In vitro antidiabetic and antityrosinase activities of methanolic extracts of four nigerian medicinal plants: A kinetic study

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Abstract

Medicinal plants provide a potential source of enzyme inhibitors to aid in diabetes and hyperpigmentation management. This research assessed the *in vitro* antidiabetic and antityrosinase effects of methanolic extracts from four Nigerian plants: *Euphorbia graminea*, *Berlinia* sp., *Tabernaemontana pachysiphon*, and *Anacardium occidentale*. Standard chromogenic tests were used to test the inhibition of diabetic enzymes (α-glucosidase and α-amylase) and the inhibitory effects on tyrosinase were monitored at 30 minutes. *Berlinia* sp. at 100 µg/mL demonstrated the highest inhibition of α-glucosidase inhibition at 83.46 ± 4.12%, like the 91.12 ± 2.34% inhibition seen with acarbose. It also showed moderate α-amylase inhibition at 66.59 ± 5.01%. *Euphorbia graminea* showed selective inhibition of α-amylase with 68.39 ± 4.87%. The inhibition of tyrosinase activity was moderate, with *Berlinia* sp. and *E. graminea* exhibiting 55.04 ± 3.98% and 53.90 ± 4.21% inhibition, respectively, both significantly less active than kojic acid (99.39 ± 1.02%). Different time-dependent inhibitory patterns were observed by kinetic analysis, indicating different modes of interaction with the enzyme. These results demonstrate the dual inhibition property of *Berlinia* sp. and reinforce the traditional uses of these plants, highlighting the potential for bioassay-guided extraction of bioactive compounds.

Keywords: Medicinal Plants; Enzyme Inhibition; Antidiabetics; Hyperpigmentation; Kinetic

1. Introduction

Nature has offered different medicinal products for many years, and several effective medicines are derived from plant species (Newman and Cragg, 2020). They have been explored for their therapeutic properties in multiple traditional healing systems and have been recognized as biological modulators over time throughout human history (Harvey *et al*. 2015). The efficacy of secondary metabolites, for example enzyme inhibitors, is based on their being biosynthetic products produced in living organisms, which boost the likelihood of interaction with therapeutic targets (Lahlou, 2013). Natural products display more rings and chiral centers than synthetic ones and contain more oxygen and can form more hydrogen bonds, which make them more drug-like than synthetic drugs (Ortholand and Ganesan, 2004; Ganesan, 2008).

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Diabetes mellitus (DM), a non-communicable disease, is a severe, chronic and complex metabolic condition that can cause serious consequences, loss of functionality and failure of several vital organs (Arumugam *et al*., 2013; Duarte *et al*., 2020). The population diagnosed with diabetes mellitus (DM) is constantly growing, reaching up to and even surpassing 600 million by 2035 as per predictions (WHO, 2021). Type 2 diabetes mellitus (T2DM) is the prevalent type of diabetes, with about 90% of all cases of diabetes being this type (WHO, 2021). T2DM is characterised by elevated blood sugar levels, decreased glucose tolerance, insulin resistance, and high lipid levels, resulting from the ineffective utilization of insulin (Mohanapriya *et al*., 2016; Duarte *et al*., 2020; Rehman *et al*., 2020). This is because obesity and inactivity are the main factors that increase T2DM and are linked to a reduced quality of life, and an higher risk of death and morbidity (WHO, 2021). People, families, health systems and countries are greatly impacted (WHO, 2021).

Inhibition of the digestion of dietary carbohydrates, especially starch to monosaccharides in the digestive system, is a viable way to manage postprandial hyperglycaemia in type 2 diabetes mellitus (T2DM), and α-amylase is an important enzyme. These are then decomposed into the glucose by the action of α-glucosidases, which after digestion is absorbed into the blood. Enzymes such as α-amylase and α-glucosidase play a role in reducing the digestion of carbohydrates, delaying the absorption of glucose and hence lowering the blood glucose level (Kajaria *et al*., 2013; Duarte *et al*., 2020). Acarbose, voglibose, and miglitol are examples of pharmaceutical inhibitors of α-glucosidase and α-amylase which are known to inhibit these enzymes, but they come with several adverse effects like bloating, abdominal discomfort, diarrhea, and flatulence (Chiasson *et al*., 2002; Van De Laar *et al*., 2005; Derosa and Maffioli, 2012; Dirir *et al*., 2022).

Melanin is a pigment that provides important photoprotection from the ultra-violet radiation (UV) damage and also serves as an important protective mechanism against elements (Boo, 2020). While melanin offers several advantages, it also can lead to conditions such as hyperpigmentation and melanoma (Pohntadavit *et al*., 2024). Increased melanin production and accumulation is characteristic of various skin problems such as age spots, freckles, and melasma (Skoczyńska *et al*., 2017). Tyrosinase is an important regulatory enzyme that performs a crucial role in the melanin biosynthesis and pigmentation in mammals (Zaidi *et al*., 2016). Tyrosinase inhibitors have been used in cosmetics to treat epidermal hyperpigmentation (Zengin *et al*., 2019). The application of cosmetics that contain synthetic tyrosinase inhibitors (hydroquinone and kojic acid) has been

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linked with a variety of undesirable effects such as post-inflammatory pigmentation, dermatitis, ochronosis and skin cancer (Garcia-Jimenez *et al*., 2017; Oiso *et al*., 2017; Pillaiyar *et al*., 2017).

While there are many existing studies that show that natural compounds have antidiabetic properties, there are still many gaps in literature. First, there is a lack of comparative studies among the many plants to be able to quantify their effect. Secondly, the medicinal use of plants regarding metabolic and dermatological treatment has not yet been extensively studied. The enzyme inhibition kinetics are not routinely studied, limiting the knowledge of the *in vitro* behavior and interactions with co-administered drugs. This study examines the gaps in existing research by conducting a comparative analysis of the *in vitro* antidiabetic and anti-tyrosinase properties of four medicinal plants. The major objective is to evaluate enzyme inhibition kinetics of this extract, with relevance to both diabetes and skin hyperpigmentation.

2. Methodology

2.1 Plant collection and identification

Four specific medicinal plant specimens were sourced from Osin-Ikole Road (located at latitude 7.8036° N and longitude 5.4175° E), Ekiti State, Nigeria. The Voucher specimens were identified by Mr. Felix Omotayo (Taxonomist, EKSU), and a copy of each plant material has been deposited at the Herbarium Unit of the Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University. The Herbarium Numbers are comprehensively documented in Table 1.

Table 1. Identification of plant materials

Plant code	Plant name	Herbarium number
11	*Euphorbia graminea*	UHA/2024/011
13	*Berlinia* sp	UHA/2024/013
17	*Tabernaemontana pachysiphon*	UHA/2024/017

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37	*Anacardium occidentale*	UHA/2024/037

2.2. Extraction of plant materials

The powdered plant materials (1.0 g) were extracted with methanol (250 mL, m/v) at room temperature (25 °C) for a period of 72 hours. The methanolic extract was then evaporated to dryness using a rotary evaporator maintained at 40 °C. The methanolic crude extract was kept in an airtight container at room temperature. The percentage yields for each plant extract are shown in Table 2.

Table 2. Percentage (%) yield of selected Plant Extracts

Plant code	% Yield
11	71.4
13	52.3
17	30.7
37	49.3

2.3. Preparation of buffers for diabetics and skin enzymatic assays

2.3.1. α-Glucosidase inhibitory assay

The α-glucosidase inhibitory activity of four selected medicinal plant extracts was evaluated using a standard method, with minor modifications (Thengyai *et al*., 2020). In a 96-well plate, the reaction mixture was prepared by combining 50 µL of phosphate buffer (100 mM, pH 6.8), 10 µL of α-glucosidase (1 U/mL), and 20 µL of different concentrations of each of the four selected medicinal plant extracts. This mixture was pre-incubated at 37 °C for 15 minutes. Subsequently,

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20 µL of p-NPG (5 mM) was introduced as a substrate and incubated at 37 °C for an additional 20 minutes. The reaction was halted by the addition of 50 µL of sodium carbonate (Na_2CO_3) at a concentration of 0.1 M. The absorbance of the released p-nitrophenol was measured at 405 nm using a Multiplate Reader (Multiskan Thermo Scientific, version 1.00.40, Vantaa, Finland). Acarbose was added at different concentrations as a standard. Each study was conducted in triplicate. The findings were presented as a percentage of inhibition, determined as follows:

Inhibitory Activity (%) = (1 − A/B) ×100 ……. Equation (1)

where A is the absorbance of the test substance and B is the control absorbance.

2.3.2. α-Amylase inhibitory assay

A standard protocol was employed in which 50 µL of phosphate buffer (0.01 M, pH 6.9), 20 µL of porcine pancreatic α-amylase (2 U/mL) solution, and 20 µL of the samples were mixed in a 96-well plate and incubated for 20 minutes at room temperature. Following the pre-incubation period, 20 µL of 1% soluble starch was added to the mixture and incubated for an additional 30 minutes. This was instantly followed by the addition of 90 µL of 3,5-dinitrosalicylic acid (DNS), which stopped the reaction. The reaction mixture was then incubated in a boiling water bath for 10 minutes prior to measuring the absorbance at 540 nm. Acarbose served as the standard, and each experiment was carried out in triplicate. Equation (2) was used to determine the percentage inhibition of α-amylase activity.

Percentage inhibition (%) = (C – T)/C x 100……. Equation (2)

where C and T are absorbance readings of the control and the treated sample, respectively.

2.4. Kinetics and end point determination of tyrosinase inhibition

This assay was conducted following the method outlined by Chompo *et al*. (2012) and Vardhan *et al*. (2014), with minor modifications applied. Samples were dissolved in DMSO (dimethyl sulphoxide) to create a stock solution with a concentration of 1 mg/mL (w/v). Subsequent dilutions were performed using a 50 mM sodium phosphate buffer at pH 6.5 for all working solutions,

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achieving a concentration of 100 µg/mL. Kojic acid served as the control drug in the study. In the wells of a 96-well plate, 70 µL of each sample working solution was mixed with 30 µL of tyrosinase (from mushroom, 500 Units/mL in sodium phosphate buffer) in triplicate. Following a 5-minute incubation at room temperature, 110 µL of a 2 mM L-tyrosine substrate was added to each well. The final concentrations of the crude extracts and the positive control were set at 100 µg/mL. The sample control consisted of each sample combined with sodium phosphate buffer. The reaction rate was monitored at 5-minute intervals over a 30-minute period. This was achieved through absorbance measurements at 490 nm using a Multiskan Plate Reader. The endpoint determination was performed at the 30-minute mark, also at 490 nm. The calculation of the percentage of tyrosinase inhibition was conducted using the following method:

$$[(A - B) - (C - D)] / (A - B) \times 100 \quad \text{..... Equation (3)}$$

Where A is the absorbance of the control with the enzyme, B is the absorbance of the control without the enzyme, C is the absorbance of the test sample with the enzyme and D is the absorbance of the test sample without the enzyme.

2.5. Statistical analysis

All experiments were conducted in triplicate, and the results were presented as mean ± standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey post hoc for multiple comparisons using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Statistically significant results were set at $p < 0.05$. The percentage inhibition values for plant extracts and standard inhibitors (acarbose for diabetic enzymes and kojic acid for tyrosinase) were subjected to analysis. All figures have error bars representing standard deviations of triplicate data.

2.5 Limitation of the study

This study has multiple limitations. All enzyme inhibition assays were performed at a consistent extract concentration of 100 µg/mL; consequently, IC_{50} values and concentration–response relationships could not be determined. The inhibition profile of tyrosinase has only been reported at one concentration and a detailed kinetic study of tyrosinase such as evaluating inhibition constants, Lineweaver-Burk plots, and Michaelis-Menten analysis was not performed. Therefore,

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the suggested inhibition modes ought to be regarded as preliminary hypotheses. Further research should include dose–response experiments, quantitative kinetic modeling, and bioassay-guided fractionation to identify the active constituents and their mode of action.

3. Results and discussion

3.1 Inhibition of diabetes enzymes

Results showed a spectrum of bioactivity, with certain selected plants exhibiting notable and selective enzyme inhibitory effects which could offer scientific justification for the plants' ethnopharmacological use. The results are illustrated in Table 3 and Figure 1.

Table 3. Inhibition of diabetic enzymes of the selected plants at 100 µg/ml

Plant code	Plant name	α-glucosidase (%)	α-amylase (%)
11	*Euphorbia graminea*	57.609 ± 4.56	68.387 ± 4.87
13	*Berlinia* sp.	83.455 ± 4.12	66.591 ± 5.01
17	*Tabernaemontana pachysiphon*	54.572 ± 5.23	62.724 ± 4.93
37	*Anacardium occidentale*	58.546 ± 4.78	58.973 ± 5.14
Standard	Acarbose	91.121 ± 2.34	88.347 ± 2.67

Values represent mean ± SD (n = 3).

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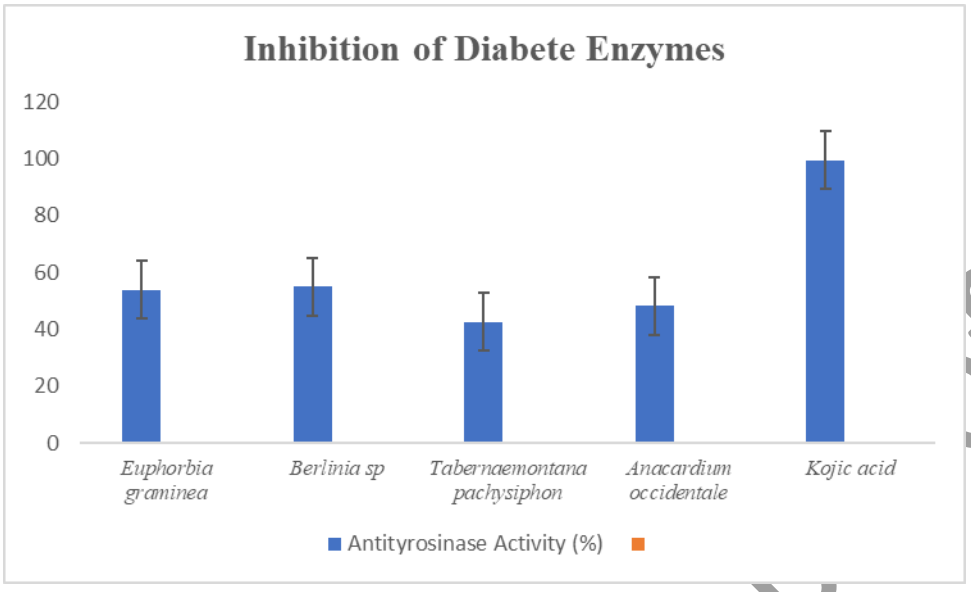

Figure 1. Inhibition of diabetic enzymes by selected plant extracts at 100 µg/mL

At a concentration of 100 µg/mL, *Berlinia* sp. exhibited the most pronounced α-glucosidase inhibition, a value of 83.46 ± 4.12%. This result was not statistically different from that of acarbose, which recorded an inhibition of 91.12 ± 2.34% ($p > 0.05$). This extract displayed a moderate percentage of α-amylase inhibition 66.59 ± 5.01%. The α-amylase inhibitory effect of *Euphorbia graminea* (68.39 ± 4.87%) was the highest, followed by its α-glucosidase inhibitory effect (57.61 ± 4.56%) among the extracts tested. As shown in Table 3, *Tabernaemontana pachysiphon* and *Anacardium occidentale* showed similar moderate inhibitory effect for both the enzymes. All extracts demonstrated an inhibition of each enzyme exceeding 50%, with *Berlinia* sp. identified as the most potent dual inhibitor (Figure 1).

3.2 Tyrosinase inhibition activity

Table 4 and Figure 2 shows the antityrosinase activity results of the selected extracts indicated varying inhibitory potentials, which might be attributed to species-specific phytochemical compositions that impact bioactivity.

Table 4. Antityrosinase activity of the selected extracts at 100 µg/ml

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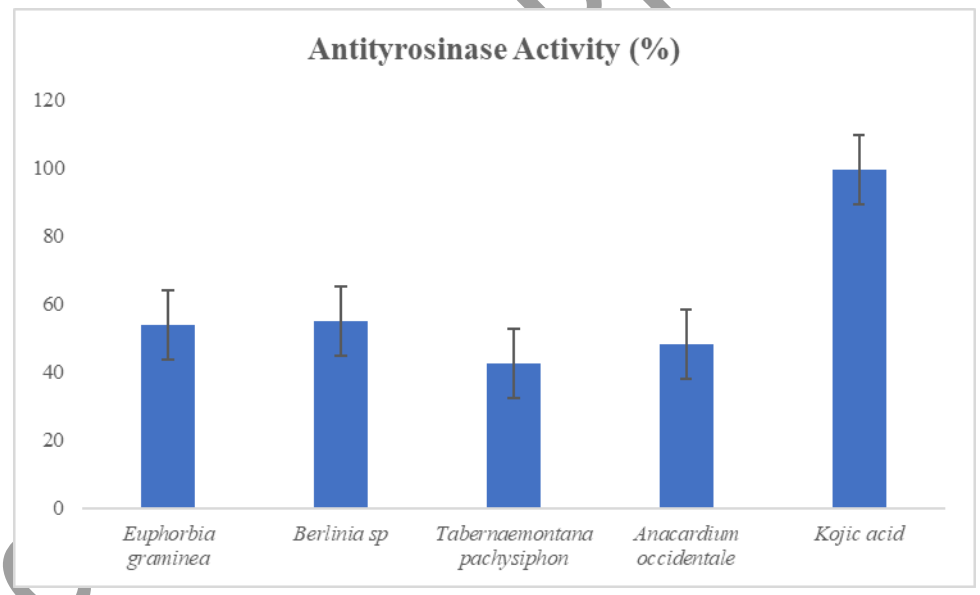
Plant code	Plant name	Antityrosinase Activity (%)
11	*Euphorbia graminea*	53.895 ± 4.21
13	*Berlinia* sp	55.038 ± 3.98
17	*Tabernaemontana pachysiphon*	42.684 ± 4.56
37	*Anacardium occidentale*	48.208 ± 4.33
Standard	Kojic acid	99.392 ± 1.02

198 Values represent mean ± SD (n = 3).



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200 **Figure 2.** Antityrosinase activity of some selected plants at 100 µg/ml

201 *Berlinia* sp. and *E. graminea* demonstrated the highest values of tyrosinase inhibition, measured
202 at 55.04 ± 3.98 % and 53.90 ± 4.21 %, respectively, with no significant difference noted between
203 the two ($p > 0.05$). *A. occidentale* exhibited moderate activity, estimated to be 48.21 ± 4.33 %,
204 while *T. pachysiphon* displayed the lowest activity at 42.68 ± 4.56 %. All extracts exhibited

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markedly reduced potency in comparison to kojic acid (99.39 ± 1.02 %; $p < 0.001$ for each comparison), indicating that further purification is required to achieve comparable efficacy (Figure 2).

3.3 Kinetics of tyrosinase inhibition

Kinetic study of the antityrosinase activity was carried out by calculating percentage inhibition of the activity as a function of time in the 30 min reaction period, which showed a different temporal profile for each of the botanical extracts in comparison to the standard, kojic acid. The result is shown in Table 5 and Figure 3.

Table 5. Time-dependent inhibition of tyrosinase by four plant extracts at 100 µg/ml

Plant codes	Kinetic Inhibition at Time (min)					
	5	10	15	20	25	30
11	3.825 ± 0.89	9.413± 1.23	29.951 ± 2.45	36.097 ± 3.01	49.883 ± 3.89	53.895 ± 4.21
13	13.271 ± 1.56	29.868± 2.34	30.177± 2.78	35.055 ± 3.12	40.382 ± 3.45	55.038 ± 3.98
17	11.943 ± 1.78	24.574 ± 2.12	31.771 ± 2.89	36.987 ± 3.23	39.111 ± 3.56	42.684 ± 4.56
37	11.714 ± 1.45	20.527 ± 1.89	26.601 ± 2.34	31.570 ± 2.89	37.252 ± 3.12	48.208 ± 4.33
Standard	56.844 ± 2.12	72.471 ± 2.45	81.939 ± 1.89	83.549 ± 1.67	96.152 ± 1.34	99.392 ± 1.02

Values represent mean ± SD (n = 3).

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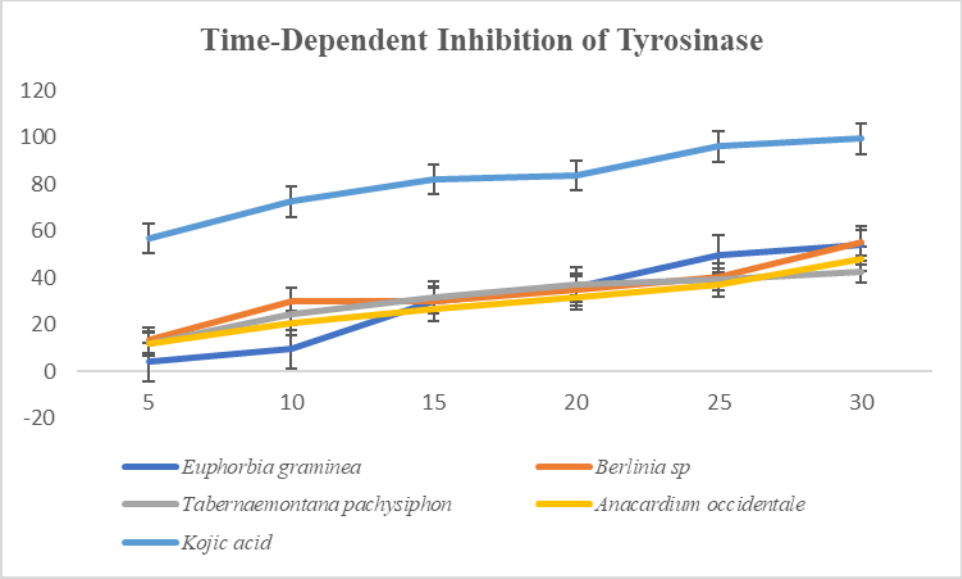

Figure 3. Time-dependent inhibition of tyrosinase by four plant extracts at 100 µg/ml

The inhibition exhibited by kojic acid was both rapid and nearly complete, achieving a 99.39% inhibition within a 30-minute timeframe. *E. graminea* had a gradual binding profile with 3.83% inhibition after 5 minutes, increasing to 53.90% inhibition after 30 minutes, whereas significant increases in the inhibition profile were observed after 10 minutes. In case of the *Berlinia* sp, a biphasic response was seen, with an initial increase of about 30% followed by a plateau between the 15- and 25-minute period before a further increase to 55.04% at 30 minutes. *A. occidentale* demonstrated a nearly linear increase, achieving 48.21% at the 30-minute mark, whereas *T. pachysiphon* exhibited one of the lowest and most linear inhibition levels at 42.68% at the same time interval. These different kinetic profiles indicate different modes of interaction between the enzyme and the inhibitors in the extracts (Figure 3).

3. Discussion

These results showed that *Berlinia* sp. exhibited the highest percentage of α-glucosidase inhibition (83.46 ± 4.12%) and moderate inhibition towards α-amylase (66.59 ± 5.01%) (Table 3, Figure 1). In comparison, acarbose demonstrated inhibition levels of 91.12 ± 2.34% and 88.35 ± 2.67%, respectively. This indicates that flavonoids and tannins are probable bioactive phytochemicals found within the extract. These classes of chemicals work by targeting the structural components

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of enzymes, which effectively inhibit the degradation of carbohydrates (Salehi *et al*., 2019; Dirir *et al*., 2022; Xiao *et al*., 2013). Structure-activity relationship studies have shown that hydroxylation at certain positions improves the binding affinity of flavonoids, which in turn suppresses the activity of these two enzymes (Lam *et al*., 2024; Tadera *et al*., 2006). Moderate α-amylase inhibition is preferable to complete inhibition, as it can help reduce some gastrointestinal effects commonly associated with the use of high doses of starch digestion inhibitors, such as bloating and diarrhea (Tundis *et al*., 2010; Pan *et al*., 2024). This helps to optimize the modulation of enzymes in modern techniques (Dirir *et al*., 2022; Pan *et al*., 2024). Specifically, *Euphorbia graminea* demonstrated the ability to inhibit both α-amylase and α-glucosidase, exhibiting values of 68.39 ± 4.87% and 57.61 ± 4.56%, respectively. This selectivity is likely explained by the differential binding affinities of diterpenes, flavonoids and phenolic compounds with these enzymes (Salehi *et al*., 2019; Dirir *et al*., 2022; McDougall *et al*., 2005). The inhibitory effects of plant extracts on these enzymes vary according to the molecular size, polarity, and functional groups present in the active compounds (Xiao *et al*., 2013; Lam *et al*., 2024). *Tabernaemontana pachysiphon* was found to be moderately and selectively inhibitory towards α-amylase (62.72 ± 4.93%), while it inhibited α-glucosidase at 54.57 ± 5.23%. This trend might be attributed to its diversified alkaloids composition, which may inhibit metabolic enzymes through both non-competitive and competitive mechanisms (Salehi *et al*., (2019). Alkaloid extracts affect pathways related to metabolism of glucose, which leads to a reduction in hyperglycaemia (Dirir *et al*., 2022; Pan *et al*., 2024). *Anacardium occidentale* showed similar percentage inhibition for both α-amylase and α-glucosidase, which were 58.55 ± 4.78% and 58.97 ± 5.14% respectively. This corroborates with results of previous studies suggesting the antidiabetic properties of cashews are due to their high levels of polyphenols, tannins, and anacardic acids which inhibit digestive enzymes and bind to glucose. Polyphenols exhibit a range of effects, including the inhibition of enzymes, enhancement of antioxidant status, and improvement of insulin sensitivity (Dirir *et al*., 2022; Febriyanti *et al*., 2025). The enzymes inhibition by *Berlinia* sp. suggests its potential as a promising subject for additional research. Selective and moderate inhibition were noted with the other extracts, highlighting the significance of phytochemical diversity on enzyme specificity and potency, as well as the necessity for bioassay-guided fractionation in the identification of active components (Dirir *et al*., 2022; Xiao *et al*., 2013). The findings indicate that all the extracts demonstrated inhibition levels exceeding 50%, thereby reinforcing their traditional medicinal

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applications. This suggests that various compounds within the plant may be beneficial in addressing the disease while possibly minimizing adverse effects, in contrast to targeting single pathways and traditional therapeutic approaches (Salehi *et al*., 2019; Pan *et al*., 2024; Febriyanti *et al*., 2025).

The highest result for antityrosinase activity as indicated in Table 4 and Figure 2, was obtained with *Berlinia* sp. at 55.04 ± 3.98%. The result showed that polyphenols in plants like flavonoids and tannins can inhibit the tyrosinase enzyme by chelating copper at the binuclear active site of the enzyme and thus modulate melanogenesis in a redox-dependent way (Zolghadri *et al*., 2019; Chang, 2009; Masum *et al*., 2019). Tyrosinase is a key enzyme in melanin biosynthesis that catalyzes the oxidation of mono- and diphenols. The inhibition of this enzyme is a major hurdle in the aspect of hyperpigmentation and enzymatic browning (Ni *et al*., 2025; Masum *et al*., 2019). *Euphorbia graminea* exhibited an inhibitory activity of 53.90 ± 4.21%. Several studies have shown that the inhibitory activity of terpenoids and phenolics against tyrosinase act as competitive, non-competitive and mixed type depending on their binding site interactions (Zolghadri *et al*., 2019; Lee *et al*., 2016 and Masum *et al*., 2019). These compounds have the capacity to interact with the active site or induce alterations in its conformation, resulting in a reduction of catalytic efficiency (Chang, 2009; Lee *et al*., 2016). *Anacardium occidentale* exhibited an inhibition of 48.21 ± 4.33%, suggesting a moderate level of activity. This might be attributed to use of crude extracts as the extraction method and solvent polarity influence the yield and the bioactivity of phytochemicals. Fractionation has been demonstrated to enrich the active phenolic compounds, subsequently leading to improved inhibitory potencies (Dirir *et al*., 2022; Masum *et al*., 2019). Synergism or antagonism between phytochemicals may affect the overall inhibitory effect, highlighting the importance of bioassay-guided fractionation for identification of potent inhibitors (Zolghadri *et al*., 2019). *Tabernaemontana pachysiphon* exhibited the lowest level of activity, recorded at 42.68 ± 4.56%. This observation may be attributed to the phytochemical profile; phenolic compounds generally show stronger tyrosinase inhibition than alkaloids, although alkaloids possess notable metal chelating and antioxidant properties (Zolghadri *et al*., 2019; Lee *et al*., 2016). The results show the relevance of the phytochemical diversity in enzyme inhibition and indicate that natural phenolic compounds may be among the more potent tyrosinase inhibitors that can be utilized for cosmetic and therapeutic purposes (Ni *et al*., 2025; Masum *et al*., 2019; Chang, 2009). *Berlinia*

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sp. and *E. graminea* present intriguing possibilities for bioassay-guided fractionation and extraction of biologically active compounds, serving as potential alternatives to various synthetic depigmenting agents such as kojic acid and hydroquinone derivatives (Zolghadri *et al*., 2019; Lee *et al*., 2016).

Time-dependent inhibition of tyrosinase activity of four plant extracts (Table 5 and Figure 3) was compared to kojic acid, which inhibited tyrosinase activity rapidly and almost fully at 30 min (99.39 ± 1.02%). This finding is constant with the competitive copper chelation mechanism, which forms a thermodynamically stable enzyme–inhibitor complex (Carcelli *et al*., 2025 and Zargaran *et al*., 2025). Slow binding was noted in *Euphorbia graminea*, showing an increase from 3.83 ± 0.89% at the 5-minute mark to 53.90 ± 4.21% at 30 minutes, with a significant inflection not observed until after the 10-minute interval. This behavior suggests a conformational change of the active site and is like the behavior of other Euphorbia species, where the phenolic components interact with the dicopper center (Sinan *et al*., 2020; Pinthong *et al*., 2025). Further studies are required for validation of this mechanism, including Michaelis-Menten kinetic studies. *Berlinia* sp. had a biphasic profile, rising to 13.27 ± 1.56% in 5 minutes and then plateauing at approximately 30% before increasing again to 55.04 ± 3.98%. This behaviour suggests a mixed type of inhibition, where the inhibitor may bind with both the free enzyme as well as the enzyme-substrate complex, as observed in the case of certain Fabaceae flavonol glycosides (Kim *et al*., 2021; Riaz *et al*., 2021). Nonetheless, a comprehensive characterization of the type of inhibition necessitates the execution of quantitative kinetic investigations. *Tabernaemontana pachysiphon* demonstrated the least degree of inhibition, ranging from 11.94 ± 1.78% to 42.68 ± 4.56%, in a nearly linear fashion when compared to other species. This is consistent with the hypothesis of possible low-affinity reversible binding. The genus is a key source of monoterpenoid indole alkaloids, and the activity reported could be due to hydrophobic interaction at the tyrosinase active site but has been rarely investigated in terms of inhibition (Marinho *et al*., 2021). The behavior of *Anacardium occidentale* showed a progressive increment, with the most pronounced rise occurring between 25 and 30 minutes (37.25 ± 3.12% and 48.21 ± 4.33%, respectively). The result indicates that there may be time dependent complexation processes. This resembles the inhibition of tyrosinase by alkylresorcinols like cardol triene, which is derived from cashew nutshell liquid (Zolghadri *et al*., 2019; Riaz *et al*., 2021). However, the claim of irreversible inhibition would

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require additional experimental proof using either dialysis experiments or dilution experiments. The extracts presented with different kinetic profiles and kojic acid showed the greatest effect. The kinetic profiles suggest there may be multiple mechanisms in the extracts, but quantitative studies of the type of inhibition (competitive, non-competitive or mixed) would require Michaelis-Menten kinetic studies and Lineweaver-Burk plots covering a range of inhibitor concentrations. The results for *Berlinia* sp. showed potential biphasic behavior, while those for *E. graminea* and *A. occidentale* were promising in late-phase increases in inhibition. These differences may help in the rational development of sustained-release topical depigmenting formulations (Zeitoun *et al*., 2020; Pillaiyar *et al*., 2017). However, this current study is limited to using single concentration data; future studies should incorporate dose-response experiments and quantitative kinetic modelling.

5. Conclusion

This research demonstrates that methanolic extracts from *Berlinia* sp., *Euphorbia graminea*, *Tabernaemontana pachysiphon*, and *Anacardium occidentale* show in vitro inhibitory effects on α-glucosidase, α-amylase, and tyrosinase activities. The dual inhibitory activity of *Berlinia* sp. was the most potent at 100 µg/mL with 83.46% α-glucosidase and 55.04% tyrosinase inhibition. The kinetic profiles showed distinct time-dependent inhibition patterns, reflecting different modes of interaction between the enzymes and inhibitors. These findings provide a scientific basis for the traditional uses of these plants and highlight *Berlinia* sp. as a significant candidate for further investigation. However, results should be interpreted with caution because all assays were performed using only one concentration of extract and thorough kinetic characterization has not been done. Consequently, IC_{50} values remain undetermined, and the proposed mechanisms of inhibition are still hypothetical. Future investigations should include dose–response studies, comprehensive kinetic analyses (encompassing the determination of K_i and the classification of inhibition), bioassay-guided fractionation for the isolation of active compounds, and an evaluation of safety and efficacy in suitable in vivo models before contemplating any therapeutic or cosmeceutical applications.

Conflict of interest

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The authors state that there is no conflict of interest.

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Author's contribution

O. K. Popoola: Administration, Design and Supervision; S. A. Oyebisi: Writing and Investigation; T. J. Fatoki: Formal analysis; O. K. Agboola: Formal Analysis

Ethical approval

Not applicable

Data availability

All data supporting the findings of this study are included within the article

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