FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 21, November, 2026, pp 268 – 273
DOI: https://doi.org/10.33003/fjs-2026-1021-5932

Comparative *In Vitro* Antioxidant and *In Vitro* Antidiabetic Activities of *Piliostigma reticulatum* Stem Bark Extract: Implications for the Management of Oxidative Stress and Diabetes Mellitus

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Received: 11 Sept. 2026
Accepted: 28 Oct. 2026
Published: 02 Nov. 2026

ABSTRACT

Medicinal plants rich in natural antioxidants have attracted considerable interest because of their ability to neutralize free radicals and protect biological systems against oxidative damage. *Piliostigma reticulatum* is widely used in African traditional medicine for managing diabetes and inflammatory disorders; however, comparative information on the antioxidant and antidiabetic activities of its solvent fractions remains limited. This study evaluated the antioxidant and antidiabetic potential of *P. reticulatum* stem bark solvent extract using *in vitro* antioxidant and carbohydrate-hydrolyzing enzyme inhibition assays. Antioxidant activity was assessed using DPPH and ABTS radical-scavenging assays, while ferric reducing antioxidant power (FRAP) evaluated reducing capacity. α-Amylase and α-glucosidase inhibitory activities were also determined. The methanol fraction exhibited the strongest DPPH scavenging activity among the solvent extract (IC_{50} = 1.353 ± 0.239 µg/mL), followed by ethyl acetate (1.717 ± 0.382 µg/mL) and n-hexane (14.023 ± 0.716 µg/mL). Similarly, methanol showed superior ABTS activity (47.420 ± 2.594 µg/mL) and FRAP capacity (1166.0 ± 2.646 µg Trolox equivalent/mL), compared with ethyl acetate and n-hexane extracts. Ethyl acetate demonstrated the strongest α-amylase (IC_{50} = 9.007 ± 0.431 µg/mL) and α-glucosidase (IC_{50} = 0.003 ± 0.001 µg/mL) inhibitory activities. All experiments were conducted in triplicate and data analysis was carried out using IBM SPSS version 24 software. Data was expressed as mean ± standard deviation (SD). Overall, *P. reticulatum* stem bark exhibited solvent-dependent antioxidant and enzyme inhibitory activities, with methanol showing superior antioxidant activity and ethyl acetate demonstrating stronger enzyme inhibition. These findings highlight *P. reticulatum* as a promising source of bioactive compounds with potential for further investigation as a source of enzyme-inhibitory bioactive compounds.

Keywords: *Piliostigma Reticulatum*, Antioxidants, DPPH, ABTS, FRAP, Diabetes Mellitus, Oxidative Stress

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, impaired insulin action, or both, having a profound impact on global health. Recent literature highlights the increasing prevalence of the condition, attributed to genetic, environmental, and lifestyle factors, with type 2 diabetes being the most common, alongside other types such as type 1 and gestational forms (Sun *et al.*, 2022) .

One of the principal mechanisms responsible for the development of diabetic complications is oxidative stress. Oxidative stress is a condition that occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system's ability to neutralize them. ROS, including free radicals and other highly reactive molecules, are natural byproducts of normal cellular metabolism. However, when their production exceeds the capacity of the body's antioxidants to counteract them, oxidative stress occurs (Forman and Zhang, 2021). This imbalance leads to a cascade of detrimental effects within the body. The excessive accumulation of ROS can damage various cellular components, including proteins, lipids, and DNA, resulting in cellular dysfunction (Ren *et al.*, 2026)

Alpha-amylase and alpha-glucosidase assays are the most common assays used to determine the antidiabetic activity of the plants *in vitro*. One of the most effective therapeutic strategies for managing type 2 diabetes mellitus involves the regulation of postprandial blood glucose levels through inhibition of carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase (Dirir *et al.*, 2022). Synthetic inhibitors such as acarbose, miglitol, and voglibose are widely used in clinical practice to inhibit these enzymes; however, their use is often limited by adverse gastrointestinal effects including bloating, flatulence, and diarrhea (Febriyanti *et al.*, 2025).

Piliostigma reticulatum (common name; English: Camel's foot Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpoatu') belongs to the family *Fabaceae* and is found in the savannah region of Nigeria. The plant is a dioeciously shrub or small tree up to 10-15 meters tall, bole short, rarely straight, up to 30 cm in diameter. The outer bark is deeply fissured, cracked grey to brown, and the inner bark pink to red crown rounded and dense. The branches are grey, waxy, and glabrous. The leaves alternate conspicuously bilobed, petiole 1-3.5 cm long, swollen at both ends, blade 5-12 cm X 4-18 cm, chordate or rounded at base, lobes rounded more or less cuneate. The flowers are unisexual, 2.5 cm in diameter, calyx 5 toothed, 15-20 cm long, petals 5 obovate, white with pink stripes, male flowers with 10 stamens, anthers brown. Fruit an oblong pod 15-30 cm X 2.5-5 cm, straight undulate woody, hard, glabrous, or sparsely pubescent, brown flat, pruinose, sometimes twisted and cracked, indehiscent and persisting, many seeded (Olorunnisola *et al.*, 2016). Research has demonstrated that extracts from the plant can directly lower

blood glucose levels. In subacute toxicity studies, a dichloromethanic extract of the stem bark administered at 500 mg/kg body weight caused significant hypoglycemia in rats from the first to the third week of treatment. Similarly, hydro-ethanolic extracts of the stem bark have shown hypoglycaemic effects at higher doses, such as 1000 mg/kg (Kone *et al.,* 2016).

Although *Piliostigma reticulatum* has received considerable attention because of its ethnomedicinal importance, limited information exists regarding the comparative antioxidant and antidiabetic activities of its stem bark solvent extract using multiple assays. Such information is essential for identifying the most bioactive fraction and establishing a scientific basis for its traditional medicinal applications. Therefore, the present study investigated and compared the antioxidant and enzymes inhibitory activities of n-hexane, ethyl acetate, and methanol extract of *Piliostigma reticulatum* stem bark wich will provide further evidence supporting the *Piliostigma reticulatum* stem bark as a natural antioxidant and antidiabetic source and its possible application in the management of oxidative stress-associated diseases, particularly diabetes mellitus.

MATERIALS AND METHODS

Sample Collection, Preparation and Identification

Fresh stem bark of *Piliostigma reticulatum* was collected from Botanical Garden of Bayero University Kano (old site), situated in Gwale Local Government Area of Kano State, Nigeria on 4th February, 2026 during harmattan season. The plant was subsequently identified by a botanist and assigned an accession number (BUKHAN 0072) at the Herbarium Unit of the Department of Plant Science and Biotechnology, Bayero University, Kano. The sample was washed, shade dried for 14 days at room temperature and then cut into small pieces and pulverized until powdered.

Extraction of *Piliostigma reticulatum* Stem Bark

A serial exhaustive extraction was carried out using n-hexane, ethyl acetate and methanol as extractants according to their order of increasing polarity as described by Ahmed, (2017) with slight modifications.

Procedure

Six hundred grams (600 g) of powdered stem bark were soaked into 2500 mL of the first solvent (n-hexane), left in a dark cupboard for 48hrs after which it was filtered through a Whattman No 1. filter paper. The residue was squeezed using a muslin cloth and airdried in order to free it of any residual n-hexane. This was subsequently soaked in the second solvent (ethyl acetate) and the procedure was observed up till the last solvent (methanol) to obtain the respective extracts. Solvent from each extract was recovered and the extract were allowed to dry. The extract was further fractionated using 100% of the solvents of varying polarities (methanol, ethyl acetate and n-hexane) to generate respective fractions.

The percentage yield of serial solvent extraction was calculated using the formula Extraction yield (%) $= \frac{\text{weight of dried extract (g)}}{\text{Initial weight of sample (g)}} \times 100\%$.

Antioxidant Assays

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay

DPPH radical scavenging activity was determined as described by (Sirivibulkovit *et al.,* 2018)with slight modification.

Procedure

1000, 500, 250, 125, and 62.5 µg/ml and quercetin in methanol solutions with concentrations of 1, 5, and 10 µg/ml were prepared. A solution of 1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with each sample and positive control. After incubation for 30 minutes at 37°C, their absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The blank contained a methanol solution plus a 1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. Percentage inhibitions of the methanol, ethyl acetate and n-hexane fractions of the extract and the standard was calculated using the formula below:

% Inhibition = $\frac{A0-A1}{A0}$ x 100%

Where; A0 = the absorbance of the blank A1 = the absorbance of the sample.

IC_{50} was analyzed using SPSS version 22 software.

FRAP (Ferric Reducing Antioxidant Power) Assay

Ferric reducing assay was carried out using the method described by Benzie and Strain, (1996) with slight modifications.

Procedure

To prepare 300 mL of acetate buffer, 300 mM sodium acetate solution and glacial acetic acid solution were mixed to achieve pH 3.6. Furthermore, 10 mM TPTZ was dissolved in 40 mM HCl, and a 20 mM $FeCl_3$ solution was also prepared. A brown bottle was then used to mix 30 mL of pH 3.6 buffer, 3 mL of 10 mM TPTZ solution, and 3 mL of 20 mM $FeCl_3$ solution to make the FRAP reagent. After the reagent dissolved homogeneously, it was incubated for 30 minutes at 37ºC. To test the *Piliostigma reticulatum* extract, they were dissolved in methanol and prepared with varying concentrations of 1000, 500, 250, 125, and 62.5 µg/mL. Then, 1200 µL FRAP reagent was mixed with each of the prepared samples. The mixtures were incubated in the dark at 37ºC for 30 minutes, after which the absorbance was measured using a UV-visible spectrophotometer at room temperature at a wavelength of 593 nm. Three measurements were taken with Trolox as a reference. The IC_{50} value is used to calculate the percentage attenuation rate using the following formula:

% Inhibition = $\frac{A0-A1}{A0}$ x 100%

Where; A0 = the absorbance of the blank A1 = the absorbance of the sample.

ABTS (2, 2-Azinobis (3-Ethylbenzothiazoline-6-Sulfonate)) Antioxidant Activity Test

The method developed by Mareček *et al.,* (2017) was used to conduct ABTS radical measurements.

Procedure

A mixture of 5 ml of 7 mM ABTS stock solution and 88 µL of 140 mM potassium persulfate solution was incubated in the dark at room temperature for 12-16 hours. Then, ABTS reagent was diluted in water (1:3, v/v) to attain an absorption value of 0.7 at 734 nm. Subsequently, *Piliostigma reticulatum* stem bark extract, dissolved in ethanol at concentrations of 1000, 500, 250, 125, and 62.5 µg/mL, were mixed with ABTS reagent (1:1) and incubated in a dark place at 37°C for 6 minutes. The absorbance of the mixture was measured with a UV-visible spectrophotometer at a wavelength of 734 nm. The measurements were performed three times, and Trolox was used as a comparison. The IC_{50} value, which represents the attenuation percentage value, was analyzed from percentage inhibition:

% Inhibition = $\frac{A0-A1}{A0}$ x 100%

Where; A0 = the absorbance of the blank A1 = the absorbance of the sample

IC_{50} was analyzed using SPSS version 22 software

Enzymes Inhibition Assay

Alpha-amylase Inhibition Assay

The α-amylase inhibitory activity was determined by assay adapted from method of (Alqahtani *et al.,* 2019).

Procedure

The reaction mixture contains 200 µL of the tested extracts of *Piliostigma reticulatum* (0.18-10 mg/mL), 200 µL of 0.02 M sodium phosphate buffer (pH 6.9; 6.7 mM NaCl) containing 1.3 U/mL of porcine pancreatic α-amylase solution (PPA). The reaction medium was pre-incubated at 37 °C for 5 min, and then 200 µL of 0.4 % starch solution in the above buffer were added and incubated at 37 °C for 10 min. 600 µL of DNSA solution was added to the reaction and placed in a boiling water bath for 7 min, then cooled down in cold water. The reaction mixture was then diluted after adding 1 mL of distilled water and the absorbance was measured at 540 nm. To eliminate the absorbance produced by plant extract, appropriate extract controls with extract and except the enzyme were also included. Commercial inhibitor acarbose was used as a positive control at a concentration range of 0.040-2.670 mg/mL. As a blank buffer solution was used instead of substrate. The tube with enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α-amylase activity (%) = $\frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}}$ x 100%

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

Alpha-glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined by assay adapted from method of (Alqahtani *et al.,* 2019)

Procedure

200 µL of a crude enzyme solution of rat intestinal α-glucosidase (adjusted to 0.2 U/mL as initial concentration in phosphate buffer 67 mM, pH 6.8) was mixed with 200 µL of the sample: plant extract (0.06-1.18 mg/mL) or acarbose, positive control, (0.001-0.118 mg/mL) solutions and 1 mL of phosphate buffer. The mixture was pre-incubated at 37 °C for 10 min after which 300 µL of p-NPG solution (10 mM) was added and the reaction mixture further incubated at 37 °C for 40 min. The reaction was terminated by adding 3 mL of sodium carbonate Na_2CO_3 (100 mM) into the mixture to stop the reaction. The absorbance of the liberated p-nitrophenol was measured at 405 nm. As a blank, buffer solution was used instead of substrate. The tube with enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α-amylase activity (%) = $\frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}}$ x 100%

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

Statistical Analysis

Data analysis was carried out using IBM SPSS version 24 software. Data was expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA and significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1: Weight and Percentage Yield of Serial Solvent Extraction of *Piliostigma Reticulatum* Stem Bark

Solvent	Weight of Sample (g)	Extract Yield (%)
N-hexane	600	1.1
Ethyl acetate	536	5.6
Methanol	482.16	12.63

Table 2: DPPH and ABTS Radical-Scavenging IC_{50} Values of *Piliostigma Reticulatum* Stem Bark Extract

Extract	DPPH (µg/mL)	ABTS (µg/mL)
n-Hexane	14.023 ± 0.716^{a}	579.68 ± 10.270^{a}
Ethyl acetate	1.717 ± 0.382^{b}	60.880 ± 3.298^{b}
Methanol	1.353 ± 0.239^{b}	47.420 ± 2.594^{b}
Gallic acid	0.477 ± 0.034^{b}	0.0430 ± 0.057^{c}

Values are expressed as mean ± SD of triplicate readings n=3. Values within the same column bearing different superscript letters are statistically ($P<0.05$) different.

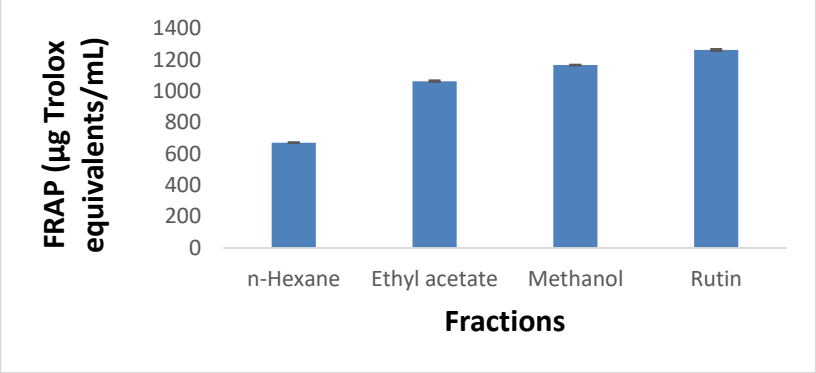


Figure 1: FRAP Antioxidant Activity of *Piliostigma reticulatum* Stem Bark Solvent Fractions

Values are expressed as mean ± SD of triplicate reading, n=3. Values within the same column bearing different Superscript letters are statistically (P<0.05) different

Table 3: Table 3. IC_{50} Values for in Vitro A-Amylase and A-Glucosidase Inhibition by *Piliostigma Reticulatum* Stem Bark Fractions

Fractions	Alpha-amylase (µg/mL)	Alpha-glucosidase (µg/mL)
N-hexane	53.68 ± 2.213^{a}	0.353 ± 0.032^{a}
Ethyl acetate	9.007 ± 0.431^{b}	0.003 ± 0.001^{b}
Methanol	12.907 ± 2.021^{b}	0.004 ± 0.001^{b}
Acarbose	0.028 ± 0.032^{c}	0.001 ± 0.0001^{b}

Values are expressed as mean ± SD of triplicate readings n=3. Values within the same column bearing different superscript letters are statistically (P<0.05) different.

RESULTS AND DISCUSSION

Serial extraction results demonstrate a clear polarity-dependent yield gradient, with methanol (12.63%) > ethyl acetate (5.6%) > n-hexane (1.1%) (Table 1). The results indicate that *Piliostigma reticulatum* stem bark is predominantly enriched in polar phytochemicals. This pattern aligns with established principles of solvent extraction efficiency and corroborates previous phytochemical screenings of *P. reticulatum*, which identified tannins and flavonoids as the major bioactive constituents (Wado *et al.,* 2022). The progressive increase in yield with solvent polarity reflects the fundamental principle that extraction efficiency is governed by the compatibility between solvent polarity and phytochemical solubility (Hashim *et al.,* 2024). Methanol, with a polarity index of 5.1, effectively solubilizes highly polar compounds including phenolic acids, flavonoid glycosides, tannins, and reducing sugars constituents previously reported as dominant in *P. reticulatum* stem bark (Dosso *et al.,* 2012). The observed yield differences may also reflect procedural factors beyond solvent polarity. Serial exhaustive extraction, where plant material is sequentially macerated with solvents of increasing polarity, ensures complete removal of compounds from each polarity class but may result in cumulative depletion of extractable material (Wado *et al.,* 2022).

The *in vitro* antioxidant results demonstrate a clear solvent polarity-dependent gradient in radical scavenging and reducing capacity, with methanol > ethyl acetate >> n-hexane across all three assays (DPPH, ABTS, FRAP) (Table 2). This pattern confirms that the antioxidant potential of *Piliostigma reticulatum* stem bark is predominantly conferred by polar phenolic phytochemicals, whose extraction efficiency and bioactivity are maximized in high-polarity solvents through enhanced solubility and preservation of hydroxyl-group-mediated redox mechanisms (Sun and Shahrajabian, 2023). The superior antioxidant activity of the methanol extract across DPPH, ABTS, and FRAP assays reflects the fundamental principle that phenolic compounds characterized by multiple hydroxyl groups on aromatic rings are preferentially solubilized in polar solvents due to hydrogen bonding and dipole-dipole interactions (Boualam *et al.,* 2021). The strong DPPH and ABTS scavenging by methanol and ethyl acetate extracts indicates abundant hydrogen-donating antioxidants capable of neutralizing stable free radicals through phenolic hydroxyl group oxidation (W. Sun and Shahrajabian, 2023). The DPPH assay measures hydrogen atom transfer capacity, where phenolics donate H- to reduce the purple DPPH- radical to yellow DPPH-H, while ABTS evaluates both HAT and SET mechanisms via formation of the ABTS- + cation radical, which is decolorized upon reduction (Dziąbowska-Grabias *et al.,* 2021). The parallel ranking (methanol > ethyl acetate >> n-hexane) across both assays suggests that the same pool of phenolic compounds likely flavonoids and phenolic acids mediate both radical scavenging mechanisms, as evidenced by strong positive correlations between total phenolic content and DPPH/ABTS activity in medicinal plants (Fahrer *et al.,* 2023).

The FRAP assay results, showing methanol > ethyl acetate >> n-hexane reducing capacity, indicate that polar extract contain abundant electron-donating compounds capable of reducing Fe^{3+}-TPTZ to Fe^{2+}-TPTZ, generating a blue chromophore (Xiang *et al.,* 2024). The antioxidant activity observed in the present study may also be relevant to the antidiabetic potential of *P. reticulatum*. Oxidative stress is closely involved in the pathogenesis and progression of diabetes, where excessive reactive oxygen species can impair pancreatic β-cell function, promote cellular damage and interfere with glucose homeostasis. Recent evidence indicates that oxidative stress contributes to β-cell dysfunction and death and interacts with other cellular stress pathways involved in diabetes development (Dinić *et al.,* 2022; Kulkarni *et al.,* 2022).

Enzymes inhibition results demonstrate that the ethyl acetate extract of *Piliostigma reticulatum* stem bark exhibits superior dual inhibitory activity against both α-amylase and α-glucosidase among the tested extract but lower than the control acarbose. This finding aligns with growing evidence that moderately polar phenolic constituents, particularly flavonoids and phenolic acids enriched in ethyl acetate extract, effectively interfere with carbohydrate digestion through competitive and non-competitive enzyme binding mechanisms (Lam *et al.*, 2024). The observation that ethyl acetate demonstrated the strongest inhibition against both enzymes, with a more pronounced effect on α-glucosidase than α-amylase, reflects structure-specific interactions between phytochemicals and enzyme active sites. The superior α-glucosidase inhibition is therapeutically advantageous, as this enzyme catalyzes the final, rate-limiting step of carbohydrate digestion hydrolysis of disaccharides and oligosaccharides to absorbable glucose in the small intestinal brush border (Febriyanti *et al.*, 2025). Dual inhibitory activity of the ethyl acetate extract likely involves multiple mechanisms: (1) competitive inhibition, where flavonoid aglycones (e.g., quercetin, kaempferol, luteolin) occupy the enzyme active site, preventing substrate binding through structural mimicry of oligosaccharide transition states; (2) non-competitive or mixed-type inhibition, where phenolic compounds bind to allosteric sites, inducing conformational changes that reduce catalytic efficiency; and (3) enzyme precipitation or aggregation through hydrophobic interactions and hydrogen bonding with surface residues (Salah *et al.*, 2022). The simultaneous inhibition of α-amylase and α-glucosidase by the ethyl acetate extract offers a strategic advantage for attenuating postprandial hyperglycemia, a critical therapeutic target in type 2 diabetes management. The present findings therefore suggest that the ethyl acetate extract of the plant extract may contain a relatively high concentration of constituents capable of interfering with carbohydrate digestion. The simultaneous inhibition of α-amylase and α-glucosidase is potentially advantageous because these enzymes act sequentially during carbohydrate digestion. α-Amylase hydrolyses starch into smaller carbohydrate fragments, whereas α-glucosidase subsequently hydrolyses these products to release glucose in the small intestine. Consequently, inhibition of both enzymes could delay the conversion of complex carbohydrates to absorbable glucose and thereby attenuate postprandial glucose elevation (Febriyanti *et al.*, 2025).

CONCLUSION

The findings demonstrate solvent-dependent *in vitro* antioxidant and carbohydrate-hydrolysing enzyme inhibitory activities of *P. reticulatum* stem bark extract. Methanol showed the strongest antioxidant activity, whereas ethyl acetate produced the strongest α-amylase and α-glucosidase inhibition under the experimental conditions. Further phytochemical characterization, confirmation of enzyme-inhibition kinetics, toxicity studies and in vivo investigations are required before therapeutic relevance to diabetes management can be established. Overall, the results indicate that *P. reticulatum* stem bark is a promising source of antioxidant and antidiabetic phytochemicals.

REFERENCES

Ahmed, R. (2017). Serial Exhaustive Extraction: Influence of Solvent Polarity on Antibacterial Activity of Extracts of Leaves of Tithonia diversifolia. Serial Exhaustive Extraction: Influence of Solvent Polarity on Antibacterial Activity of Extracts of Leaves of Tithonia Diversifolia, (1), 2987–2996. https://doi.org/10.19240/njpas.2017.A05

Alqahtani, A. S., Hidayathulla, S., Rehman, M. T., ElGamal, A. A., Al-Massarani, S., Razmovski-Naumovski, V., Alqahtani, M. S., El Dib, R. A., and AlAjmi, M. F. (2019). Alpha-Amylase and Alpha-Glucosidase Enzyme Inhibition and Antioxidant Potential of 3-Oxolupenal and Katononic Acid Isolated from Nuxia oppositifolia. Biomolecules, 10(1), 61. https://doi.org/10.3390/biom10010061

Azzahra, V. O., Mardiana, S., Suharyadi, S., Sianipar, R. J., and Ramadhan, D. S. (2025). Effect of Solvent Polarity on Extraction Yield, Phytochemical Composition, and Antioxidant Activity of Curcuma xanthorrhiza Roxb. And Moringa oleifera Lam. Biology, Medicine, and Natural Product Chemistry, 14(2), 1273–1284. https://doi.org/10.14421/biomedich.2025.142.1273-1284

Benzie, I. F. F., and Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry, 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Boualam, K., Ndiaye, B., Harhar, H., Tabyaoui, M., Ayessou, N., and Taghzouti, K. (2021). Study of the Phytochemical Composition, the Antioxidant and the Anti-Inflammatory Effects of Two Sub-Saharan Plants: Piliostigma reticulatum and Piliostigma thonningii. Advances in Pharmacological and Pharmaceutical Sciences, 2021, 1–8. https://doi.org/10.1155/2021/5549478

Dinić, S., Arambašić Jovanović, J., Uskoković, A., Mihailović, M., Grdović, N., Tolić, A., Rajić, J., Đorđević, M., and Vidaković, M. (2022). Oxidative stress-mediated beta cell death and dysfunction as a target for diabetes management. Frontiers in Endocrinology, 13, 1006376. https://doi.org/10.3389/fendo.2022.1006376

Dirir, A. M., Daou, M., Yousef, A. F., and Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. Phytochemistry Reviews, 21(4), 1049–1079. https://doi.org/10.1007/s11101-021-09773-1

Dosso, K., N'guessan, B., Bidie, A., Gnangoran, B., Méite, S., N'guessan, D., Yapo, A., and Ehilé, E. (2012). Antidiarrhoeal Activity of an Ethanol Extract of the Stem Bark of Piliostigma reticulatum (Caesalpiniaceae) in Rats. African Journal of Traditional, Complementary and Alternative Medicines, 9(2), 242–249. https://doi.org/10.4314/ajtcam.v9i2.9

Dziąbowska-Grabias, K., Sztanke, M., Zając, P., Celejewski, M., Kurek, K., Szkutnicki, S., Korga, P., Bulikowski, W., and Sztanke, K. (2021). Antioxidant Therapy in Inflammatory Bowel Diseases. Antioxidants, 10(3), 412. https://doi.org/10.3390/antiox10030412

Fahrer, J., Wittmann, S., Wolf, A.-C., and Kostka, T. (2023). Heme Oxygenase-1 and Its Role in Colorectal Cancer. Antioxidants, 12(11), 1989. https://doi.org/10.3390/antiox12111989

Febriyanti, R. M., Indradi, R. B., Maisyarah, I. T., Iskandar, Y., Susanti, R. D., and Lestari, D. (2025a). Alpha-amylase and Alpha-glucosidase enzymes inhibition and antioxidant

potential of selected medicinal plants used as anti-diabetes by Sundanese community in West Java, Indonesia. BMC Complementary Medicine and Therapies, 25(1), 426. https://doi.org/10.1186/s12906-025-05144-x

Febriyanti, R. M., Indradi, R. B., Maisyarah, I. T., Iskandar, Y., Susanti, R. D., and Lestari, D. (2025b). Alpha-amylase and Alpha-glucosidase enzymes inhibition and antioxidant potential of selected medicinal plants used as anti-diabetes by Sundanese community in West Java, Indonesia. BMC Complementary Medicine and Therapies, 25(1), 426. https://doi.org/10.1186/s12906-025-05144-x

Febriyanti, R. M., Indradi, R. B., Maisyarah, I. T., Iskandar, Y., Susanti, R. D., and Lestari, D. (2025c). Alpha-amylase and Alpha-glucosidase enzymes inhibition and antioxidant potential of selected medicinal plants used as anti-diabetes by Sundanese community in West Java, Indonesia. BMC Complementary Medicine and Therapies, 25(1), 426. https://doi.org/10.1186/s12906-025-05144-x

Forman, H. J., and Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery, 20(8), 652–652. https://doi.org/10.1038/s41573-021-00267-5

Hashim, N. A., Mahmod, N. H., Lema, A. A., Ho, L.-H., and Khandaker, M. M. (2024). Effects of Extraction Solvents and Drying Techniques on Antioxidant Content and Radical Scavenging Activity of Roselle (Hibiscus sabdariffa) Calyx. Sarhad Journal of Agriculture, 40(s1). https://doi.org/10.17582/journal.sja/2024/40/s1.110.121

Kone, M., Yao, K. E., Gbogbo, M., N'Dri, N. M., and Yapo, A. P. (2016). Effects of dichloromethanic extract of Piliostigma reticulatum D.C. (Hochst) stem bark on some biochemical parameters of Wistar albino rats. 05, 11–23.

Kulkarni, A., Muralidharan, C., May, S. C., Tersey, S. A., and Mirmira, R. G. (2022). Inside the β Cell: Molecular Stress Response Pathways in Diabetes Pathogenesis. Endocrinology, 164(1), bqac184. https://doi.org/10.1210/endocr/bqac184

Kumari, K., Adhikari, P., Pandey, A., Samant, S. S., Lal, M., and Pande, V. (2024). Influence of Solvent Polarity on Phytochemicals, Antioxidants, and Antimicrobial Properties of Delphinium denudatum: A Medicinal Herb from Sainj Valley, Himachal Pradesh, India. Bioactivities, 2(1), 30–40. https://doi.org/10.47352/bioactivities.2963-654X.214

Lam, T.-P., Tran, N.-V. N., Pham, L.-H. D., Lai, N. V.-T., Dang, B.-T. N., Truong, N.-L. N., Nguyen-Vo, S.-K., Hoang, T.-L., Mai, T. T., and Tran, T.-D. (2024). Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: A systematic review of in vitro studies. Natural Products and Bioprospecting, 14(1), 4. https://doi.org/10.1007/s13659-023-00424-w

Mareček, V., Mikyška, A., Hampel, D., Čejka, P., Neuwirthová, J., Malachová, A., and Cerkal, R. (2017). ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. Journal of Cereal Science, 73, 40–45. https://doi.org/10.1016/j.jcs.2016.11.004

Olorunnisola, O. S., Adetutu, A., Owoade, A. O., Oluranti, O., Oyewo, E. B., and Adegbola, P. (2016). Ethno-pharmacological and in-vitro anti-diabetic study of some medicinal plants commonly used in Ogbomoso, South Western Nigeria.

Ren, Y., Li, J., and Dai, X. (2026). Reactive oxygen species in health and disease. Molecular Biomedicine, 7(1), 30. https://doi.org/10.1186/s43556-026-00419-2

Salah, A., Nwafor, C. C., Han, Y., Liu, L., Rashid, M., Batool, M., El-Badri, A. M., Cao, C., and Zhan, M. (2022). Spermidine and brassinosteroid regulate root anatomical structure, photosynthetic traits and antioxidant defense systems to alleviate waterlogging stress in maize seedlings. South African Journal of Botany, 144, 389–402. https://doi.org/10.1016/j.sajb.2021.08.012

Sirivibulkovit, K., Nouanthavong, S., and Sameenoi, Y. (2018). Paper-based DPPH Assay for Antioxidant Activity Analysis. Analytical Sciences, 34(7), 795–800. https://doi.org/10.2116/analsci.18P014

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., and Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice, 183, 109119. https://doi.org/10.1016/j.diabres.2021.109119

Sun, W., and Shahrajabian, M. H. (2023). Therapeutic Potential of Phenolic Compounds in Medicinal Plants—Natural Health Products for Human Health. Molecules, 28(4), 1845. https://doi.org/10.3390/molecules28041845

Wado, T. E., Suleman, S., and Mohammed, T. (2022). Antimicrobial Evaluation of Sequentially extracted Leaf of Vernonia auriculifera Hiern (Rejicho). BMC Complementary Medicine and Therapies, 22(1), 219. https://doi.org/10.1186/s12906-022-03690-2

Xiang, Z., Liu, L., Xu, Z., Kong, Q., Feng, S., Chen, T., Zhou, L., Yang, H., Xiao, Y., and Ding, C. (2024). Solvent Effectson the Phenolic Compounds and AntioxidantActivity Associated with Camellia polyodonta FlowerExtracts. ACS Omega, 9(25), 27192–27203. https://doi.org/10.1021/acsomega.4c01321

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