappa and Ocimum gratissimum.

DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) Radical Scavenging Activity.

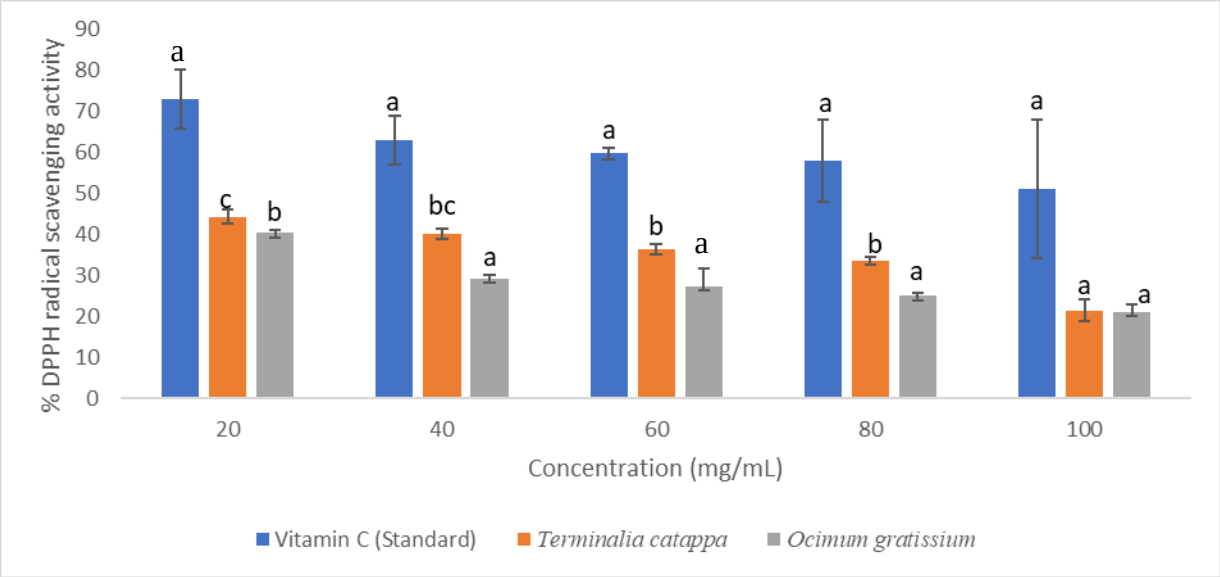

Figure 4.0: DPPH's Radical Scavenging capacity of Extracts of *Terminalia catappa* and *Ocimum gratissimum* Leaf extracts.

As shown in Figure 4.0, both extracts demonstrated concentration-dependent DPPH radical scavenging activities. *T. catappa* had a lower IC_{50} (1.346 ± 0.157 mg/mL) than *O. gratissimum* (2.653 ± 0.174 mg/mL), indicating higher antioxidant potency. Vitamin C showed the highest activity across all concentrations**.**

Table 1.0: IC_{50} values of Vitamin C, *Terminalia catappa* and *Ocimum gratissimum*

	IC_{50} Value (mg/mL)
Vitamin C	3.114 ± 0.973
Terminalia catappa	1.346 ± 0.157
Ocimum gratissimum	2.653 ± 0.174

Reducing Power Assay

Figure 5.0 shows the reducing power of both extracts. There was no significant difference ($p > 0.05$) in reducing power between the extracts and the standard (ascorbic acid), although the activity was dose-dependent.

DISCUSSION

Carbohydrates are the primary source of energy the body consumes (Campos *et al*., 2022).
In terms of nutritional composition, the absence of significant differences in crude fiber and total carbohydrate content between *T. catappa* and *O. gratissimum* underscores their comparable dietary value. However, the slightly higher carbohydrate content in *O. gratissimum* positions it as a valuable source of slow-digesting energy, especially beneficial for diabetic and obese individuals (Olumide *et al*., 2019).
Conversely, the higher crude fiber content in *T. catappa* may enhance gastrointestinal function and reduce the risk of metabolic disorders such as hyperglycemia and hypercholesterolemia (Oluwole *et al*., 2019).Both leaves exhibited a good content of crude fiber; though *T. catappa* had the greater concentration.

The most abundant antioxidants in plants, phenolic compounds have been proven to be crucial in scavenging free radicals and thereby lowering oxidative damage. Consistent with past results by Suryavanshi *et al*., (2019), and Ibeabuchi *et al*. (2023), *T. catappa* had a considerably greater total phenol content than *O. gratissimum*. The presence of these phenolic molecules greatly enhance the antioxidant action of plant extracts.

Also serving several biological purposes including anti-inflammatory, cardioprotective, and antidiabetic actions, flavonoids are polyphenolic antioxidants recognized for scavenging free radicals.The two plants leaves had almost same flavonoid concentration. Both species have flavonoids present as reported also by Moneme *et al*. (2025) and Nnaoma (2024).

Using TAC and FRAP tests measuring electron-donating capacity, *T. catappa* exceeded *O. gratissimum* in antioxidant assessments. These results, which match those of Mwangi *et al.* (2024) and Annegowda *et al.* (2010),suggests that *T. catappa*'s antioxidant ability increases simultaneuosly with its phenolic content , however this generalization may not be true for all plants. This is because different plants have varying amounts of phytochemicals.

The standard (Vitamin C), exhibited the highest DPPH radical scavenging activity test activity at all doses.The DPPH scavenging in the plant leaf extracts, *T. catappa* exceeded *O. gratissimum*. The low IC50 value of *T. catappa* underlines its great antioxidant capacity (Sadeer *et al*., 2020).High phenolic and flavonoid content has been linked to antioxidant properties, Ibeabuchi *et al*. (2023) and Mwangi *et al*. (2024)

The reducing power test, which also gauges antioxidant activity, showed dose−dependent findings in both extracts. The leaf extracts and the standard (ascorbic acid) showed similar antioxidant activity and no appreciable variations.

Whereas *O. gratissimum* α -amylase inhibition potential grew with increasing concentrations, *T. catappa*'s inhibition of α -amylase decreased with increasing concentration. Given its potential to be more effective in preventing carbohydrate digestion, *O. gratissimum* appears appropriate for glycemic control. These results align with Sharma *et al*. (2022), whereas another study by Iheagwam *et al*. (2019) indicated concentration-dependent inhibition by *T. catappa* leaves.

The antioxidant advantage of *T. catappa* is thus supported by its higher phenolic load, while *O. gratissimum* excels in α -amylase inhibition−a key enzyme in carbohydrate digestion. This suggests that *O. gratissimum* could be more effective in glycemic regulation. This is consistent with *in vivo* findings by Iyare *et al.* (2018), who demonstrated that this plant leaves significantly attenuates postprandial hyperglycemia without inducing hypoglycemia.

Collectively, these findings highlight the nutritional and therapeutic potential of both plants. *T. catappa* may serve as a potent antioxidant supplement, while *O. gratissimum* shows promise in managing type 2 diabetes through its enzyme-inhibitory effects. Including these plants leaves in functional meals or herbal formulations might help to prevent or manage metabolic diseases brought on by glucose metabolism problems and oxidative stress.

CONCLUSION

This study provides a comparative insight into the nutritional composition, phytochemical constituents, antioxidant potential, and α-amylase inhibitory activities of methanol leaf extracts of *Terminalia catappa* and *Ocimum gratissimum*. The findings reveal that *T. catappa* possesses higher total phenolic content and more antioxidant properties. At the same time, *O. gratissimum* exhibits more effective α-amylase inhibitory activity, suggesting its potential use in glycemic control. These results affirm both plants' therapeutic potential in managing oxidative stress-related disorders and type 2 diabetes mellitus. Given the rising prevalence of diabetes in Nigeria, particularly in urban and high-risk populations, these plants could serve as accessible, affordable, and natural sources for developing functional foods or phytomedicines for disease prevention and management.

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