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Mechanistic Evaluation of the Antidiabetic Potential of *Newbouldia laevis*: Evidence from Phytochemical Profiling and Enzyme Inhibition Studies

***[1]Ebhohon, Shirley Onagbonfeoana, [2]Akubuiro, Paschal Chikama, [1]Edward, Udoka Isimhenmhen and [3]Adarki, Pongri Wazakarda**

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.
[2]Department of Chemistry, University of South Dakota, Vermillion, South Dakota, United States of America
[3]National Agency for Food and Drug Administration and Control, Lagos Nigeria
*Corresponding Author's email: so.ebhohon@mouau.edu.ng

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ABSTRACT
Newbouldia laevis is widely utilized in traditional medicine for the management of metabolic and inflammatory disorders; however, its pharmacological properties require scientific validation. This study evaluated the phytochemical composition and *in vitro* antioxidant, anti-inflammatory, and antidiabetic activities of the crude aqueous leaf extract of *Newbouldia laevis*. Standard phytochemical screening methods and spectrophotometric assays, including DPPH, nitric oxide scavenging, ferric reducing antioxidant power (FRAP), protein denaturation, proteinase inhibition, α-amylase, and α-glucosidase inhibition assays, were employed, with IC_{50} values determined. The extract contained alkaloids (12.52 mg/100 g) in moderate amounts and lower levels of flavonoids (8.62 mg/100 g), saponins (5.95 mg/100 g), tannins (3.42 mg/100 g), phenols, terpenoids, cardiac glycosides, and steroids. It exhibited notable antioxidant activity in DPPH (IC_{50} = 6.33 µg/ml) and FRAP (IC_{50} = 7.73 µg/ml) assays, indicating high radical scavenging and reducing capacity comparable to ascorbic acid/BHT under similar conditions, alongside moderate nitric oxide scavenging (IC_{50} = 28.45 µg/ml), and anti-inflammatory activities. The extract also demonstrated notable α-amylase (IC_{50} = 9.29 µg/ml) and α-glucosidase inhibition (IC_{50} = 17.88 µg/ml); however, it exhibited lower potency than acarbose, with approximately 3-fold and 2.8-fold higher IC_{50} values for α-amylase (9.29 vs. 3.17 µg/ml) and α-glucosidase (17.88 vs. 6.37 µg/ml), respectively. These findings suggest that the extract could possess multi-target bioactivity mediated by its phytochemical constituents. It may be concluded that *Newbouldia laevis* has significant therapeutic potential; therefore, further *in vivo* studies and isolation of active compounds are recommended, contributing novel evidence to its pharmacological validation.

CITATION
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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both (Antar *et al.,* 2023). Its global prevalence continues to rise, particularly in low- and middle-income countries, where access to effective and affordable therapies remains limited (Hossain *et al.,* 2024). Postprandial hyperglycemia, driven largely by the rapid digestion and absorption of dietary carbohydrates, is a key contributor to disease progression and complications (Maffettone *et al.,* 2018). Enzymes such as α-amylase and α-glucosidase play central roles in carbohydrate hydrolysis; thus, their inhibition represents a validated therapeutic strategy for glycemic control (Alqahtani *et al.,* 2019). Although synthetic inhibitors (e.g., acarbose) are clinically effective, their use is often associated with gastrointestinal side effects, necessitating the search for safer, plant-based alternatives (Du & Zhao, 2025).

Newbouldia laevis is a widely used African medicinal plant for treating diabetes, infections, inflammation, pain, and reproductive disorders, with several traditional uses scientifically validated (Ogunlesi *et al.,* 2009; Iwu, 2000). Its ethnomedicinal relevance suggests the presence of bioactive phytochemicals capable of modulating key biochemical pathways. Phytoconstituents such as flavonoids, alkaloids, tannins, and saponins have been reported to exhibit antioxidant properties and enzyme inhibitory activities, thereby contributing to glycemic regulation (Ansari *et al.,* 2024). However, despite its widespread traditional use, there remains a paucity of systematic studies integrating phytochemical profiling with mechanistic evaluation of enzyme inhibition to substantiate its antidiabetic potential.

The rationale for this study is grounded in the need to bridge this gap by providing a biochemical basis for the therapeutic claims associated with *N. laevis*. Specifically, elucidating the relationship between its phytochemical constituents and its effect on carbohydrate-metabolizing enzymes and oxidative stress will enhance its pharmacological relevance. This integrated approach reflects a growing emphasis on therapeutics that concurrently address multiple pathological processes involved in diabetes.

This study is therefore justified by the limited mechanistic data linking the phytochemical composition of *N. laevis* to its antidiabetic effects. Previous investigations have largely focused on isolated biological activities without establishing a coherent relationship between phytoconstituents and enzyme inhibition. Consequently, the present study aims to evaluate the phytochemical composition of the crude aqueous leaf extract of *N. laevis* and investigate its inhibitory effects on α-amylase and α-glucosidase, alongside its antioxidant potential.

The specific objectives of this study are to qualitatively and quantitatively characterize the phytochemical constituents of the extract; assess its *in vitro* antioxidant capacity; evaluate its inhibitory activities against key carbohydrate-hydrolyzing enzymes; and establish mechanistic links between phytochemical composition and observed bioactivities. By addressing these objectives, this study contributes to closing the knowledge gap and provides scientific evidence supporting the development of *N. laevis* as a potential natural therapeutic agent for diabetes management.

MATERIALS AND METHODS

Chemicals and Reagents

Porcine α-amylase, yeast α-glucosidase, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), p-Nitrophenyl-α-glucopyranoside (pNPG) and acarbose were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Collection of Plant Material

Fresh leaves of *Newbouldia laevis* were collected in April 2025 from a farm settlement located in Umuahia North, Abia State, Nigeria. The plant was identified and authenticated by Mr. Pipi of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen (Ref. No. 20/395NL) was deposited in the department's herbarium for future reference.

Preparation of Extract

The collected leaves were washed with distilled water and shade-dried for seven days. The dried material was pulverized into a coarse powder using a Waring blender and stored in a sterile, airtight container. A portion of the powdered sample (120 g) was soaked in 500 mL of distilled water in a glass container and stirred intermittently to facilitate extraction. The mixture was left at room temperature for 24 h, then filtered sequentially through clean muslin cloth and Whatman No. 1 filter paper. The resulting filtrate was concentrated using a hot air oven at 40 °C. The dried extract was stored in a refrigerator at 4 °C in a sterile, airtight bottle until further use in biochemical assays. The percentage yield of the extract was 11.20% (w/w) relative to the initial dry plant material.

Phytochemical Analysis of Aqueous *Newbouldia laevis* Leaf Extract

The presence or absence of some specific phytochemicals (Alkaloids, tannins, flavonoids, phenol, terpenoids, saponins, cardiac glycosides, and steroids) were examined qualitatively and quantitatively on aqueous *Newbouldia laevis* leaf extract, using standard phytochemical screening procedures described by

(Harborne, 1973, 1984, 1998; Sofowora, 1993; Trease & Evans, 2002).

Assessment of *In Vitro* Antioxidant Activity

DPPH Radical Scavenging Assay

The free radical scavenging ability of the crude aqueous *Newbouldia laevis* leaf extract, against DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical was evaluated according to the method of Gyamfi *et al.,* (1999). Two milliliters of the diluted extract (10, 20, 40, and 80 µg/mL) was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 25 minutes and the absorbance was taken at 516 nm using methanol as the blank and reagent blank as the reference. The same procedure was adopted for the reference standard- ascorbic acid.

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power of the crude aqueous leaf extract of *Newbouldia laevis*, was evaluated according to the method of Benzie and Strain, (1996). It is based on the ability of the extract to reduce ferric tripyridyltriazine (Fe (III) TPTZ) complex to ferrous tripyridyltriazine (Fe (II) TPTZ) at low pH. (Fe (III) TPTZ) has an intensive blue colour which can be read at 593 nm. 1.5 ml of freshly prepared FRAP solution, containing 25 ml of 300 mM acetate buffer p H 3.6, 2.5 ml of 10 mM 2,4,6-tripyridylstriazine (TPTZ) in 40 mM HCl and 2.5 ml of 20 mM ferric chloride ($FeCl_3.6H_2O$) solution was mixed with 1 ml of the extract (10, 20, 40, and 80 µg/mL), and the absorbance was read at 593 nm.

The FRAP value of the extract was obtained by plotting a graph of standard curve of FeSO4. $7H_20$ at concentrations between 0 and 1000 µm. Results were expressed in µm Fe (II)/µg extract and compared with that of ascorbic acid.

$$\%\ Radical\ Scavenging\ activity\ (RSA) = \frac{(A_0 - A_1)}{(A_0)} \times 100$$

Nitric Oxide Radical Scavenging Assay

The nitric oxide (NO.) radical scavenging activity of the crude aqueous leaf extract of *Newbouldia laevis*, was evaluated according to the method of Garrat, (1964). In this study, naphthyl ethylenediamine dihydrochloride (0.1% w/v) was used. The reaction mixture containing sodium nitroprusside (10 m M, 2ml), phosphate buffer saline (0.5 ml), and extract solution (10, 20, 40, and 80 µg/mL) was incubated at 25 ºC for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted into new sets of test tubes and mixed with 1 ml sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for complete diazotization. The 1 ml naphthyl ethylenediamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 minutes. A pink-coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against corresponding blank solutions. Vitamin C was used as positive standard.

Results were expressed as percentage radical scavenging activity (% RSA).

$$\%\ Radical\ Scavenging\ activity\ (RSA) = 1 - \frac{Abs\ of\ sample}{Abs\ of\ control}$$

***In Vitro* Evaluation of Hypoglycemic Activities**

Alpha-amylase Inhibition Assay

The alpha-amylase inhibitory activity of the crude aqueous leaf extract of *Newbouldia laevis* was performed according to the method described by Worthington, 1993. Briefly, varying concentrations of the extract as well as the standard drug; acarbose (5 µ g/mL – 100 µ g/mL) were incubated in enzyme porcine pancreatic solution at 37 ℃ for 10 min, the reaction was initiated by further incubation with adding starch solution for another 30 min at 37 ℃. The reaction was terminated with 10 µL of HCl (1 M), followed by colour development with an iodine reagent. Change in absorbances was measured at 580 nm and the per centage inhibitory activity was calculated by using the following equation:

$$\%\ inhibition = \frac{(Abs\ of\ blank) - (Abs\ of\ sample)}{(Abs\ of\ blank)} \times 100$$

α-Glucosidase Inhibitory Activity Assay

The α-glucosidase inhibitory activity of the crude aqueous leaf extract of *Newbouldia laevis* was determined following a standard *in vitro* protocol with minor modifications. α-Glucosidase derived from *Saccharomyces cerevisiae* was dissolved in 100 mM phosphate buffer (0.1 M, pH 6.8) and was used as the enzyme extract. p-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Different concentrations of plant extracts (10 µ g/mL – 30 µ g/mL) were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 ºC for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula (Bray and Greenway, 1999)

$$\%Inhibition = \frac{Abs_{410}(control) - Abs_{410}(extract)}{Abs_{410}(control)} \times 100$$

***In vitro* anti-inflammatory assays**

Protein Denaturation Inhibition Assay

Inhibition of protein denaturation was determined according to the method of Mizushima and Kobayashi, 1968. The reaction mixture contained different concentrations of the extract and 1% BSA (aqueous solution). Aspirin was used as the positive control. The samples were heated at 55 ◦ C for 30 min and allowed to cool. The turbidity of the samples was measured at 660 nm. The percentage inhibition of protein denaturation was calculated as follows:

$$\%\ Inhibition = \frac{Abs\ of\ blank - Abs\ of\ sample}{Abs\ of\ blank} \times 100$$

Proteinase Inhibition Assay

The proteinase inhibitory assay was performed as described by Oyedepo and Femurewa, 1995. The reaction mixture (2 mL) contained 0.06 mg trypsin, 1 mL Tris-HCl buffer and 1 mL of the extract at different concentrations. Aspirin was used as the positive control. The reaction mixture was incubated at 37 ℃ for 5 mins followed by the addition of 0.8% (w/v) casein and incubated for 20 mins. Perchloric acid (2 mL of 70%) was added to stop the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 210 nm against Tris-HCl buffer as blank.

$$\%\ Inhibition = \frac{Abs\ of\ blank - Abs\ of\ sample}{Abs\ of\ blank} \times 100$$

Statistical Analysis

All analysis were conducted in triplicate and results expressed as Mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey post-hoc test with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1: Qualitative and Quantitative Phytochemical Composition of the Crude Aqueous Leaf Extract of *Newbouldia*

Phytochemical parameter	Qualitative	Quantitative (mg/100g)
Alkaloids	++	12.52±0.015
Tannins	+	3.42±0.025
Flavonoids	+	8.62±0.020
Phenols	+	1.54±0.015
Terpenoids	+	0.93±0.017
Saponins	+	5.95±0.020
Cardiac glycosides	+	0.82±0.020
Steroids	+	0.21±0.017

Key: ++ = moderate amount, + = low amount

Phytochemical screening of the aqueous leaf extract of *Newbouldia laevis* (Table 1) revealed a diverse array of bioactive constituents. Alkaloids were present in moderate amounts (12.52 mg/100 g), whereas flavonoids (8.62 mg/100 g), saponins (5.95 mg/100 g), tannins (3.42 mg/100 g), phenols (1.54 mg/100 g), terpenoids (0.93 mg/100 g), cardiac glycosides (0.82 mg/100 g), and steroids (0.21 mg/100 g) were detected in low amounts.

Table 2: Half-maximal inhibitory concentrations (IC_{50}) of the crude aqueous leaf extract of *Newbouldia laevis* in *in vitro* antioxidant and anti-inflammatory assays

Assay	Extract IC_{50} (µg/ml)	Standard	Standard IC_{50} (µg/ml)	Activity Classification
DPPH radical scavenging	6.33	BHT	6.05	Strong antioxidant
Nitric oxide scavenging	28.45	Gallic acid	7.06	Moderate antioxidant
FRAP (reducing power) *	7.73	Ascorbic acid	6.54	Strong reducing capacity
Protein denaturation inhibition	89.63	Aspirin	81.69	Moderate anti-inflammatory
Proteinase inhibitory assay	80.45	Aspirin	67.67	Moderate anti-inflammatory
α-Amylase inhibition	9.29	Acarbose	3.17	Strong enzyme inhibition
α-Glucosidase inhibition	17.88	Acarbose	6.37	Moderate enzyme inhibition

DPPH; 1, 1-diphenyl-2-picrylhydrazyl, FRAP; ferric reducing antioxidant power

The crude aqueous leaf extract of *Newbouldia laevis* demonstrated notable antioxidant and anti-inflammatory activities across multiple *in vitro* models (Table 2). The extract exhibited strong radical scavenging activity in the DPPH assay (IC_{50} = 6.33 µg/ml), comparable to BHT (6.05 µg/ml). In contrast, nitric oxide scavenging activity was moderate (IC_{50} = 28.45 µg/ml) relative to gallic acid (7.06 µg/ml). The FRAP assay indicated substantial reducing power (IC_{50} = 7.73 µg/ml), closely approaching that of ascorbic acid (6.54 µg/ml). Anti-inflammatory potential assessed via protein denaturation and proteinase inhibition assays yielded IC_{50} values of 89.63 µg/ml and 80.45 µg/ml, respectively, which were higher than those of aspirin. Furthermore, the extract demonstrated appreciable antidiabetic potential via α-amylase (IC_{50} = 9.29 µg/ml) and α-glucosidase inhibition (IC_{50} = 17.88 µg/ml), although less potent than acarbose.

Discussion

The present study provides a systematic evaluation of the *in vitro* antioxidant, anti-inflammatory, and antidiabetic potentials of the crude aqueous leaf extract of *Newbouldia laevis*, supported by its phytochemical composition. Phytochemical analysis revealed that the extract contains a diverse array of bioactive constituents, including alkaloids, flavonoids, tannins, phenols, saponins,

terpenoids, cardiac glycosides, and steroids. This finding corroborates the report of Osigwe *et al*. (2017). Alkaloids were present in moderate amounts, while other bioactive compounds were detected in lower concentrations. These classes of secondary metabolites are well-documented for their pharmacological activities. For example, flavonoids and phenolic compounds are potent free radical scavengers and metal chelators (Tungmunnithum *et al.,* 2018), contributing to the extract's strong antioxidant activity observed in DPPH and FRAP assays. Saponins, terpenoids, and alkaloids can modulate enzymatic pathways and inflammatory cascades (Singh *et al.,* 2022), providing a mechanistic basis for the moderate anti-inflammatory activity evidenced by protein denaturation and proteinase inhibition.

The extract exhibited strong radical scavenging and reducing power, which are indicative of its ability to donate electrons or hydrogen atoms to neutralize reactive oxygen species (Chandimali *et al.,* 2025). Reactive oxygen species (ROS) are implicated in oxidative stress-mediated tissue injury and the progression of chronic metabolic disorders (Lindsay and Rhodes, 2025). Moderate nitric oxide scavenging further suggests the extract can mitigate nitrosative stress, a key factor in inflammation and vascular dysfunction (Venkatesan *et al.,* 2026). The antioxidant activity is consistent with the presence of flavonoids, phenols, and tannsins, which act synergistically to stabilize radicals and chelate transition metals (Tungmunnithum *et al.,* 2018). The present results align with the findings documented by Lawal *et al*. (2022) and Alqahtani *et al*. (2019).

Inflammation is a central component in the pathogenesis of metabolic syndrome, diabetes, and cardiovascular disorders (Sun *et al.,* 2021). The extract's inhibitory effects on protein denaturation and proteolytic activity suggest that its bioactive constituents could stabilize protein structures and inhibit inflammatory enzymes, such as proteases. Alkaloids, saponins, and terpenoids are known to suppress inflammatory mediators and modulate NF-κB signaling (Singh *et al.,* 2022), providing a plausible mechanistic explanation for the observed anti-inflammatory effects. Although the extract exhibited lower potency than aspirin, its moderate activity suggests a therapeutically relevant profile, particularly as a multi-target natural agent with potentially fewer side effects. These findings are consistent with reports by Lawal *et al*. (2022) and Alqahtani *et al*. (2019).

Carbohydrate-hydrolyzing enzyme inhibition is a validated approach for controlling postprandial hyperglycemia (Mohammed *et al.,* 2025). The extract demonstrated α-amylase and α-glucosidase inhibitory activity, although lower than acarbose. These results suggest that the extract could reduce starch and disaccharide hydrolysis, thereby attenuating glucose absorption. Flavonoids and saponins may have contributed to these effects by binding to the enzymes' active sites or by modifying the substrate's affinity. The extract's dual antioxidant and enzyme-inhibitory activities could indicate a synergistic mechanism, targeting both oxidative stress and carbohydrate metabolism, a strategy increasingly recognized in diabetes management. Similar results have been reported by Lawal *et al*. (2022) and Alqahtani *et al*. (2019).

The above findings highlight the synergistic multi-target potential of the extract, driven by diverse phytochemicals acting on complementary pathways. These metabolites may have modulated oxidative stress, inflammatory signaling, and carbohydrate metabolism, producing enhanced therapeutic effects. Such synergy may improve efficacy at lower doses while reducing toxicity, supporting *Newbouldia laevis* as a promising holistic agent for managing diabetes and metabolic syndrome.

Furthermore, the observed pharmacological activities provide scientific support for the traditional use of *N. laevis* leaves in managing diabetes, inflammation, and oxidative stress-related disorders. The combination of antioxidants, anti-inflammatory agents, and enzyme inhibitors makes the extract a promising candidate for multi-target interventions, consistent with the holistic approach of herbal medicine.

CONCLUSION

The crude aqueous leaf extract of *Newbouldia laevis* exhibits robust antioxidant, moderate anti-inflammatory, and notable antidiabetic activities, reinforced by its rich phytochemical composition. These findings validate its ethnomedicinal applications and highlight its potential as a natural multi-target therapeutic agent. Further *in vivo* and mechanistic studies are necessary to identify active constituents and optimize its pharmacological potential.

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REFERENCES

Alqahtani, A. S., Hidayathulla, S., Rehman, M. T., ElGamal, A. A., Al-Massarani, S., Razmovski-Naumovski, V., Alqahtani, M. S., El Dib, R. A., & 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

Ansari, P., Khan, J. T., Chowdhury, S., Reberio, A. D., Kumar, S., Seidel, V., Abdel-Wahab, Y. H. A., & Flatt, P. R. (2024). Plant-based diets and phytochemicals in the management of diabetes mellitus and prevention of its

complications: A review. *Nutrients, 16*(21), 3709. https://doi.org/10.3390/nu16213709

Antar, S. A., Ashour, N. A., Sharaky, M., Khattab, M., Ashour, N. A., Zaid, R. T., Roh, E. J., Elkamhawy, A., & Al-Karmalawy, A. A. (2023). Diabetes mellitus: Classification, mediators, and complications: A gate to identify potential targets for the development of new effective treatments. *Biomedicine & Pharmacotherapy, 168*, 115734. https://doi.org/10.1016/j.biopha.2023.115734

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bray, G. A., & Greenway, F. L. (1999). Pharmacological treatment of the overweight patient. *Endocrine Reviews, 20*, 805–775.

Chandimali, N., Bak, S. G., Park, E. H., Lim, H.-J., Won, Y.-S., Kim, E.-K., Park, S.-I., & Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: A review. *Cell Death Discovery, 11*, 19. https://doi.org/10.1038/s41420-024-02278-8

Du, C., & Zhao, Z. (2025). Characteristic analysis of adverse reactions to natural and synthetic alpha-glucosidase inhibitors: A descriptive analysis using World Health Organisation–VigiAccess. *Journal of Chromatography B, 1267*, 124800. https://doi.org/10.1016/j.jchromb.2025.124800

Garrat, D. C. (1964). *The quantitative analysis of drugs* (3rd ed., pp. 456–458). Chapman and Hall.

Gyamfi, M. A., Yonamine, M., & Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. *General Pharmacology, 32*, 661–667.

Harborne, J. B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis* (pp. 40–75). Chapman & Hall.

Harborne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis* (2nd ed.). Chapman & Hall.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (2nd ed., pp. 88–185). Chapman & Hall.

Hossain, M. J., Al-Mamun, M., & Islam, M. R. (2024). Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports, 7*(3), e2004. https://doi.org/10.1002/hsr2.2004

Iwu, M. M. (2000). *Handbook of African medicinal plants*. CRC Press.

Lawal, B., Sani, S., Onikanni, A. S., Ibrahim, Y. O., Agboola, A. R., Lukman, H. Y., Olawale, F., Jigam, A. A., Batiha, G. E., Babalola, S. B., Mostafa-Hedeab, G., Lima, C. M. G., Wu, A. T. H., Huang, H. S., & Conte-Junior, C. A. (2022). Preclinical anti-inflammatory and antioxidant effects of *Azanza garckeana* in STZ-induced glycemic-impaired rats, and pharmacoinformatics of its major phytoconstituents. *Biomedicine & Pharmacotherapy, 152*, 113196. https://doi.org/10.1016/j.biopha.2022.113196

Lindsay, R. T., & Rhodes, C. J. (2025). Reactive oxygen species (ROS) in metabolic disease—Don't shoot the metabolic messenger. *International Journal of Molecular Sciences, 26*(6), 2622. https://doi.org/10.3390/ijms26062622

Maffettone, A., Rinaldi, M., & Fontanella, A. (2018). Postprandial hyperglycemia: A new frontier in diabetes management? *Italian Journal of Medicine, 12*(2), 108–115. https://doi.org/10.4081/itjm.2018.961

Mizushima, Y., & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology, 20*, 169–173.

Mohammed, A., Saidu, B. S., Aliyu, N., & Simon, M. P. (2025). Antioxidant activity and inhibition of carbohydrate-hydrolyzing enzymes by underutilized parts from some plant-based foods and their effect on postprandial hyperglycemia. *Food Chemistry International*, 1–10. https://doi.org/10.1002/fci2.70045

Ogunlesi, M., Okiei, W., Ofor, E., & Awonuga, O. (2009). Determination of the concentration of zinc and vitamin C in oysters and some medicinal plants used to correct male factor infertility. *Journal of Natural Products, 2*, 89–97.

Osigwe, C. C., Akah, P. A., & Nworu, C. S. (2017). Biochemical and haematological effects of the leaf extract of *Newbouldia laevis* in alloxan-induced diabetic rats. *Journal of Biosciences and Medicines, 5*, 18–36. https://doi.org/10.4236/jbm.2017.56003

Oyedapo, O., & Famurewa, A. J. (1995). Antiprotease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Olax subscorpioides* and *Tetrapleura*

tetraptera. *International Journal of Pharmacognosy, 33*, 65–69.

Panjamurthy, K., Manoharan, S., & Ramachandran, C. R. (2005). Lipid peroxidation and antioxidant status in patients with periodontitis. *Cellular and Molecular Biology Letters, 10*, 255–264.

Singh, S., Bansal, A., Singh, V., Chopra, T., & Poddar, J. (2022). Flavonoids, alkaloids and terpenoids: A new hope for the treatment of diabetes mellitus. *Journal of Diabetes & Metabolic Disorders, 21*(1), 941–950. https://doi.org/10.1007/s40200-021-00943-8

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd.

Sun, Y., Rawish, E., Nording, H. M., & Langer, H. F. (2021). Inflammation in metabolic and cardiovascular disorders—Role of oxidative stress. *Life, 11*(7), 672. https://doi.org/10.3390/life11070672

Thenmozhi, V., Elango, V., & Sadique, J. (1989). Anti-inflammatory activity of some Indian medicinal plants. *Ancient Science of Life, 8*, 258.

Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders Publishers.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines, 5*(3), 93. https://doi.org/10.3390/medicines5030093

Venkatesan, S., Smirne, C., Aquino, C. I., Surico, D., Remorgida, V., Ola Pour, M. M., Pirisi, M., & Grossini, E. (2026). Nitric oxide signaling in cardiovascular physiology and pathology: Mechanisms, dysregulation, and therapeutic frontiers. *International Journal of Molecular Sciences, 27*(2), 629. https://doi.org/10.3390/ijms27020629

Worthington, K. (1993). Alpha-amylase. In *Worthington enzyme manual* (pp. 36–41). Worthington Biochemical Corporation.