GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Pharmacognostic and phytochemical analysis, and evaluation of *Newbouldia laevis* and *Garcinia kola* on advanced glycation and carbohydrate metabolizing enzyme

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GSC Biological and Pharmaceutical Sciences, 2025, 30(03), 372-384

Publication history: Received on 29 January 2025; revised on 09 March 2025; accepted on 11 March 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.30.3.0066

Abstract

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, leading to complications such as oxidative stress and inflammation due to the formation of Advanced Glycation End Products (AGEs). The inhibition of AGEs formation and the modulation of carbohydrate-metabolizing enzymes, particularly α-amylase, are key therapeutic strategies in diabetes management.

Objective: This study evaluates the pharmacognostic and phytochemical properties of *Newbouldia laevis* and *Garcinia kola* and investigates their effects on AGE formation and amylase inhibition.

Results: Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, terpenoids, and other bioactive compounds, which contribute to their pharmacological properties. The study demonstrated a progressive decrease in IC50 values for AGEs inhibition, with G. kola showing significant efficacy over four weeks. Similarly, the combination of G. kola and N. laevis exhibited enhanced amylase inhibition compared to individual extracts, suggesting potential synergistic effects.

Conclusion: These findings provide insights into the traditional use of these plants in diabetes management and highlight their potential as natural therapeutic agents for controlling postprandial hyperglycemia and preventing glycation-related complications.

Keywords: Diabetes; *Newbouldia laevis*; *Garcinia kola*; AGE; Amylase

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). A significant consequence of prolonged hyperglycemia is the non-enzymatic glycation of proteins, leading to the formation of advanced glycation end-products (AGEs) (2). AGEs contribute to the pathogenesis of various diabetic complications, including neuropathy, nephropathy, and retinopathy, by promoting oxidative stress and inflammation (3). Additionally, the activity of carbohydrate-metabolizing enzymes, such as α-

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amylase and α-glucosidase, plays a crucial role in postprandial blood glucose levels. Inhibiting these enzymes can effectively manage postprandial hyperglycemia, a therapeutic strategy in diabetes management (4,5).

Medicinal plants have been explored for their potential to inhibit AGE formation and modulate carbohydrate-metabolizing enzymes (6). *Newbouldia laevis* and *Garcinia kola* are two such plants traditionally used in African medicine for various ailments. Phytochemical analyses have revealed that *Newbouldia laevis* leaves are rich in flavonoids, saponins, and other bioactive compounds, which may contribute to its pharmacological activities, including anti-inflammatory and antioxidant effects (7). Similarly, *Garcinia kola* seeds contain bioactive compounds with significant pharmacological properties, such as antimicrobial, anti-inflammatory, and antidiabetic activities (8).

Previous studies have indicated that extracts from these plants exhibit antidiabetic properties. However, the specific mechanisms underlying their antidiabetic effects, particularly concerning the inhibition of AGE formation and modulation of carbohydrate-metabolizing enzymes, remain underexplored.

This study aims to conduct a comprehensive pharmacognostic and phytochemical analysis of *Newbouldia laevis* and *Garcinia kola*, focusing on their potential inhibitory effects on AGE formation and key carbohydrate-metabolizing enzymes. Understanding these mechanisms may provide insights into their traditional use in managing diabetes and offer potential avenues for developing novel therapeutic agents.

2. Methods

2.1. Extraction

The powdered plants (3 kg each) was extracted separately with distilled water (10 L) at 40°C for 24 h under constant shaking. Filtration was done thereafter using muslin cloth. The filtrate was concentrated using Lyoquest Plus 55 Freeze dryer at -40°C. The percentage yield was determined. The dried extract was stored in a refrigerator at 4°C

2.2. Qualitative Phytochemical Analysis

The qualitative phytochemical, physicochemical, and microscopic analyses were carried out using standard methods described by Odoh et al. (2019) (9)

- Test for Alkaloids: 0.5 g of each of the plant extracts was placed in labeled test tubes and warmed in 10 mL of 5% HCl solution in a hot water bath for about 2 min. The mixture was allowed to cool and filtered using filter paper. The resulting filtrates were used for the following tests.
- Dragendorff's Test: A few drops of Dragendorff's reagent were added to 2 mL of the filtrates and mixed by shaking. The formation of a red/reddish brown precipitate indicated the presence of alkaloids.
- Wagner's Test: A few drops of Wagner's reagent were added to 2 mL of the filtrates and mixed by shaking. The formation of a red/ reddish brown precipitate indicates the presence of alkaloids.
- Mayer's Test: A few drops of the Mayer reagent were added to 2 mL of the filtrates and mixed by shaking. The formation of a white/milk-coloured precipitate indicates the presence of alkaloids.
- Test for Saponins: A 10 mL volume of distilled water was added to 0.5 g of the plant extracts, boiled in a hot bath for 2 min and filtered. The filtrates were allowed to cool and then used for the following tests;
- Frothing Test: A 5 mL volume of each of the samples were placed in labeled test tubes and then diluted with 5 mL of distilled water; the mixtures were shaken vigorously and observed for the formation of stable froth
- Emulsion Test: Two drops of olive oil were added to the frothing solution, and the contents were shaken vigorously and observed to form an emulsion.

2.3. Test for Tannins

A 0.5 g of the plant extracts were each boiled in 10 mL of distilled water for 2 min. The solutions were allowed to cool and then filtered using filter paper (Whatman No 1). To 2 mL of each of the samples few drops of 1% Ferric chloride solution was added and observed for dirty green or a blue-black coloration.

2.4. Test for Flavonoids

One gram quantity of the plant extracts was heated in 20 mL of ethyl acetate on a hot water bath for 2 min. The resulting mixtures were cooled and filtered. The filtrates were used for the following tests;

- Shinoda Test: A few magnesium turnings (ribbons) were added to 2 mL of each of the filtrates, followed by 3 drops of concentrated HCl to the inclined test tube containing the filtrate. The test tubes were observed for a pink, scarlet, crimson red, or occasionally green to blue effervescence colour, which indicates the presence of flavonoids.
- Ammonium hydroxide Test: A 2 mL volume of 10% ammonia solution was added to 5 mL each of the filtrates and shaken. Then the two layers were allowed to separate. The test tubes were observed for yellow color at the lower ammoniacal layer, which indicates the presence of flavonoids.
- Test for Steroids: A 0.5 g of the plant extracts was placed in labeled test tubes, 5 mL of chloroform was added to each sample and shaken. The resulting solutions were filtered, and the filtrates were used for the following tests;
- Salkowski Test: A few drops of concentrated H2SO4 solution were added to each of the filtrates. Then, the test tubes were observed for the formation of a reddish-brown color, which indicates the presence of terpenes.
- Liebermann-Burchard Test: Two mL of acetic anhydride was added to each of the filtrates, and three drops of H2SO4 were carefully added to the resulting mixture from the side of the test tubes. The test tubes were observed for the formation of first red and then green colour, which indicates the presence of steroids.

2.5. Test for Terpenoids

Copper acetate Test: The plant extracts were dissolved in methanol individually and the resulting solutions were used for the test. A few drops of copper acetate solution were added to of each of the samples and the test tubes observed for the formation of green colour which indicates the presence of Diterpenes.

2.6. Test for Cardiac Glycosides

0.5 g of the plant extracts were dissolved in 5 mL of methanol individually and were treated with 5 mL each of distilled water and strong lead acetate solution, respectively. The resulting solution was filtered, and 10% H2SO4 solution was added dropwise until no further precipitate was formed. The mixture was filtered and shaken with an equal volume of chloroform. The chloroform part (chloroform layer) was washed with water, separated from the water, and then filtered using a small cotton wool plug. The chloroform part obtained was used for the test.

Keller-Kiliani Test: the plant extract and 0.4 mL of glacial acetic acid containing traces of ferric chloride was added, then the mixture was transferred to a test tube, and carefully, 0.5 ml of concentrated H_2SO_4 solution was poured down the side of the test tube. The test tube was observed for the formation of bluish green color in the upper acetic acid layer and a reddish-brown ring at the interface, which indicates a deoxysugar characteristic of cardenolides.

2.7. Determination of Total Phenolic Content of the Plants by Folin Ciocalteu's Assay

The total phenolic content of the extracts were determined using the method described by Kim *et al*., (2003). One milliliter of the extracts (62.5 µg/mL) was mixed with 0.2 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 1 mL of 7.6% Na_2CO_3 solution was added to the mixture, followed by 2 mL of distilled water. The mixture (in duplicate) was incubated at 40°C for 30 min, after which the absorbance was read at 760 nm using a UV-VIS spectrophotometer (Model 752, China). The total phenolic content was estimated from the calibrated curve made by preparing the gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extracts.

2.8. Qualitative Microscopy

2.8.1. Microscopic examination of fresh plant material

The fresh leaf sample was washed, cut into smaller pieces and placed in 70% chloral hydrate solution in a test tube and heated in a water bath to clear the cells. The cleared leaf sample was then placed on a slide and viewed under the microscope

2.8.2. Microscopic Examination of Powdered Leaves

A small quantity of the powdered crude drug was placed on a slide and few drops of chloral hydrate solution were added to it. The mixture was passed across the flame of a Bunsen burner repeatedly until bubbles occurred and allowed to cool for proper clearing of the sample. Two drops of glycerine were added to the slide as mountant, and the slide was covered with a cover slip and viewed under the microscope. The microscopic characters were observed and noted

2.8.3. Transverse Section of the Leaf

A sledge microtome machine was used to get a thin transverse section of a fresh leaf that was collected in a petri dish containing 70% ethanol.

The sectioned materials were transferred into a staining jar containing safranin solution and allowed to stand for 5 min after which the safranin was drained off and the section washed 3 times in distilled water. The section was washed twice in 97% alcohol and rewashed with absolute ethanol for additional two times to achieve dehydration.

The section was counterstained in 1% fast green for 5 min and washed in absolute alcohol and clove oil at a ratio of 3:1 for 3-4 times at a 2-minute interval. The section was transferred into another staining jar containing a 50/50 alcohol/xylene solution for preliminary clearing. Pure xylene was finally used to clear the section, and Canada balsam was used as a mountant for permanent slide preparation of the sectioned leaf material.

2.8.4. Inhibition of Advanced glycation end product (AGE) formation

The glycated BSA formation was determined using a method previously described by Adisakwattana *et al*., 2012 (10) with slight modification. Bovine serum albumin (BSA) was used as the model protein in a concentration of 40 mg/mL corresponding to physiological albumin concentration in human blood; similarly, 0.5 M of glucose was used as the glycating agents. Briefly, BSA (40 mg/mL) was incubated with 0.5M glucose in 0.1 M phosphate buffer saline (PBS) (pH 7.4), containing0.02 % sodium azide in the dark at 37 °C for 1, 2, 3, and 4 weeks. Before incubation, the solution containing the extracts and their combinations dissolved in PBS were added to the mixtures. All incubations were performed under sterile conditions. A small drop of chloroform was added to the solution and the corks were moistened with toluene to inhibit bacterial growth. The glycated BSA formation was measured by using fluorescent intensity at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Aminoguanidine (AG, 1 mg) was used as a positive control for the study.

$$\text{AGE inhibition (\%)} = [1 - \frac{Fs - Fsb}{Fc - Fcb}] \times 100$$

Where Fs – Fsb is the difference between the fluorescent intensity of sample + BSA incubated with or without glucose while Fc - Fcb is the difference between the fluorescent intensity of BSA incubated with or without glucose.

2.9. Inhibition of carbohydrate metabolizing enzyme

2.9.1. Measurement of α-amylase enzyme inhibition by starch-iodine assay

This method is based on the binding of iodine to terminals of the starch's polymeric chain, resulting in a blue-colored complex that can also be quantitatively monitored by UV–vis-spectrophotometry (11). The dark blue color indicates the presence of starch only and the yellow color indicates the absence of the starch. The brown color indicates the partially degraded starch in the reaction mixture. The ability of the extracts and their combinations to inhibit alpha-amylase enzyme was analyzed by the iodine-starch method as described by the standard protocol of Ferosekhan *et al*., 2016 (12), with slight modification.

Preparation of iodine solution (5 mM)

At the beginning, 5 mM potassium iodide (KI) was prepared by dissolving 0.208 grams in distilled water in a 250 mL volumetric flask. Then, 5 mM iodine solution was prepared by dissolving 0.0634 grams of iodine in 2 mL of ethanol, and finally, 100 mL iodine solution was prepared by dissolving in KI solution.

Preparation of phosphate buffer (0.02M)

Initially, 0.04 M Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) and (0.04 M) sodium dihydrogen phosphate ($NaH_2PO4.2H_2O$) solution were prepared by dissolving 0.712 gram and 0.624 gram in 100 mL of distilled water respectively. Phosphate buffer (0.02 M) was prepared by mixing 50 mL of 0.04 M disodium hydrogen phosphate and 50 mL of0.04M sodium dihydrogen phosphate. Then the mixture of phosphate solution was stabilized by further adding 0.072 gram of sodium chloride (NaCl) and P^H6.9 was adjusted. Finally, a mixture of phosphate buffer was diluted with 100 mL of distilled water to obtain 200 mL of 0.02 M phosphate buffer.

Preparation of 0.5% of a soluble starch solution

The soluble starch solution of 0.5% was prepared by dissolution of 0.05 g of soluble starch in 10 mL phosphate buffer at pH 6.9. The solution was slightly warmed at 50ºC.

Preparation of 0.2 Unit α-amylase enzyme

Under the detailed information given, a stock solution of 9 unit α-amylase was prepared by taking 1 mg in 1 mL phosphate buffer in a vial of 2 mL and diluted to 0.4 unit and then to 0.2 unit.

Preparation of stock solution of the extracts and their combination

Graded doses of the extract and their combinations was prepared using tween 20. Similarly, a standard stock solution of Acarbose was prepared by dissolving 1 mg in 2 mL of Phosphate buffer at pH 6.9. Different concentrations 160, 80, 40, 20, and 10µg/mL were diluted from stock Acarbose solution of 500 µg/mL in phosphate buffer.

Preparation of 1M Hydrochloric acid 5% Tween 20

The actual strength of Hydrochloric was determined from this. 1 M hydrochloric acid was prepared by mixing appropriate quantity of Hydrochloric acid (HCL) in distilled water. Similarly, 5% Tween 20 was also prepared by mixing 0.5 mL of Tween 20 in 9.5 mL distilled water.

Measurement of α-amylase enzyme inhibition by starch-iodine assay

Screening of the extracts and their combinations for α-amylase inhibition was carried out according to the method described by Ferosekhan *et al*., 2016 (12), with slight modifications. To 250 µL of each extract and their combination concentrations in a test tube in triplicates, the following was added sequentially: 250 µL of phosphate buffer (0.02 M, pH 6.9 and containing 6 mM sodium chloride), phosphate-buffered α-amylase (250 µL, 0.05 mg/ml), and starch (250 µL, 1% w/v), and the reaction mixture was incubated for 15 min at 37°C. One molar HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5 mM I2 and 5 mM KI). The color change was noted, and the absorbance was read at 625 nm on a 1 mL cuvette at exactly 1 min after adding the iodine/iodide solution. The control reaction representing 100% enzyme activity contained no test extract or reference standard. To eliminate the absorbance produced by the extracts, appropriate extracts controls without the enzyme were also included. Inhibition of enzyme activity was calculated as follows:

$$\text{Inhibition (\%)} = (C - S) / C \times 100$$

Where S is the absorbance of the sample and C is the absorbance of control.

The concentration of the samples that will result in 50% inhibition of enzyme activity (IC 50)

Was determined graphically.

2.10. Gas Chromatographic-Mass Spectroscopic (Gc-Ms) Assay of Extracts

The GC–MS analysis of extracts was done to find the phytocompounds present using using Agilent Technologies GC systems with GC-220 model (Varian, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high-energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with an increasing rate of 3 °C/min and a holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. The relative quantity of the chemical compounds present in each of the extracts was expressed as a percentage based on the peak area produced in the chromatogram.

2.11. Identification of Phytochemical Constituents of the Extracts

Bioactive compounds from the different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

3. Results

3.1. Phytochemical test

The result of the qualitative phytochemical test is presented in Table 1 below. The result reveals the presence and absence of different classes of phytochemicals.

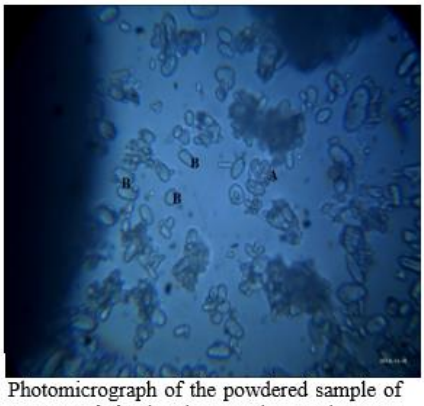
Table 1 Phytochemical test result

Phytocompounds	***G. kola***	***N. laevis***
Saponins	+++	+++
Cardiac glycosides	+++	+++
Tannins	++	++
Flavonoids	+++	++
Alkaloids	-	-
Steroids	+++	+++
Terpenoids	+++	+++
Total Phenolic content (mg/g GAE)	855.82	373.91

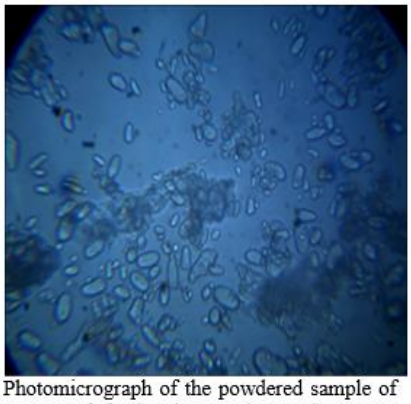
+ = trace; ++ moderately abundant; +++ abundant, - = absent

3.2. Photomicroscopy

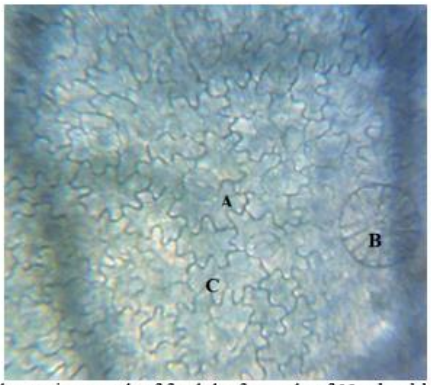
Figures 1 –2 show photomicrographs of some microscopic parameters of the plant samples. G. kola seed revealed the presence of important diagnostic features like starch granules and stone cells, while N. laevis leaf revealed anomocytic stomata, prismatic calcium oxalate crystal, and schizogenous cavity. The transverse section of the leaf revealed the tissue arrangement of a dicot plant (dorsiventral).



Photomicrograph of the powdered sample of *Garcinia kola* showing starch granules. A = Stone cell, B = Starch granules

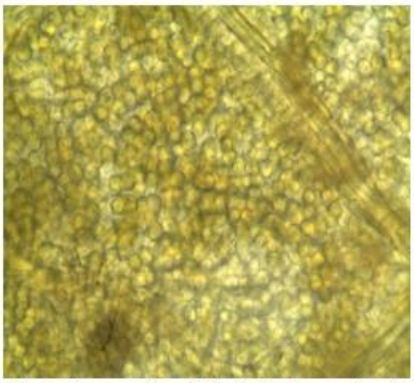


Photomicrograph of the powdered sample of *Garcinia kola* showing starch granules

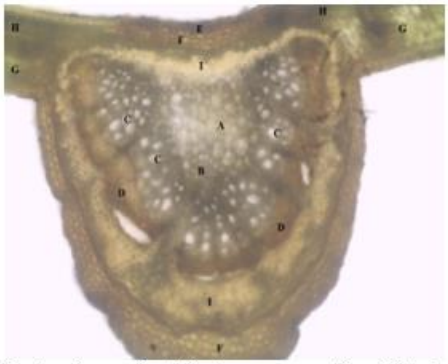


Photomicrograph of fresh leaf sample of *Newbouldia laevis* showing wavy wall epidermal cells, anomocytic stomata and schizogenous cavity. A = Anomocytic stoma, B = Schizogenous cavity, C = Wavy wall epidermal cell

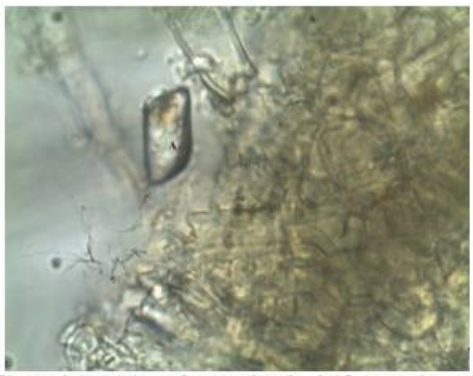
Figure 1 Photomicrograph of powdered *Garcinia kola* and fresh leaf of *Newbouldia laevis*



Photomicrograph of fresh leaf sample of *Newbouldia laevis* showing palisade mesophyll cells of the upper epidermis



Photomicrograph of the transverse section of *Newbouldia laevis* leaf. A = Xylem, B = Phloem, C = Xylem vessel, D = Bundle Sheath, E= Epidermal cells, F = Collenchyma, G = Spongy mesophyll, H= Palisade mesophyll, I = Parenchyma cells



Photomicrograph of powdered leaf sample of *Newbouldia laevis* showing prismatic calcium oxalate crystal. A = Prismatic calcium oxalate crystal

Figure 2 Photomicrograph of fresh leaf of *Newbouldia laevis*

3.3. GC-MS analysis

From the result of the analysis, the compounds from *Newbouldia laevis* extracts with peak area above 4% include: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-(6.22%), (E)-.beta.-Famesene(4.33%), beta. Bisabolene(10.82%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S (R*,S*)]-(7.58%), 1-Piperonyl-3,5-diamino-1,2,4-triazole(4.68%) and (E)-5 (Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one (9.13%). *N. laevis* contains Linalool, aromadendrene, caryophyllene oxide, Linoleic acid

On the other hand, from G. kola seed extract, the 5 compounds with peak area above 4 include: Gamma-Terpinene(4.07%), Heptadecane, 2,6,10,14 tetramethyl(9.44%), Tetradecane(5.13%) and Undecane(8.0%), 2,4-Di-tert-butylphenol(9.27%). Present in G. Kola include gamma-terpene and para-cymene.

3.4. AGE inhibition determination

The AGE inhibition analysis result is presented in Figures 3,4,5 and 6 below. The result shows an increase in the percentage of inhibition with increasing concentration over four weeks. The result of the IC_{50} of the AGE inhibitions is presented in Figure 7 and Table 1. The results show that the IC_{50} decreases over time, except for the *N. laevis*

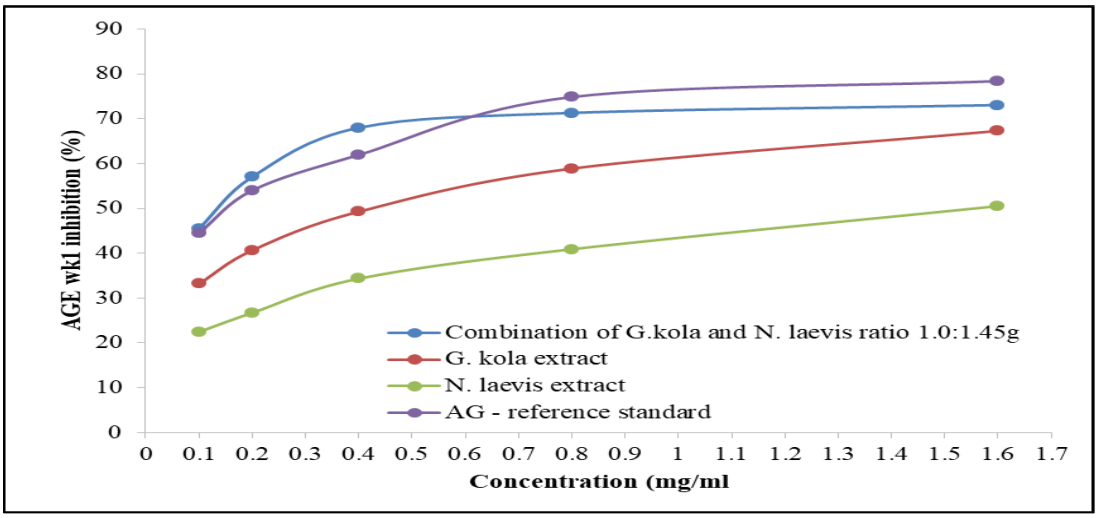


Figure 3 Week one of age inhibition determination

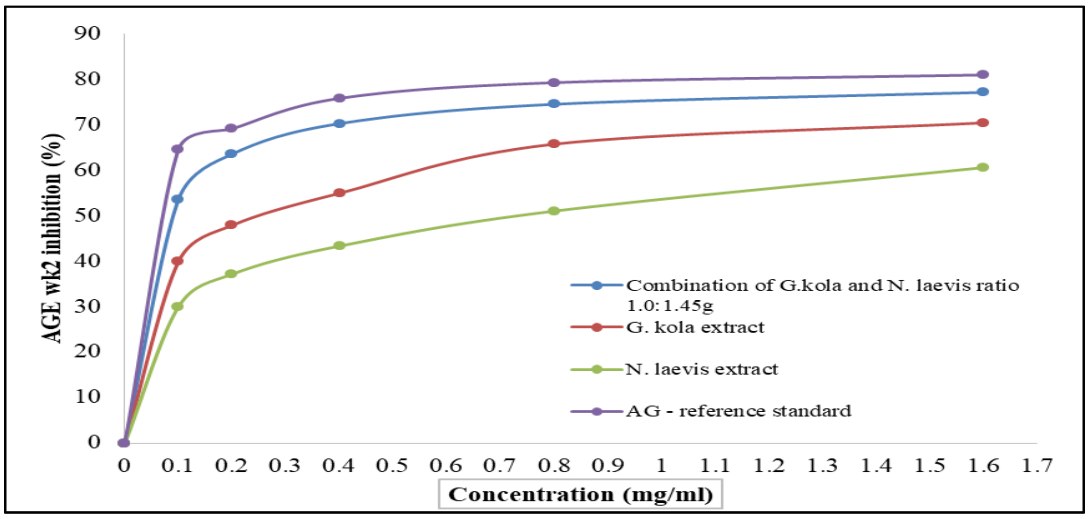


Figure 4 Week two of age inhibition determination

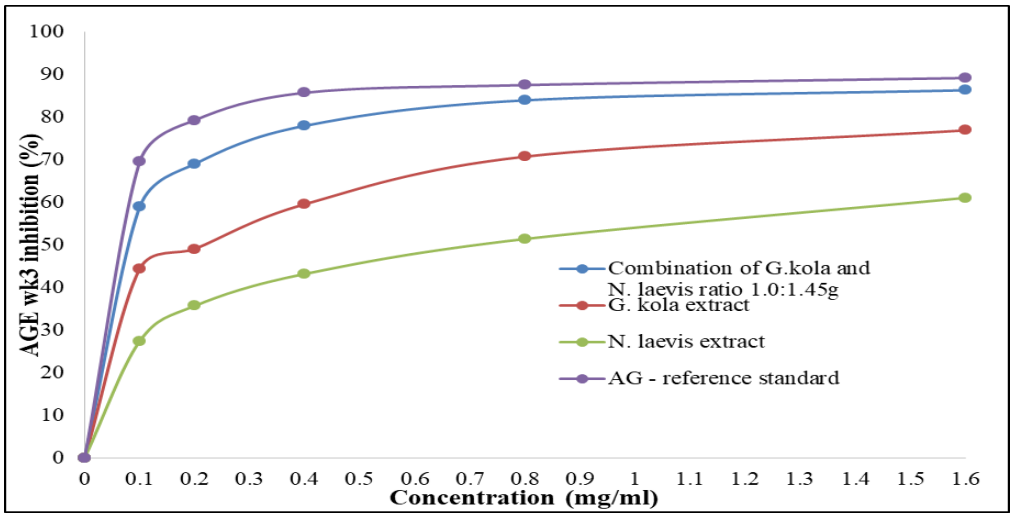


Figure 5 Week three of age inhibition determination

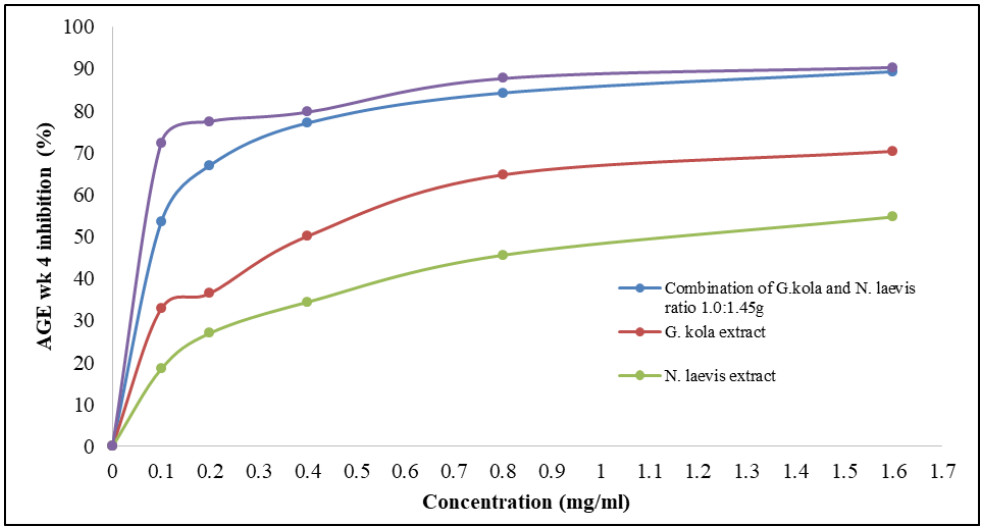


Figure 6 Week four of age inhibition determination

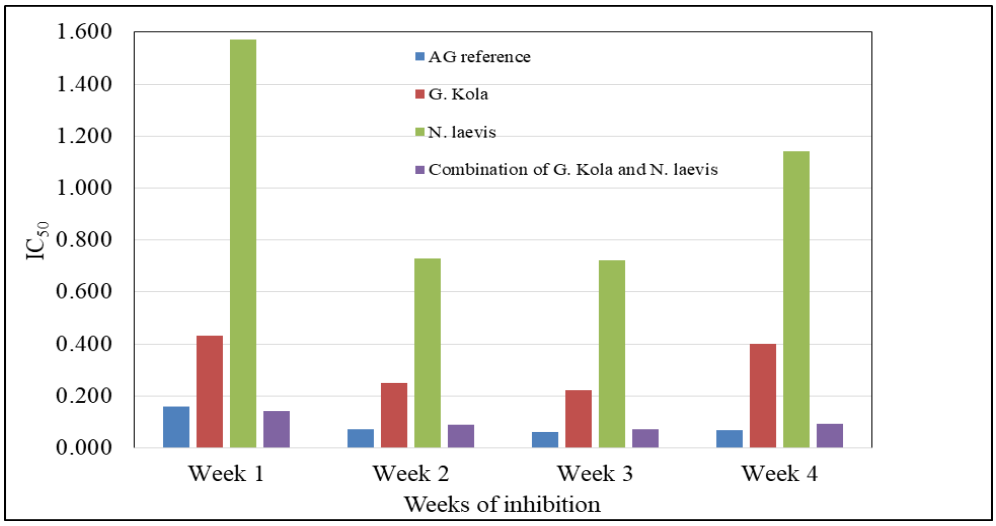


Figure 7 IC_{50} of the AGE inhibitions

Table 2 IC_{50} of the age inhibitions

	AG reference	***G. Kola***	***N. laevis***	**Combination of *G. Kola and N. laevis***
Week 1	0.160	0.430	1.570	0.141
Week 2	0.070	0.250	0.730	0.090
Week 3	0.060	0.220	0.720	0.070
Week 4	0.067	0.400	1.140	0.094

3.5. Amylase inhibition determination

The amylase inhibition analysis result is presented in Figures 8,9,10,11 and 12 below. The result shows an increase in the percentage of inhibition with increasing concentration over four weeks. Figure 12 shows the graphical presentation of the IC50 comparison of the reference, G. kola, *N. laevis,* and a combination of *G. kola* and N. laevis.

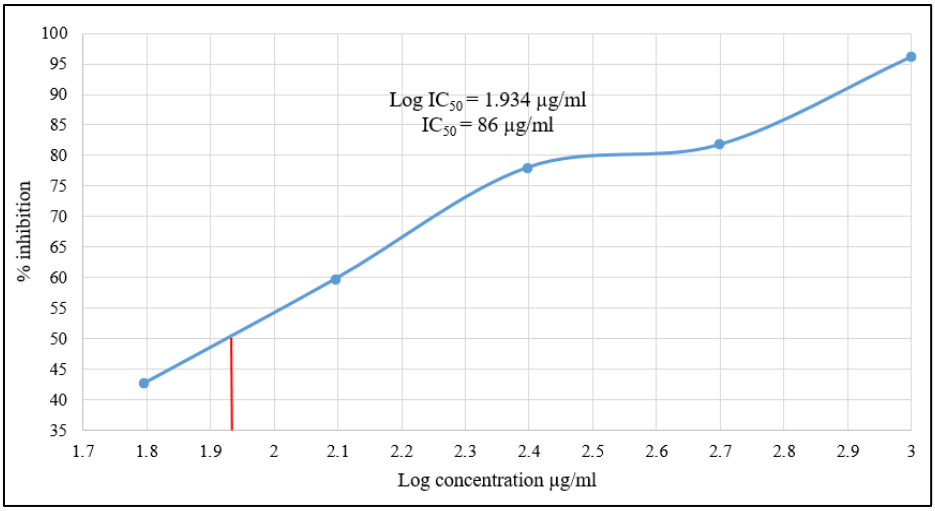


Figure 8 Acarbose amylase inhibition activity

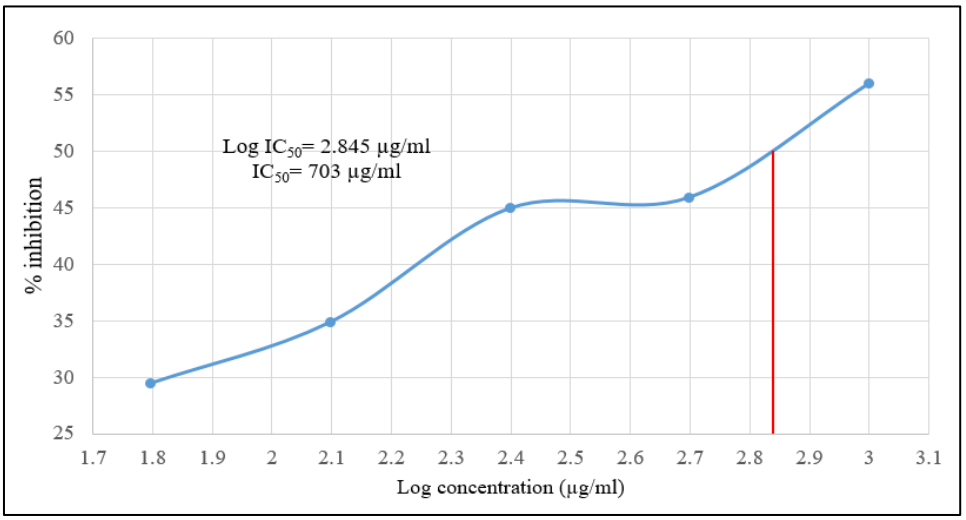


Figure 9 *N. laevis* extract amylase inhibition activity

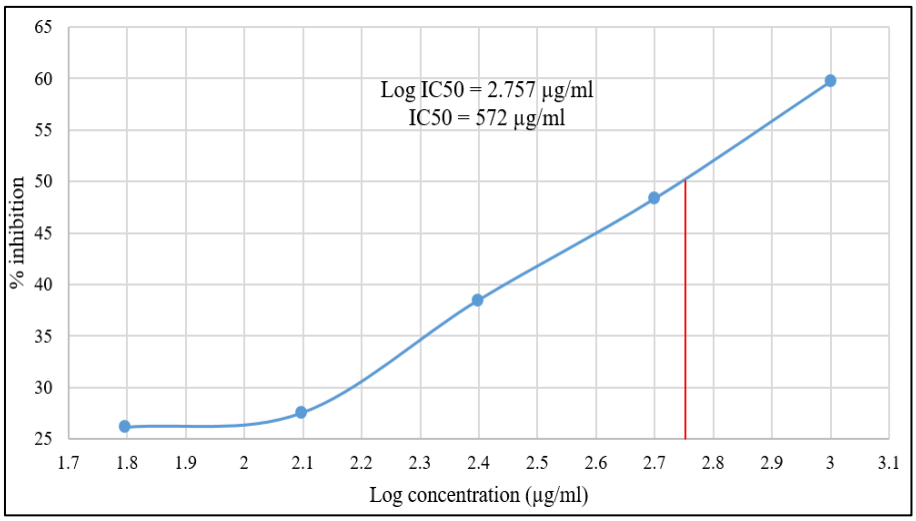


Figure 10 *G. kola* extract amylase inhibition activity

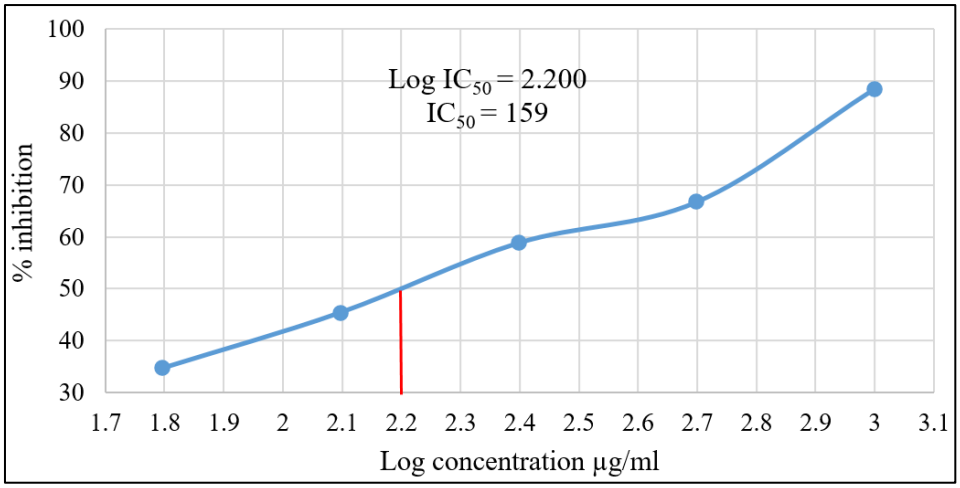


Figure 11 Combination of G.kola and N. laevis ratio 1.0:1.45g amylase inhibition activity

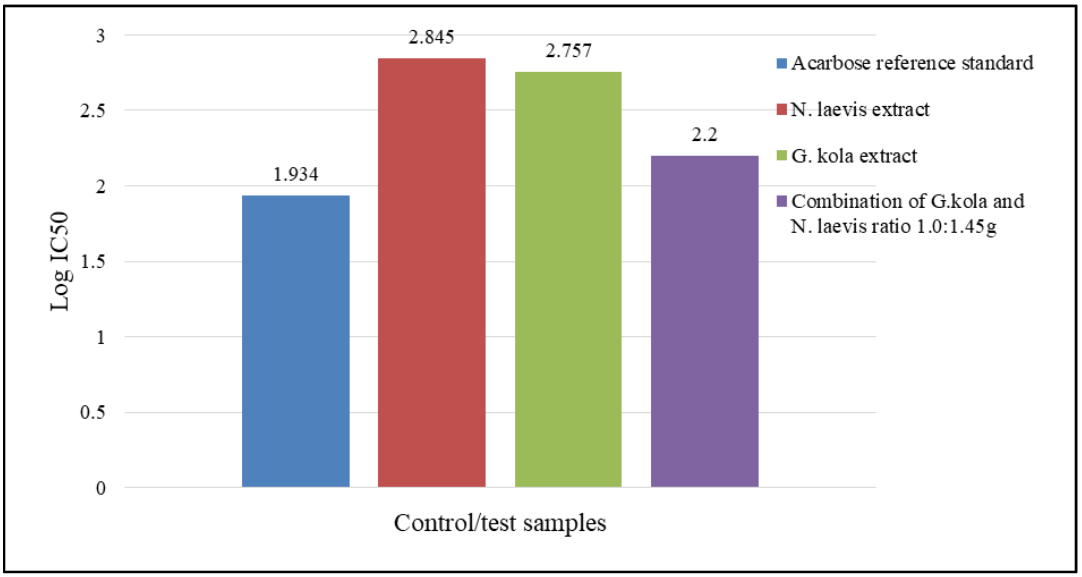


Figure 12 Graphical plot of IC_{50} values

4. Discussion

Advanced glycation end products (AGEs) are potentially harmful heterogeneous molecules of irreversible products derived from nonenzymatic glycation. Reactions involving nonenzymatic glycation occur between the reactive carbonyl group of a reducing sugar and nucleic acids, lipids, or proteins. AGEs can be formed endogenously or provided by exogenous sources under normal and pathological conditions (12). The pathological implications of AGEs are ascribed to their ability to produce reactive oxygen (ROS) and nitrogen (RNS) species, as well as oxidative stress and inflammation, leading to structural and functional protein alterations, cellular dysfunction and apoptosis, and ultimately multitissue/organ injuries3

The assessment of Advanced Glycation End Products (AGEs) inhibition through the administration of herbal extracts in rats over a four-week period reveals significant insights into the efficacy of *Garcinia kola* (*G. kola*) and *Newbouldia laevis* (*N. laevis*). The IC50 values, which represent the concentration required to inhibit 50% of AGEs formation, demonstrate a trend of decreasing values over the weeks, indicating a progressive enhancement in the inhibitory effects of these herbal extracts.

In week one, the IC50 values for G. kola, N. laevis, and their combination were recorded at 0.160, 0.430, and 1.570, respectively, with the reference AG showing an IC50 of 0.141. The notable potency of G. kola in the initial week aligns with findings that highlight its antidiabetic properties and its ability to mitigate oxidative stress, which is crucial in the

context of AGEs formation (13). The phytochemical constituents of G. kola, particularly its flavonoids and tannins, have been linked to its antioxidant capabilities, which may contribute to its effectiveness against AGEs (13).

As the weeks progressed, the IC50 values for G. kola decreased to 0.067 by week four, while N. laevis exhibited a slight increase to 0.400, and the combination showed a decrease to 0.094. This suggests that G. kola maintains its efficacy over time, while N. laevis may require a longer duration to exhibit its full potential. The decline in IC50 values for G. kola is consistent with the literature, which indicates that prolonged exposure to its extracts can enhance its bioactive effects, particularly in the context of glycation inhibition (14). The combination of these two herbs may also indicate a synergistic effect, as suggested by previous studies that have reported enhanced pharmacological activities when combining herbal extracts. The observed results can also be contextualized within the broader framework of glycation and its implications in diabetic complications. AGEs are known to exacerbate conditions such as diabetic retinopathy and nephropathy, and thus, the inhibition of their formation is a critical therapeutic target (15). The protective actions of plant extracts against AGEs have been documented, with specific compounds exhibiting chaperone-like activities that suppress receptor signaling pathways associated with AGE-induced toxicity (14). This aligns with the observed results where the herbal extracts not only inhibit AGEs formation but may also mitigate the downstream effects associated with glycation.

The results of the AGEs inhibition assessment underscore the potential of G. kola and N. laevis as effective herbal interventions in the management of glycation-related complications. The progressive decrease in IC50 values, particularly for G. kola, highlights its sustained efficacy, while the combination with N. laevis suggests a promising avenue for enhanced therapeutic strategies. Future studies should focus on elucidating the specific mechanisms of action and the potential synergistic effects of these herbal extracts in the context of glycation inhibition.

On the other hand, the amylase inhibition activity of *N. laevis* (*Newbouldia laevis*) and *G. kola* (*Garcinia kola*) extracts, both individually and in combination, compared to Acarbose. Acarbose exhibits a known and potent amylase inhibitory effect. It serves as a reference point to evaluate the efficacy of the plant extracts. The N. laevis extract shows amylase inhibition activity. Similarly, G. kola extract also demonstrates amylase inhibition activity. Its IC_{50} value can be compared to that of N. laevis and Acarbose to assess its relative effectiveness. The combination of the two extracts exhibits amylase inhibition. From Figure 12, it can be observed that even though the combined extract has better IC50 than the reference compound, the combined extracts have lower IC50 than the individual extracts, suggesting a possible antagonism effect

5. Conclusion

This study provides scientific validation of the antidiabetic potential of *Newbouldia laevis* and *Garcinia kola*, particularly in their ability to inhibit AGE formation and α-amylase activity. The progressive decline in IC50 values for AGEs inhibition, especially in G. kola, underscores its sustained efficacy, while the combination of both plants demonstrated a synergistic effect on carbohydrate metabolism regulation. The phytochemical composition of these plants, rich in flavonoids, tannins, and terpenoids, may contribute to their antioxidant and antidiabetic properties. These findings suggest that G. kola and N. laevis could be explored further as complementary therapeutic agents in diabetes management. Future studies should focus on in vivo evaluations and the molecular mechanisms underlying their glycation and enzyme inhibitory activities to support their integration into conventional diabetes treatment strategies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest

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