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Total Alkaloid Content, Total Phenolic Content, *In Vitro* Antioxidant Activity, and Preliminary Antiglycemic Potential of the Methanol Extract from Mozambican Wild Fruits of *Vangueria infausta*

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Abstract

This study investigated the antioxidant activity and alpha-amylase inhibitory potential of the methanolic extract from *Vangueria infausta* fruits with the aim of exploring its therapeutic and nutritional applications. Bioactive compounds such as total phenolics (TPC) and total alkaloids (TAC) were analyzed. Antioxidant activity was assessed using the DPPH, ABTS, FRAP, Ferrozine, Phosphomolybdate, and Folin-Ciocalteu methods, while alpha-amylase inhibitory activity was determined using the 3,5-Dinitrosalicylic Acid (DNSA) and 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) methods. The results revealed significant variations in antioxidant activity across the methods used, with IC_{50} values ranging from 318.42 to 1255.56 µg/mL. Alpha-amylase inhibition was also significant, with IC_{50} values ranging from 140.85 to 121.06 µg/mL. The TPC was found to be 95.6 mg of gallic acid equivalent per 100 g of dry powder, and the TAC was 0.188 mg of atropine equivalent per 100 g of dry powder. These findings suggest that *Vangueria infausta* possesses promising antioxidant and alpha-amylase inhibitory potential, indicating its potential application in antioxidant therapies and in the management of carbohydrate digestion.

Keywords

Vangueria infausta, Antioxidant Activity, Alpha-Amylase Inhibition, TPC, TAC

1. Introduction

Diabetes mellitus is a complex metabolic condition characterized by chronic hyperglycemia resulting from defects in insulin secretion or action [1]. Among its forms, type 2 diabetes is the most prevalent and can often be managed by reducing postprandial hyperglycemia through the inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase [2] [3]. Diabetes management involves a balanced diet, regular exercise, constant glucose monitoring, medication as prescribed, education about the disease, and regular health care to prevent complications [4].

Acarbose, an alpha-amylase inhibitor, is widely used for this purpose [5]. However, the search for plant-derived alpha-amylase inhibitors has gained prominence due to their therapeutic potential, with high efficacy and fewer side effects [6]. Medicinal plants not only offer a therapeutic alternative with fewer side effects, reduced costs, and sustainability but also promote biodiversity and the potential for new pharmaceutical discoveries [7]-[10]. Recent research has demonstrated that extracts from polyphenol-rich fruits significantly impact the inhibition of enzymes such as alpha-amylase and alpha-glucosidase, suggesting their use as natural antidiabetic agents [11] [12]. For instance, studies show that strawberry and raspberry extracts are particularly effective in inhibiting alpha-amylase, while blueberry and blackcurrant extracts exhibit greater inhibitory activity against alpha-glucosidase [11]. Additionally, the antioxidant activity of medicinal plants attracts considerable scientific interest, given its crucial role in neutralizing free radicals and preventing various chronic diseases [13] [14].

Vangueria infausta, native to various regions of Africa, is traditionally valued for its therapeutic properties. Its fruits are particularly recognized for their nutraceutical potential [15]-[17]. Although some studies have reported on the quantitative phytochemistry, as well as the antioxidant [15] [18] and anti-inflammatory activities *in vitro* of *V. infausta* fruits, additional research is still needed to explore other potential biological activities of these fruits. To date, the quantification of total alkaloids and the evaluation of the antidiabetic activity *in vitro* of *V. infausta* fruits have not been reported. Thus, this work offers a valuable opportunity to establish primary information on the antiglycemic activity of *V. infausta* fruits, opening new perspectives for future research on the applicability of these fruits. This could contribute to the validation of traditional claims and the identification of other potential health benefits.

2. Materials and Methods

2.1. Total Alkaloid Content (TAC)

For the extraction of TAC, 10 g of dried pulp powder was extracted for 8 hours using a Soxhlet apparatus. The resulting extract was filtered and concentrated using a rotary evaporator, and the residue was dissolved in 2% H_2SO_4. The solution was washed three times with 50 mL of diethyl ether. The aqueous phase was then alkalinized with ammonia to pH 9, and the TAC was extracted with 3 × 50 mL of chloroform. The chloroform phase was dried with anhydrous sodium sulfate and

concentrated to dryness, being redissolved in hydrochloric acid at pH 2.5. The TAC was then analyzed using a UV/visible spectrophotometric method based on the reaction with Bromocresol Green (BCG) at 470 nm [19], and the TAC was expressed as mg equivalents of atropine sulfate per 100 grams of powder.

2.2. Total Phenolic Content (TPC)

For the extraction of TPC, 0.5 g of powder was extracted with 25 mL of methanol (90%) and agitated for 30 minutes. The mixture was centrifuged at 4000 rpm for 20 minutes at 40˚C, decanted, and filtered. The volume was adjusted to 25 mL with ultrapure water. The TPC was then measured using the Folin-Ciocalteu spectrophotometric assay at 760 nm [20] and expressed as mg equivalents of gallic acid per 100 g of dry powder.

2.3. Alpha-Amylase Inhibition (DNSA Method)

Alpha-amylase inhibition was conducted according to the method of [21], with minor modifications. Briefly, 0.5 mL of extract (0.8 mg/mL - 4 mg/mL) or acarbose (50 µg/mL - 500 µg/mL) was mixed with 200 µL of alpha-amylase solution and 400 µL of phosphate buffer (pH 6.9), followed by incubation for 10 minutes at room temperature. After the addition of 400 µL (microliters) of 1% starch solution, the mixture was incubated again for 10 minutes. The reaction was stopped by adding 1 mL of DNSA acid, and the tubes were heated in a boiling water bath for 5 minutes. The samples were cooled and adjusted to 10 mL with ultrapure water, and absorbance was measured at 540 nm. The control was prepared by replacing the extract with ultrapure water. Alpha-amylase inhibition (%) was calculated, and IC_{50} was determined by extrapolation of the inhibition corresponding to 50%. All assays were performed in triplicate.

2.4. Alpha-Amylase Inhibition (CNPG3 Method)

The alpha-amylase inhibition assay was conducted following the CNPG3 method as described by Kumar *et al.* (2011), with slight modifications. Briefly, 200 µL of alpha-amylase solution was mixed with 200 µL of buffer (pH 6.2) and 500 µL of the working solution of the methanol extract in test tubes. The mixture was incubated at 37˚C for 5 minutes. Afterward, 500 µL of the chromogenic substrate (p-nitrophenyl-2-chloro-4-nitrophenyl-*α*-D-maltotrioside, CNPG3) was added, and the mixture was incubated for an additional 25 minutes at 37˚C. The reaction was terminated by placing the tubes in a boiling water bath for 5 minutes. The final volume was adjusted to 10 mL with ultrapure water, and the absorbance was measured at 405 nm. A control sample was prepared using the same procedure, substituting the extract with 500 µL of ultrapure water.

2.5. Antioxidant Assays

The antioxidant activity of the methanol extract was evaluated at final dilutions ranging from 50 µg/mL to 800 µg/mL using the DPPH [22], FRAP [23], ABTS

[24], Phosphomolybdate [25], Folin-Ciocalteu [26], and Ferrozine [27] methods. The percentage of inhibition or reduction was calculated using Equation (1) for the DPPH or ABTS assays and Equation (2) for the FRAP, Ferrozine, Phosphomolybdate, and Folin-Ciocalteu methods. The assays were performed in triplicate. Calibration curves were plotted based on the percentages of antioxidant activity, allowing for the calculation of IC_{50}.

$$\text{Inibition}(\%) = \frac{\text{OD}(\text{control}) - \text{OD}(\text{samples})}{\text{OD}(\text{control})} \times 100 \quad (1)$$

$$\text{Inibition}(\%) = 100 - \left[\left(1 - \text{OD}(\text{samples})\right)\right] \times 100 \quad (2)$$

where ODcontrol is the optical density of the control solution, and ODsample is the optical density of the sample at each dilution. The IC_{50} values (the concentration required to inhibit 50% of the enzymatic activity) were determined by interpolating the inhibition data obtained for different extract concentrations.

2.6. Statistical Analysis

The antioxidant activity and alpha-amylase inhibition graphs were generated and analyzed using Python Compiler. The inhibition percentages were calculated in Excel 2016 (Microsoft Corporation), and the data were fitted to a sigmoid curve using the curve_fit function from the scipy.optimize library, version 3.12.5150.0. IC_{50} was determined as the concentration required to inhibit 50% of the activity based on the fitted curve. All statistical calculations were performed in triplicate with a significance level of 0.05.

3. Results and Discussion

3.1. Total Phenols Content

Total phenols are bioactive compounds in plants with notable therapeutic activities, including antioxidant properties. In the study of *Vangueria infausta* fruits, the quantification of total phenols was carried out using the gallic acid calibration curve (**Figure 1**), resulting in a significant concentration of 95.6 mg/100g of dry powder. This high concentration suggests a potent antioxidant capacity of the extract. The accuracy of the method was confirmed by an excellent linear correlation between the concentration of gallic acid and absorbance, with a determination coefficient (R^2) of 0.9989. Phenols, with their potent antioxidant properties, neutralize free radicals through the donation of electrons or hydrogens [28], and their high concentration in the *Vangueria infausta* extract suggests a significant antioxidant capacity, as the antioxidant efficacy of various plant extracts is highly correlated with compounds of this nature [29]. Additionally, these phenols can inhibit the enzyme alpha-amylase, a valuable mechanism in controlling blood glucose levels, especially in the treatment of type 2 diabetes. Studies suggest that phenolic compounds from plant extracts can bind to alpha-amylase, reducing its activity and consequently slowing down the digestion of carbohydrates and the absorption of glucose [11] [12].

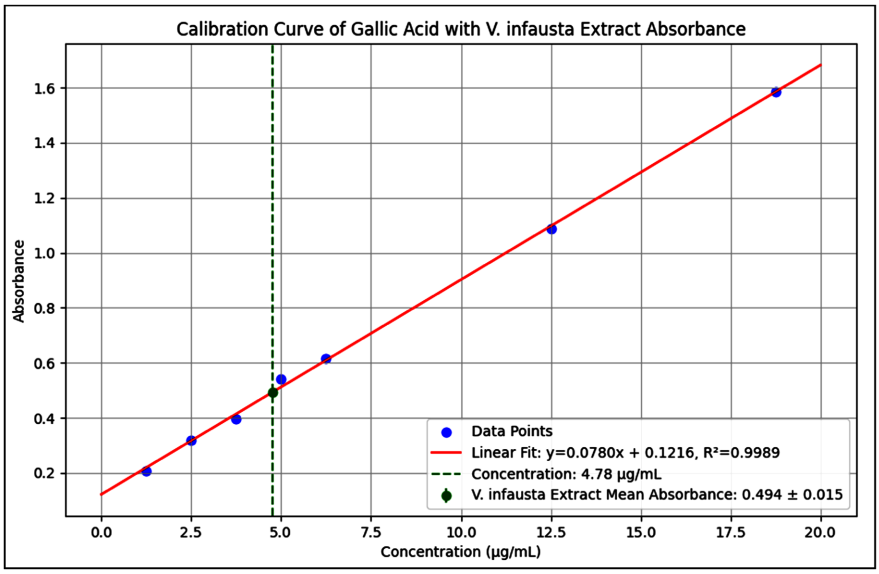

Figure 1. The presented graph illustrates the gallic acid calibration curve. The red line represents the linear fit line. The green dot represents the average absorbance of the *V. infausta* extract (0.494 ± 0.015). The green dotted line indicates the corresponding concentration of 4.78 µg/mL.

3.2. Total Alkaloid Content

Although traditionally less recognized for their antioxidant properties compared to phenols, certain alkaloids play a crucial role as indirect antioxidants, protecting cells against oxidative stress and acting as inhibitors of enzymes such as alpha-amylase and alpha-glucosidase [30]. In the extract of *Vangueria infausta*, the atropine calibration curve (**Figure 2**) was generated using known concentrations of atropine and their respective measured absorbances, and the total alkaloids were expressed in mg of atropine equivalent per 100 g of dry powder. The alkaloid concentration (0.188 mg/100 g of dry powder), although lower compared to other plant species such as *Khaya grandifoliola*, whose bark, root, and leaf extracts showed TAC ranging between 10.67 ± 0.22 and 3.78 ± 0.13 mg of atropine equivalent per 100 mg of extract [31], can still significantly contribute to the extract's overall antioxidant capacity and exert relevant pharmacological activities, particularly in the inhibition of digestive enzymes [32].

3.3. Antioxidant Activity

The graph presented in **Figure 3** illustrates the inhibitory activity of different methods at various concentrations of the *Vangueria infausta* extract. The methods used include Folin-Ciocalteu's, ABTS, FRAP, DPPH, Ferrozine, and Phosphomolybdate. The inhibition curve for each method was plotted based on the percentage of inhibition at different concentrations, and the IC_{50} values were determined for each method. The graph presented in **Figure 4** (Scatter Plot) illustrates the distribution of IC_{50} values (the concentration required to inhibit 50% of activity) obtained through different analytical methods. On the horizontal axis (x), the

methods used. On the vertical axis (y), the IC_{50} values are represented in μg/mL. This graph allows for a visual comparison of the inhibitory efficacy of the different methods, highlighting the variations in the concentration needed to achieve 50% inhibition of activity in each case.

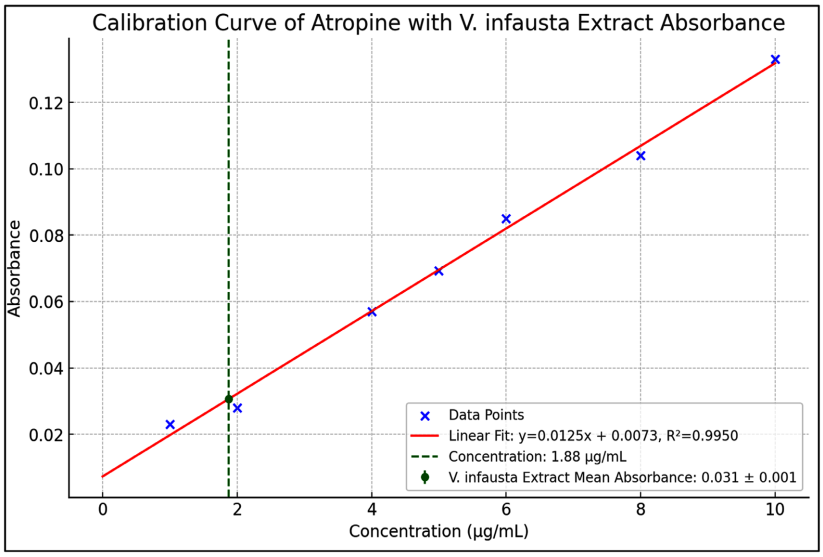

Figure 2. The calibration curve of atropine with the average absorbances of the *V. infausta* extract plotted. The green dashed line represents the corresponding concentration of 1.88 μg/mL. The green dot shows the average absorbance (0.031 ± 0.001). The red line represents the linear fit of the data points. The equation of the fit line is y = 0.0125x + 0.0073, with an R^2 of 0.9950.

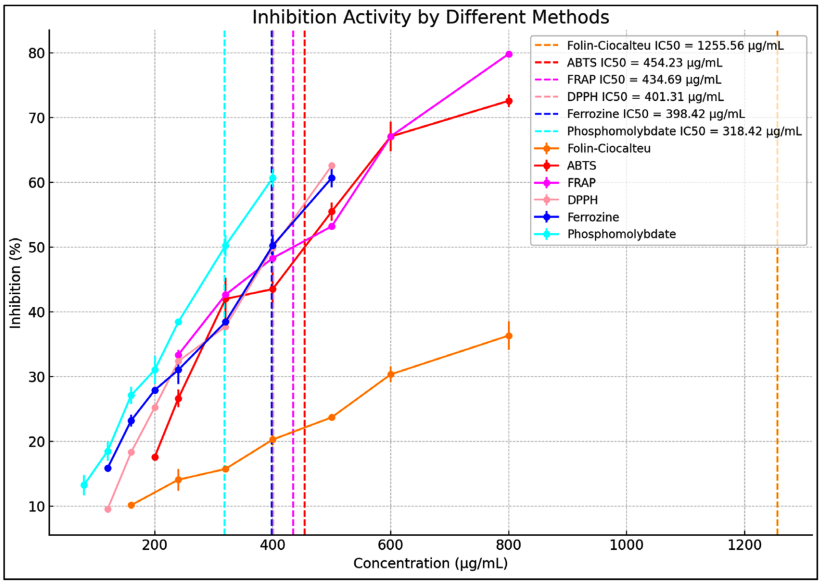

Figure 3. The presented graph illustrates the inhibition activity of different methods at various concentrations of the *V. infausta* extract. On the horizontal axis (x), the concentrations are shown in μg/mL, while on the vertical axis (y) the inhibition percentage (%) is displayed. The different colored lines represent the methods used: Folin-Ciocalteu, ABTS, FRAP, DPPH, Ferrozine, and Phosphomolybdate.

3.3.1. Phosphomolybdate Assay

This assay is based on the reduction of phosphate-molybdenum (VI) ions, initially yellow in color, in the presence of an antioxidant, resulting in the formation of a green phosphate/Mo (V) complex, which is measured spectrophotometrically at 695 nm [25]. In the conducted assay, the methanol extract of *V. infausta* demonstrated remarkable antioxidant activity, as evidenced by the low IC_{50} value of 318.42 µg/mL. This activity can be attributed to its effectiveness in reducing molybdenum, indicating an excellent Total Antioxidant Capacity of *V. infausta* fruits, as this method simultaneously assesses both liposoluble and hydrosoluble antioxidants. The strong activity demonstrated by the extract of *Vangueria infausta* fruits is clear evidence of the fruit's ability to neutralize reactive species, which are widely described in the literature as the main agents responsible for oxidative stress. This stress is linked to numerous degenerative disorders, ranging from premature aging and chronic inflammatory processes to diseases such as cancer, Alzheimer's, and Parkinson's [33].

3.3.2. Ferrozine Method

Ferrozine is a water-soluble organic compound, available in the form of a disodium salt, that forms a stable magenta-colored complex with ferrous ions (Fe^{2+}). This complex, with an absorption peak at 562 nm, is widely used as an indicator reagent to detect the presence of ferrous ions, which arise from the reduction of ferric ions (Fe^{3+}) mediated by antioxidants [27]. In the conducted assay, the methanol extract of *V. infausta* demonstrated remarkable antioxidant activity, as evidenced by the low IC_{50} value of 398.42 µg/mL, the second lowest among the methods tested. This result suggests that the extract possesses enhanced sensitivity in neutralizing free radicals and reactive species in food and biological systems, highlighting its potential as an antioxidant agent in food applications and in the prevention of oxidative processes associated with the development of chronic diseases.

3.3.3. ABTS and DPPH Radical Scavenging Assay

The ABTS assay measures the relative capacity of antioxidants to neutralize the ABTS radical cation, generated in the aqueous phase by the reaction of ABTS salt with a strong oxidizing agent, such as potassium persulfate [34]. This radical cation presents a violet coloration that absorbs at 734 nm. The assay evaluates the rate of elimination of this radical cation, which is indicative of the antioxidant activity of the extract under analysis. In the ABTS assay, the methanol extract of *V. infausta* showed an IC_{50} of 454.23 µg/mL, a moderate value that suggests a reasonable capacity of the extract to neutralize ABTS radical cations. This method is widely used to measure the antioxidant capacity of various substances, thus providing a useful benchmark for comparisons. Similarly to ABTS, the DPPH assay, which also generates a violet-colored radical absorbing at 517 nm, showed a moderate IC_{50} of 401.31 µg/mL, indicating that the *V. infausta* extract has a reasonable antioxidant capacity against DPPH radicals.

3.3.4. FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay, developed by Benzie and Strain in 1996, was originally designed to measure the ferric reducing power of human plasma. This method was later adapted to assess the antioxidant capacity of plant extracts, specifically their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). In the FRAP assay, the key reaction involves the reduction of the Fe(III)-TPTZ complex (ferric [III]-2,4,6-tripyridyl-s-triazine) by a reducing agent, forming a ferrous/TPTZ complex with a Prussian blue color that absorbs at 593 nm [35]. The intensity of this color is directly proportional to the antioxidant capacity of the sample under study. For the methanol extract of *V. infausta*, the FRAP assay revealed an IC_{50} value of 434.69 µg/mL. Although this value is relatively high compared to other antioxidant methods, it is still significant for characterizing the antioxidant capacity of the extract. This result suggests that, while the extract possesses antioxidant activity, its effectiveness in reducing ferric ions is lower compared to other methods evaluated.

3.3.5. Folin-Ciocalteu Antioxidant Capacity Assay

The Folin-Ciocalteu's assay is widely used to determine the total phenolic content in a sample. This method relies on the reduction of Folin-Ciocalteu's reagent, leading to a color change measurable by spectrophotometry. The IC_{50} value for the methanol extract of Vangueria infausta was found to be 1255.56 µg/mL, the highest among all methods tested. This elevated IC_{50} value in the Folin-Ciocalteu assay suggests a lower concentration of phenolic compounds in the extract compared to other antioxidant agents, such as ascorbic acid [15].

3.3.6. Overview of the Antioxidant Activity of *V. infausta* Fruits

The graph in **Figure 3** demonstrates that the antioxidant activity of the *Vangueria infausta* extract is dose-dependent, exhibiting a linear trend where activity intensifies with increasing concentration. These results indicate that the extract has significant antioxidant capacity, although its efficacy varies depending on the method used. This variation in results highlights the complexity of antioxidant activity, which may involve different mechanisms, such as electron donation or hydrogen atom transfer to neutralize free radicals.

The scatter plot in **Figure 4** complements these observations by illustrating the IC_{50} values (half-maximal inhibitory concentration) obtained from the different methods used to assess the antioxidant activity of the extract. The X-axis represents the evaluation methods, while the Y-axis displays the IC_{50} values in micrograms per milliliter (µg/mL). Each blue dot on the graph corresponds to the IC_{50} value for a specific method, thereby revealing the variation in antioxidant efficacy of the *Vangueria infausta* extract depending on the method employed.

The Ferrozine and Phosphomolybdate methods stood out as the most effective, showing the lowest IC_{50} values, while the Folin-Ciocalteu's method was the least efficient, with the highest IC_{50} value. These results suggest that the *V. infausta* extract contains a complex mixture of bioactive compounds that contribute

differently to antioxidant activity, depending on the mechanism of action evaluated by each method.

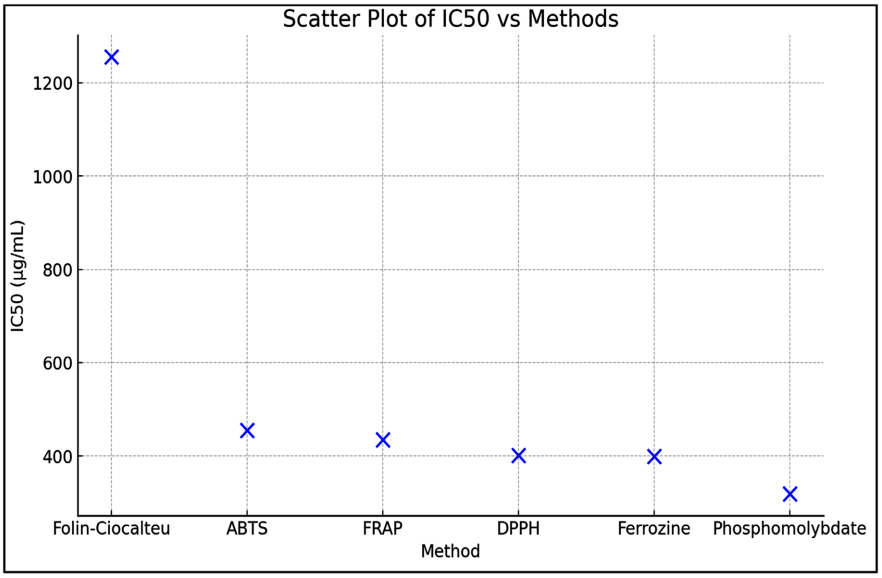

Figure 4. The presented graph is a "Scatter Plot" that shows the relationship between IC50 values (the concentration required to inhibit 50% of activity) and different analytical methods.

Previous studies on extracts from native African fruits, such as *V. infausta*, have demonstrated significant antioxidant potential. For example, [21] reported similar antioxidant activities in extracts from *Strychnos madagascariensis* fruits. Similarly, [36] identified antioxidant activity in *Strychnos spinosa* seeds, while [15] demonstrated the antioxidant activity of *Mimusops caffra* fruits. These findings, along with the results obtained for the methanol extract of *Vangueria infausta*, reinforce the effectiveness of plant extracts in antioxidant applications.

The comparison of IC_{50} values obtained by different methods suggests that the methanol extract of *V. infausta* possesses significant antioxidant properties, with notable efficacy in free radical neutralization and overall antioxidant capacity. These results are promising for the application of the extract in preserving perishable foods, where oxidation is a constant challenge. Furthermore, the confirmation of these findings by other studies underscores the importance of native plant extracts in the search for effective natural antioxidants. The use of such extracts could contribute to the development of natural preservatives, reducing the reliance on synthetic additives and promoting sustainability.

3.4. Inhibition of Alpha-Amylase

The inhibition of α-amylase is a key strategy in controlling blood glucose levels, especially in the treatment of conditions such as diabetes mellitus. This study investigated the inhibitory potential of *V. infausta* fruits against α-amylase using two different methods: DNSA and CNPG3. The DNSA method measures the

amount of reducing sugars released by the action of α-amylase on starch. The reduction of DNSA to 3-amino-5-nitrosalicylic acid produces a color change, which is measured at 540 nm. The reaction mixture contains starch, α-amylase, and the plant extract. After incubation, the DNSA reagent is added, and the mixture is boiled. The absorbance of the resulting solution is measured to determine the α-amylase activity. On the other hand, the CNPG3 (2-chloro-4-nitrophenyl-β-D-maltotrioside) assay uses a chromogenic substrate that is hydrolyzed by α-amylase, releasing 2-chloro-4-nitrophenol, which can be measured spectrophotometrically at 405 nm. The rate of formation of 2-chloro-4-nitrophenol is proportional to the α-amylase activity, allowing the determination of enzyme activity in the presence of inhibitors.

α-Amylase Inhibition by Methanol Extract of *V. infausta* Fruits

The alpha-amylase inhibition data for the *V. infausta* extract are presented in Table 1. It was observed that alpha-amylase inhibition at the lowest concentrations (40 µg/mL) ranged between approximately 26.55% and 36.20% for the CNPG3 and DNSA methods, respectively. The initial inhibitory activity (40 µg/mL) is significant, indicating that even at low concentrations, the extract has a notable inhibitory effect on the alpha-amylase enzyme. The gradual increase in inhibition with increasing concentration suggests a dose-response relationship, where higher concentrations of the extract result in greater enzyme inhibition.

Table 1. Inhibition of alpha-amylase by the methanolic extract of *V. infausta* fruits.

Conc. (µg/mL)	Inibição (%) DNSA	Inibição (%) CNPG3
40.0	36.20 ± 0.44	26.55 ± 0.29
60.0	35.93 ±0.67	27.15 ± 0.06
80.0	34.67 ± 0.70	29.75 ± 0.16
100	41.13 ± 0.66	32.10 ± 0.48
120	49.83 ± 0.36	38.30 ± 0.60
160	56.13 ± 0.94	60.75 ± 1.81
200	66.30 ± 0.94	75.56 ± 0.19

At intermediate concentrations, inhibition increases more markedly, reaching 41.13% at 100 µg/mL and 32.10% at 120 µg/mL. Significant increases in inhibition are observed, demonstrating the extract's growing efficacy in inhibiting alpha-amylase activity. These intermediate values are important for identifying concentration ranges where the extract is highly effective but has not yet reached maximum inhibition levels.

At higher concentrations, alpha-amylase inhibition by the *V. infausta* extract increases substantially, reaching 66.30% at 200 µg/mL and 75.56% for both methods, suggesting that the extract has significant potential to inhibit the enzyme at elevated concentrations. An analysis of these data shows that, at higher concentrations, the extract is highly effective and may be useful in applications where

maximum alpha-amylase inhibition is desired.

The IC_{50}, or the concentration required to inhibit 50% of enzymatic activity, was calculated at approximately 121.06 μg/mL (CNPG3) and 140.85 μg/mL (DNSA). These values indicate the potency of the *V. infausta* extract, showing that a relatively moderate concentration is sufficient to achieve significant alpha-amylase inhibition. The low standard deviation associated with the IC_{50} (±3.67) demonstrates the consistency of the results and the reliability of the measurements.

3.5. *α*-Amylase Inhibition by Acarbose

The alpha-amylase inhibition by the acarbose standard, as detailed in Table 2, begins at relatively high levels at low concentrations. For example, at 2.5 μg/mL, inhibition is 33.10% in the DNSA method and 29.79% in the CNPG3 method. Acarbose demonstrates significant efficacy even at low concentrations, indicative of its strong inhibitory potential.

Table 2. Inhibition of alpha-amylase by acarbose (positive control).

Conc. (μg/mL)	Inibição (%) DNSA	Inibição (%) CNPG3
2.50	33.10 ± 1.35	29.79 ± 1.48
5.00	45.59 ± 0.19	41.03 ± 0.21
7.50	50.27 ± 0.61	45.24 ± 0.67
10.0	56.29 ± 0.18	50.66 ± 0.19
12.5	57.90 ± 0.22	52.11 ± 0.24
15.0	63.57 ± 0.34	57.21 ± 0.38
17.5	66.36 ± 0.65	59.72 ± 0.71
20.0	67.65 ± 0.19	60.89 ± 0.21
22.5	69.93 ± 0.25	62.94 ± 0.28
25.0	69.74 ± 0.20	62.77 ± 0.22

As the concentration increases, inhibition also increases consistently. At 10 μg/mL, inhibition is 56.29% in the DNSA method and 50.66% in the CNPG3 method. These data show a clear dose-dependent response, with a continuous increase in inhibition as acarbose concentration increases. At higher concentrations, inhibition approaches a plateau. For example, at 25 μg/mL, inhibition is 69.74% in the DNSA method and 62.77% in the CNPG3 method. Acarbose remains effective in inhibiting alpha-amylase at high concentrations, but the increase in inhibition diminishes, indicating possible saturation of the inhibitory effect.

The IC_{50} for the DNSA method is 7.360 μg/mL with a standard deviation of 0.26, indicating high efficacy and precision. The IC_{50} for the CNPG3 method is 9.70 μg/mL with a standard deviation of 0.21, also indicating high efficacy and precision, although slightly less effective than the DNSA method.

The IC_{50} values clearly show that acarbose is a significantly more potent inhibitor of alpha-amylase than the methanol extract of *V. infausta*. The IC_{50} values for

acarbose are about 20 times lower than the values for *V. infausta*, indicating that a much lower concentration of acarbose is required to achieve 50% inhibition.

3.6. Overview of the Alpha-Amylase Inhibition

Both assay methods (DNSA Assay and CNPG3 Assay) provided consistent results, although there are small differences. For *V. infausta*, the CNPG3 Assay method showed a slightly lower IC_{50}, suggesting slightly more effective inhibition compared to the DNSA Assay. In the case of acarbose, the IC_{50} values are very close between the two methods, indicating high precision in the results.

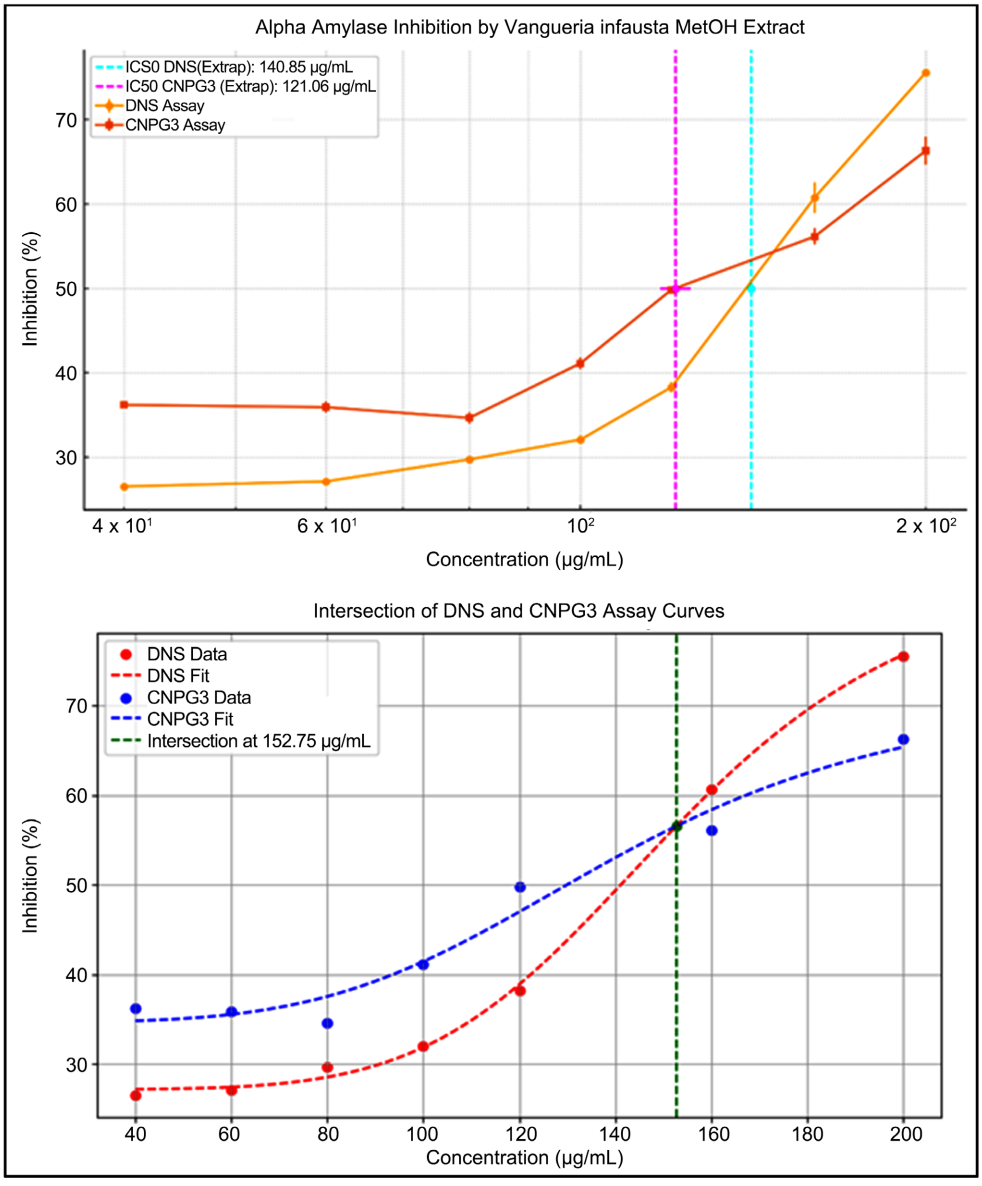

Figure 5. *α*-Amylase inhibition by *V. infausta* extract using DNSA and CNPG3 methods. The fitted curves represent the percentage of inhibition as a function of the extract concentrations, with fitted lines and IC_{50} values indicated by dotted vertical lines. The intersection is highlighted by a green vertical line in the lower part of the graph, indicating the concentration of 152.75 µg/mL.

Acarbose demonstrates high efficacy in alpha-amylase inhibition, with a very low IC_{50}, standing out as a potent inhibitor and an effective tool in glycemic control. The *V. infausta* extract, although less potent, still shows significant alpha-amylase inhibition, suggesting its potential as a natural inhibitor. The exploration of plant extracts like *V. infausta* may open new possibilities for natural and complementary treatments for diabetes management and blood glucose control.

The graphs in **Figure 5** show that alpha-amylase inhibition by the *V. infausta* extract and acarbose increases in a dose-dependent manner. The curves represent the percentage of enzyme inhibition as a function of concentration, with fitted lines and IC_{50} values indicated by dotted vertical lines. The highest inhibition observed for the extract was at a concentration of 200 µg/mL and 25 µg/mL for acarbose. It can also be observed that there is an intersection of the inhibition curves of *V. infausta* for the DNSA and CNPG3 methods, occurring at a specific concentration. This intersection is highlighted by a green vertical line in **Figure 5**, indicating the concentration of 152.75 µg/mL. This intersection is a critical point for understanding the consistency and correlation between the two methods. This suggests that, to a certain extent, the DNSA and CNPG3 methods are consistent and can be used interchangeably to measure alpha-amylase inhibition by *V. infausta* extracts.

5. Conclusion

We conclude that the fruits of Vangueria infausta possess significant antioxidant and antiglycemic activities, with both phenols and alkaloids contributing to these properties. While phenols are the primary contributors to the antioxidant activity, alkaloids also play an important role, particularly in the inhibition of alpha-amylase. These findings suggest that the fruits of *Vangueria infausta* have great potential for therapeutic applications, both in the prevention of oxidative stress and in the management of carbohydrate digestion, making it a promising natural ingredient for dietary supplements and treatments aimed at glycemic control.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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