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Research Article

In Vitro Antidiabetic, Antioxidant, and Cytotoxic Evaluation of Honeybush Tea (*Cyclopia genistoides*) Extracts

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Background. This study evaluated *in vitro* antidiabetic and antioxidant properties of different extracts (n-hexane, dichloromethane (DCM), and 70% ethanol) of honeybush tea (*Cyclopia genistoides*). Over a period of 28 days, antiprotein glycation was evaluated and some antidiabetic indicators (α-amylase, α-glucosidase, and pancreatic lipase inhibitory effects) and antioxidant activities (DPPH, ABTS, hydroxyl radical, metal ion chelating, and reducing power) for each of the crude extracts were also investigated. The results showed that all of the tested *C. genistoides* extracts had strong α-amylase and lipase inhibitory activity in a concentration-dependent manner with IC_{50} values from 0.018 μg/ml (DCM extract) to 9.93 μg/ml (n-hexane extract), respectively. The extracts also displayed inhibitory effects on protein glycation between the 14^{th} and 28^{th} days. The DCM and ethanolic extracts further exhibited strong antioxidant activities as they effectively scavenged most of the radicals tested, with IC_{50} values ranging from 0.014–0.048 mg/ml to 0.019–0.043 mg/ml. Two hundred and seventy-four chemical constituents had been identified by GC-MS, with the n-hexane extract having the highest number of peaks (127) followed by the DCM extract (107). Six compounds were identified across all the following three extracts: decane (RT: 6.4), undecane (RT: 7.7), dodecane (RT: 9.00), phytol (RT: 21.32), heptadecanoic acid, 9-methyl, methyl ester (RT: 21.65), and 9-octadecenamide (RT: 24.30). The cytotoxicity of the extracts against C3A cell lines was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay, which demonstrated that honeybush tea had a toxicity effect ranging from 66.3–88.4 μg/ml on C3A cell lines. The results showed that honeybush has antioxidant and antidiabetic activities, which could be partially attributed to the phytochemical compounds identified within the extracts.

1. Introduction

Diabetes mellitus (DM), a group of chronic disorders characterised by hyperglycemia, has emerged as one of the most prevalent metabolic syndromes with a high morbidity and mortality rate globally [1]. DM arises from either insufficient secretion of insulin by the body or the degradation of secreted insulin, both resulting from cellular alterations induced by a multitude of internal and external factors, including but not limited to obesity, sedentary lifestyle, and oxidative stress [2].

Hyperglycemia induces a pathophysiological state characterized by an upregulation in the biosynthesis of reactive oxygen species (ROS) of both nonmitochondrial and mitochondrial origin. Surplus in ROS leads to the activation of several prooxidative signalling pathways including polyol pathway flux, hexosamine pathway flux, protein kinase C (PKC) isoforms, and advanced glycation

end-products (AGE), also referred to as Maillard reaction products [3]. The early stages of the Maillard reaction emanate from a nonenzymatic nucleophilic addition reaction of the carbonyl residue of sugar with the free amino group of proteins forming a reversible Schiff base [4]. Schiff bases further undergo N-glycoside rearrangements to form more stable corresponding ketoamines, referred to as Amadori products, such as fructosamine. Amadori products proceed to be converted into dicarbonyl intermediates which are liable to form AGE over a period of time [4, 5].

Antioxidants serve a crucial function in safeguarding the human body against the damaging effects of ROS. Consequently, compounds possessing both hypoglycemic and antioxidant properties represent a promising avenue for the development of effective antidiabetic agents. Currently, there are a number of synthetic drugs used in the treatment of diabetes and their primary objective is to decrease postprandial hyperglycemia by inhibiting the activity of digestive enzymes or by the stagnation of the intestinal absorption of glucose. However, these drugs are very expensive, not easily available in developing countries, and they are associated with adverse effects, which limit their use [6]. Therefore, searching for alternative drugs from natural resources such as plants with fewer side effects that are easily accessible has gained much interest.

Honeybush (*Cyclopia* sp.) is a local, naturally occurring shrub found in selected regions and widely used as honeybush tea [7]. Out of 23 of the available *Cyclopia* species endemic to the fynbos biome in South Africa, only five species (*Cyclopia intermedia*, *Cyclopia maculata*, *Cyclopia subternata*, *Cyclopia sessiflora,* and *Cyclopia genistoides*) are vastly commercially made into honeybush tea [8]. Honeybush has been traditionally used against a number of ailments including the treatment of colds and fevers, intestinal complications, and skin ointment [9]. The final product of honeybush products is usually oxidised to honeybush tea; hence, the nonoxidized honeybush materials are often recommended as food nutrient sources due to their higher phenolic content and antioxidant capacity [10]. The recently observed popularity and the high demand of honeybush have gained much attention in the field of research in the discovery of its novel bioactive compounds as they are used as nutraceuticals. Even though the chemical characterization, antioxidant potentials, age-related neurodegenerative disorder, and antihyperglycemic activities of different extracts of *Cyclopia genistoides* have been reported [11], Agapouda et al., 2020 [12], more studies are still required to further support its beneficial properties. Therefore, this study aimed at screening the phytochemical constituents of varying extracts of *Cyclopia genistoides* through GC-MS analysis, investigating the *in vitro* antidiabetic properties of the extracts on some key linked diabetic enzymes, as well as their antioxidant and cytotoxicity potentials.

2. Materials and Methodology

2.1. Materials and Methods

2.1.1. Chemical Reagents. All chemicals and reagents utilized in this study were procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA, and were of analytical grade unless explicitly mentioned otherwise.

2.2. Methodology

2.2.1. Collection and Extraction of Honeybush Material. The oxidised material of honeybush tea (*Cyclopia genistoides)* was commercially bought at the Cape Town Honeybush Company (LTD). It was ground into fine powder (grinding machine: IKA-Werke, Polychem suppliers) and further sequentially extracted with an increasing polarity of different solvents (in a ratio of 1:5 w/v), namely, n-hexane, dichloromethane (DCM), and 70% ethanol (incubated for 24 h at the shaker at 180 rpm, 30°C); each extract was rinsed twice with the same solvent of extraction, respectively. Thereafter, the extracted extracts were subjected to filtration via Whatman No. 1 filter paper and subsequently concentrated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extracts were then preserved in sterile brown bottles at 4°C to prevent light-induced degradation until used. Thereafter, the concentrated extracts were then dissolved in methanol for use. A stock solution of 0.1 mg/ml was prepared from all three extracts, from which various working concentrations between 0 and 0.05 mg/ml were prepared for determination of biological activities.

2.3. Phytochemical Profiling of Honeybush Extracts by GC-MS. Phytochemical characterization of honeybush tea extracts was performed by gas chromatography-mass spectrometry (GC-MS), using the protocol illustrated by Iordache et al. [13] with some amendments. An Agilent 7890A gas chromatography-system coupled with a VL-MSD model 5975C with a triple-axis detector was used. The GC-column profile of the GC-MSD used will be Aligent 190915-433: 325°C: 30 m length $x250\,\mu$m diameter x 0.25 µm film thickness. A suitable stationary-phase, eluting solvent (methanol, methylene, chloride, hexane, ethyl acetate, or acetone) and carrier gas (He or N^2) for the mobile phase was identified and applied to the Gas Chromatography (GC) system. A suitable temperature program (50°C for 2 minutes; then increased to 250°C at a rate of 8 °C·min-1; then increased to 310°C at a rate of 30°C·min-1; with 10 minutes of keeping) was used based on the stationary phase and isolate properties, prescribed on the stationary-phase profile. A suitable carrier gas flow rate was determined (1 ml·min-1). Subsequently, 3 μL of each honeybush extract sample were introduced into the column at an injector temperature of 250°C. The initial oven temperature was set to 60°C, with an automated temperature ramp of 10°C per minute until reaching a final temperature of 280 °C. The column was held at each temperature increment for 3 minutes. Mass spectrometry (MS) was carried out in the electron ionization mode with a voltage of 70 eV and an electron multiplier voltage of 1859 V. Additional MS parameters were set as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 minutes, and scan range 50–700 amu. The compounds present in the samples were identified through comparison of the mass spectrum and the retention time of each analyte with those of reference standards listed in the 2011 National Institute of Standards

and Technology (NIST) library. The area percentage of each component was then determined by comparing its average peak area to the total area obtained.

2.4. Determination of the Total Phenolic Content (TPC). The Folin–Ciocalteu method was used to determine the TPC of honeybush extracts [14]. Briefly, 1.5 ml of diluted (1 : 10 v/v) Folin–Ciocalteu reagent and 1.2 ml of 7.5% sodium carbonate solution were mixed with each extract (0.2 mg/ml) and then kept in the dark for 30 minutes at 21°C. The absorbance of the coloured mixture was read at 765 nm. The TPC of honeybush was calculated as gallic acid equivalent to a calibration curve of garlic acid and expressed as mg/g dry crude extract.

2.5. Determination of the Total Flavonoid Content (TFC). The method previously described by Ordon-Ez et al. [15] was used to determine the TFC of honeybush extracts. Briefly, 0.2 mg/ml of each of the extract added with 0.5 ml of 2% alcoholic aluminium chloride solution was incubated at a standard room temperature for 1 hour. The absorbance of the colour change was read at 420 nm against the blank containing alcoholic aluminium chloride. The TFC of the extract was calculated from a standard curve of quercetin and expressed as mg/g dry crude extract.

2.6. In Vitro Antioxidant Assays. The antioxidant activity of honeybush extracts (n-hexane, DCM, and ethanol) was assessed against various inorganic free radicals, including 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS), and hydroxyl radicals. Unless specified otherwise, ascorbic acid and butylated hydroxyl anisole (BHA) were employed as standard compounds. The experiment was replicated in triplicate ($n = 3$). The free radical scavenging activity of each extract was calculated according to the following formula:

$$\text{Scavenging activity } (\%) = \frac{(A_c - A_t)}{(A_c \times 100)}, \quad (1)$$

where A_c is the absorbance of the control sample and A_t is the absorbance of the sample in the presence of the tested extract.

Absorbance was measured with Microplate Ready (Synergy HT, BioTek Instruments supplies).

2.7. DPPH Radical Scavenging Activity. DPPH radical scavenging activity of the plant extracts was determined following the method of Brand-Williams et al. [16]. A mixture (of a ratio of 1 : 1) of the plant extracts at different concentrations (0.0–0.05 mg/ml) and DPPH (0.2 mg/ml) was incubated for 30–60 minutes in tenebris, and the absorbance was read at 517 nm. Methanol and ascorbic acid were used as negative and positive controls, respectively. The percentage of scavenging was then calculated.

2.8. ABTS Radical Scavenging Activity. ABTS radical scavenging activity of the plant extracts was evaluated as described by Re et al. [17]. ABTS radicals were generated by incubating 7 mM of ABTS with 2.45 mM potassium persulfate in tenebris for 16 hours. Thereafter, the ABTS radical produced was diluted with methanol (1 ml ABTS: 60 ml methanol) to prepare a working solution. One ml of diluted ABTS radical was added to 1 ml of various concentrations (0.0–0.05 mg/ml) of plant extracts and incubated at room temperature for 6 minutes. The absorbance of the mixture was read at 734 nm. Ascorbic acid and methanol were used as positive and negative controls, respectively.

2.9. Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activity of *Cyclopia genistoides* crude extracts was evaluated according to a method previously described by Osawa and Kawakishi [20]. A mixture containing $FeSO_4{\cdot}H_2O$ (10 mM), extracts (0.0–0.05 mg/ml), H_2O_2 (10 mM), EDTA (10 mM), and 100 mM phosphate buffer (pH 7.4) was incubated at 37°C for 2 hours. Following incubation, the mixture was treated with trichloroacetic acid (TCA) (2.8%) and 1% 2-thiobarbituric acid (TBA), followed by the boiling of the reaction mixture for 10 minutes. The reaction mixture was cooled on ice, prior to absorbance measurement. The absorbance of the resulting solution was then measured at 520 nm.

2.10. Reducing Power. The reduction potential of *Cyclopia genistoides* crude extracts was determined using the method previously described by Oyaizu [20]. Briefly, 2.5 ml of each extract at varying concentrations (0–0.05 mg/ml), potassium ferricyanide (1% w/v), and 200 mM phosphate buffer (pH 6.6) were combined and incubated for 20 minutes at 50°C. Following incubation, the mixture was centrifuged at 1000 rpm for 10 minutes and 0.1% ferric III chloride ($FeCl_3$) was added to the supernatant. The reduction potential of the extracts was then determined by measuring the absorbance at 700 nm.

2.11. Metal Ion (Fe^{2+}) Chelating Activity Assay. The method by Decker and Welch [19] was followed to determine the ability of the honeybush extracts to chelate Fe^{2+}. The mixture containing ferrozine (5 mM), 2 mM $FeCl_2$, different concentrations of extract (0–0.05 mg/ml), and deionized water was incubated at 25°C for 10 minutes. After incubation, absorbance was read spectrophotometrically at 562 nm and EDTA was used as a standard.

2.12. Enzyme Inhibition Assay. The inhibitory activity of the extracts on carbohydrate digestive enzymes was tested against α-amylase and α-glucosidase. The inhibitory effect of the extracts was also tested on pancreatic lipase, a dietary triglyceride-digesting enzyme. The experiments were triplicated, and percentage inhibition was calculated using the formula as follows:

$$\text{Inhibitory activity } (\%) = \left(\frac{(A_c - A_t)}{A_c \times 100}\right), \quad (2)$$

where A_c is the absorbance of the control and A_t is the absorbance in the presence of the tested extract.

2.13. Alpha Amylase Activity. A modified version of the Kandra et al. [21] assay was employed to determine the inhibitory potential of honeybush extracts on α-amylase activity. The reaction mixture was composed of 0.2 ml of 2 units/ml α-amylase solution and crude extract (0–0.2 μg/ml) and incubated for 10 minutes at 30°C. Subsequently, 0.2 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added and incubated for 5 minutes. The reaction was terminated with 1.0 ml of 3.5-dinitrosalicylic acid (DNSA) as a colorimetric indicator (1 g of DNSA in 50 ml of reagent grade water and 30 g of sodium potassium tartrate tetrahydrate in 20 ml of 2 M NaOH) and boiled for 10 minutes in a water bath at 85–90°C. The mixture was cooled and diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm. The control sample containing saline buffer instead of *Cyclopia* extracts was regarded as having 100% enzyme activity.

2.14. Alpha Glucosidase Activity. The inhibitory effect of honeybush extracts on α-glucosidase activity was determined using the method described by Adisakwattana et al. [22]. Specifically, Eppendorf tubes containing 50 μl of 10 mM phosphate buffer (pH 6.8), 10 μl of α-glucosidase solution (0.5 units/ml) extracted from rat intestinal acetone powder (Sigma-Aldrich), and 20 μl of different concentrations (0–2 μg/ml) of honeybush extracts were incubated for 15 minutes at 37°C. Thereafter, 20 μl of 2.5 mM 4-nitrophenyl-β-d glucopyranoside was added, and the reaction mixture was further incubated for 20 minutes at 37°C. After incubation, 50 μl of 0.1 M sodium carbonate was added to the reaction mixture. Control experiments were carried out by replacing the crude extracts with an equal volume of buffer. The percentage inhibitory activity of the extracts on α-glucosidase activity was calculated by measuring the absorbance at 405 nm.

2.15. Pancreatic Lipase Activity. The pancreatic lipase inhibitory effect of honeybush crude extract was evaluated according to a method previously described by Slanc et al. [23]. Briefly, 2.5 mg/ml of porcine pancreatic lipase was prepared in 10 mM morpholine propanesulphonic acid and 1 mM EDTA buffer solution (pH 6.8). A mixture of 75 μl of different concentrations of honeybush (0–50 μg/ml), 50 μl of lipase (2.5 mg/ml), and 150 μl of Tris-HCl buffer (75 mM, pH 8, 4) was incubated at 37°C for 15 minutes. Then, 25 μl of 3.3 mM p-nitrophenyl palmitate was added, and the mixture was incubated at room temperature for 30 minutes. A blank containing a mixture (1 : 1 v/v) of methanol and water was used, and orlistat was used as the standard. The release of 4-nitrophenol at 405 nm was measured to estimate the enzyme inhibitory activity of the extract, and the percentage inhibition of pancreatic lipase activity was calculated.

2.16. In vitro Antiprotein Glycation Activity- BSA-Fructose Assay

2.16.1. Bovine Serum Albumin (BSA)-Fructose Assay. The bovine serum albumin (BSA)-fructose assay was utilized to determine the honeybush crude extracts' protein anti-glycation potential [24]. A total volume of 2 ml containing 10 mg/ml BSA, plant extract (1–100 μg/ml), and 0.5 M fructose in 0.1 M phosphate buffer saline (PBS, pH 7.4) was incubated in test tubes. Sodium azide (0.02%) was added to the reaction mixture as a bacteriostatic agent. The reaction mixture was then incubated in the dark at 37°C for 7, 14, 21, and 28 days. Aminoguanidine (AG) was used as a reference standard. After incubation, the formation of AGEs was measured fluorometrically at excitation and emission wavelengths of 355 nm and 460 nm, respectively. The percentage inhibition of BSA glycation was then calculated using the following formula:

$$\text{Percentage inhibition of fluorescent AGE } (\%) = \left[\frac{\left(F_{\text{control}} - F_{\text{Tsample}}\right)}{F_{\text{control}}}\right] \times 100, \quad (3)$$

where $F_{control}$ represents fluorescence of the control sample and $F_{Tsample}$ represents fluorescence of the test samples.

2.17. Estimation of Fructosamine Content. The concentration of fructosamine in BSA-fructose reaction samples containing honeybush crude extracts was determined using a nitroblue tetrazolium (NBT) assay for each sampling interval (7, 14, 21, and 28 days), as described by Adisakwattana et al. [24]. Briefly, 1 ml of glycated BSA was incubated with 1 ml of 0.5 mM NBT in 2 M sodium carbonate buffer (pH 10.4) at 37°C for 10–15 minutes. The absorbance was measured at a wavelength of 590 nm. The concentration of fructosamine (μg/ml) in the respective samples was determined from the standard curve of 1-deoxy-1-morpholino-fructose (1-DMF).

2.18. Cytotoxicity Assay. The cytotoxicity of *C. genistoides* extracts against the hepatic (C3A) cell line was assessed using the MTT assay as previously described by Mosman [25]. The cells were seeded in a noncell-binding 96-well plate at a concentration of 1.1 × 105 cells/ml and allowed to attach for 48 hours before being treated with different concentrations of *C. genistoides* extracts (0–100 μg/ml) via serial dilutions. The extracts were administered in media containing fetal bovine serum (1%) and reincubated for 48 hours. After incubation, the spent medium was removed and 2 mg/ml of tetrazolium salt was added as

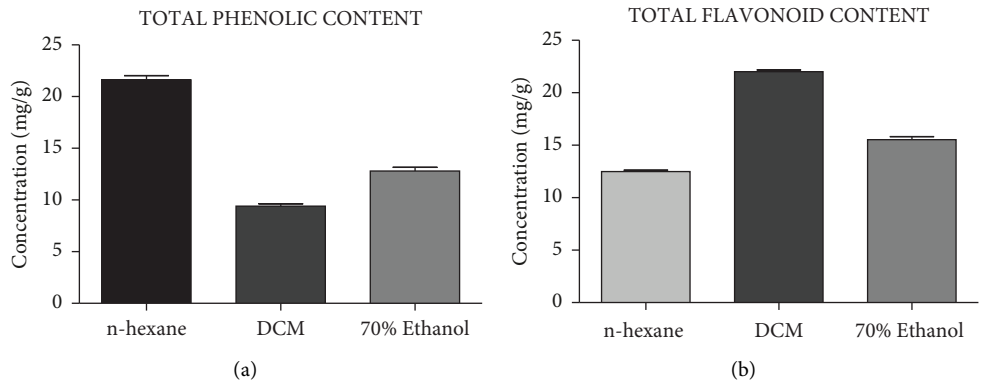

FIGURE 1: Phenolic (a) and flavonoid (b) contents of honeybush (*Cyclopia genistoides*). Data are expressed as the mean ± SD ($n = 3$). Statistical significance at $p < 0.05$.

a cytotoxicity indicator to determine cell viability. A fresh medium (100 μl) was added to each well and incubated at 37°C for 1 hour. The media containing MTT were aspirated from the wells, and the formed formazan crystals were solubilized in 100 μl of dimethyl sulfoxide (DMSO). The optical density of the solutions was measured at 570 nm using a SpectraMax i3 microplate reader. The percent inhibition of cell viability was calculated using the following formula:

$$\% \text{ Cell death} = \left[\frac{(A_c - A_t)}{A_c \times 100}\right], \quad (4)$$

where A_c represents the absorbance of the control and A_t represents the absorbance in the presence of the tested extract.

2.19. Statistical Analysis. In this study, the results were presented as the mean ± standard deviation (SD), unless otherwise stated. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with the Dunnett post hoc test and two-way ANOVA with Tukey's multiple comparisons test. The IC_{50} values were calculated using Graph Pad Prism version 6 (V6). The statistical difference was considered significant where $p < 0.05$.

3. Results

3.1. Percentage Yield. Powdered material from honeybush was sequentially extracted with n-hexane, dichloromethane, and ethanol. The yield from each extract was 0.69%, 0.75%, and 12%, respectively.

3.2. TPC and TFC. Figure 1 shows the results of TPC and TFC on n-hexane, DCM, and ethanol extracts. All the extracts demonstrated the presence of both phenolics and flavonoids content. While n-hexane extract showed the highest phenolic content (21 ± 1.04 mg/ml) when compared to ethanol and DCM extracts (9.7 ± 0.46 mg/ml), DCM extract (13.4 ± 1.04 mg/ml) demonstrated the highest flavonoid when compared to n-hexane and ethanol extracts. It is also worth noting the lower concentrations of phenolics and flavonoids displayed by ethanolic extract (when compared to DCM and n-hexane extracts, respectively).

3.3. Chromatographic Chemical Characterization of Honeybush Extracts. In the GC-MS analysis of honeybush extracts, a total of 274 compounds were detected after being sequentially extracted with n-hexane (127/274), DCM (107/274), and 70% ethanol (65/274) (Table 1). Six compounds such as decane (RT: 6.4), undecane (RT: 7.7), dodecane (RT: 9.00), phytol (RT: 21.32), heptadecanoic acid, 9-methyl, methyl ester (RT: 21.65), and 9-octadecenamide (RT: 24.30) were detected to be present in all three extracts. Hence, the presence of 9,12-octadecadienoic acid (RT: 22.17) and 6-octadecenoic acid (RT: 22.73) was observed only in n-hexane and ethanolic extracts. Furthermore, the abundance of pentane 2,3,3-trimethyl (RT: 5.01), oxalic acid, cyclohexyl ethyl ester (RT: 6.15), benzene 1,2,4-trimethyl (RT: 6.46), cyclohexasiloxane, dodecamethyl (RT: 10.16), 3,5-dimethyl-2-octanol (RT:12.02), 1 hexacosene (RT: 17.25), isopropyl palmitate (RT: 18.69), and n-heptadecanol-1 (RT: 20.79) was also observed in hexane and DCM extracts.

3.4. Antioxidant Activity

3.4.1. Free Radical Scavenging Activity. The antioxidant activity of the honeybush extracts was determined (Table 2). The honeybush extracts showed, to varying degrees of efficacy, concentration-dependent free radical scavenging and Fe^{2+} chelating activities. The IC_{50} values of the extracts ranged from 0.014–0.047 mg/ml to 0.019–0.043 mg/ml for free radical scavenging and metal ion (Fe^{2+}) chelating activities, respectively. The lowest IC_{50} values from the extract were observed for DPPH (0.021 mg/ml) and OH (0.014 mg/ml), which were comparable to those of ascorbic acid ($IC_{50} = 0.035$ mg/ml) and BHA ($IC_{50} = 0.017$ mg/ml). The reducing power of the extracts was also determined, and the results are given in Figure 2. The extracts showed concentration-dependent reducing power. It is also worth noting that the highest activity displayed by hexane extract is relatively similar to that of the standard ascorbic acid used.

3.5. Enzyme Activity

3.5.1. Carbohydrate Digesting Enzymes' Inhibitory Activity. The results of the effect of the plant extracts on α-amylase and α-glucosidase activity are presented in Figure 3. All three

Table 1: Selected compounds from different extracts of honeybush (*Cyclopia genistoides*) tea identified by GC-MS.

Compounds names	RT	*n*-hexane (%)	DCM (%)	70% ethanol (%)
Decane	6.4	0.7	2.0	1.3
Undecane	7.7	3.0	3.6	1.1
Dodecane	9.0	1.2	2.9	1.0
Phytol	21.32	13.2	4.5	1.6
Heptadecanoic acid, 9-methyl-, methyl ester	21.65	1.8	1.3	0.2
9-Octadecenamide	24.30	1.2	1.7	1.5
Pentane, 2, 3, 3-trimethyl	5.01	1.9	2.6	—
Benzene, 1, 2, 4-trimethyl	6.46	2.2	2.6	—
n-heptadecanol-1	20.79	4.5	4.4	—
1-penten-3-ol, 2-methyl	3.68	1.9	2.1	—

Table 2: IC_{50} values of different extracts of *Cyclopia genistoides*.

Extracts	DPPH	ABTS	OH	Metal chelating	Ascorbic acid	BHA
n-hexane IC_{50} (mg/ml)	<5	<5	0.047 ± 0.00	0.043 ± 0.00	0.018 ± 0.00	0.017 ± 0.01
DCM IC_{50} (mg/ml)	<5	<5	0.217 ± 0.00	0.048 ± 0.01	0.025 ± 0.01	0.047 ± 0.00
70% ethanol IC_{50} (mg/ml)	0.021 ± 0.01	<5	0.014 ± 0.01	0.019 ± 0.00	0.035 ± 0.01	0.017 ± 0.00

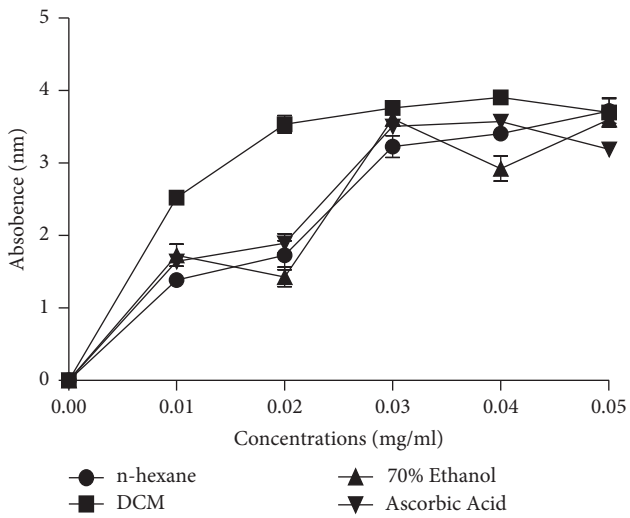

Figure 2: The graph showing the reducing power of the extracts. Data are expressed as mean ± standard deviation. ($n = 3$). Statistical significance at $p < 0.05$.

extracts exhibited a more convincing concentration-dependent inhibitory effect only on α-amylase activity with DCM extract IC_{50} value of 0.063 μg/ml which was less when compared to acarbose's IC_{50} value of 0.14 μg/ml. Moreover, it was also observed that all extracts did not show any inhibitory activity on α-glucosidase enzyme.

3.6. Effects of Cyclopia genistoides Extracts on Pancreatic Lipase Activity. Figure 4 presents the results of the effect of *Cyclopia genistoides* on pancreatic lipase activity. All the extracts (n-hexane, DCM, and ethanol) demonstrated the inhibitory effects on lipase activity. However, the hexane extract exhibited a twice lower IC_{50} value (9.93 μg/ml) against pancreatic lipase when compared to that of the standard orlistat (26.5 μg/ml).

3.7. Bovine Serum Albumin Glycation. The antiglycation of honeybush extracts was evaluated on BSA over a period of 28 days at 7 days of interval. The obtained results are presented in Figure 5. All the extracts exhibited a concentration-dependent inhibitory effect on BSA glycation during the 14 to 28 days of incubation. Their antiprotein glycation activity within this period was comparable to that of aminoguanidine which was used as a standard.

3.8. Fructosamine Content. Results in Table 3 present the effect of honeybush (*Cyclopia*) extracts on fructosamine content. The level of fructosamine in glycated BSA was gradually increased during 28 days of incubation. However, only ethanolic extract showed a significant reduction in fructosamine levels in glycated BSA during the 07 to 28-day incubation period which was even greater when compared to aminoguanidine.

3.9. Cytotoxicity. Table 3 shows the results of the crude extract cytotoxic activity against hepatic human cell lines (C3A). The results showed that honeybush extracts reduced the cell viability of C3A cells in a concentration-dependent manner, with the LC_{50} of n-hexane extract, DCM extract, and ethanol extract being calculated as 72.6 μg/ml, 66.3 μg/ml, and 88.4 μg/ml, respectively.

4. Discussion

Diabetes mellitus, which is characterised by hyperglycemia, continues to be a life-threatening chronic ailment that grows globally at an alarming rate [26, 27]. Hence, the control of postprandial hyperglycemia has prompted the search for alternative approaches in the discovery of novel antidiabetic agents [28]. Plant-derived products, which serve as sources of bioactive constituents, have shown a significant role in the development of new therapeutic agents [2, 29]. In this study, the phytochemical profiles of different extracts of honeybush

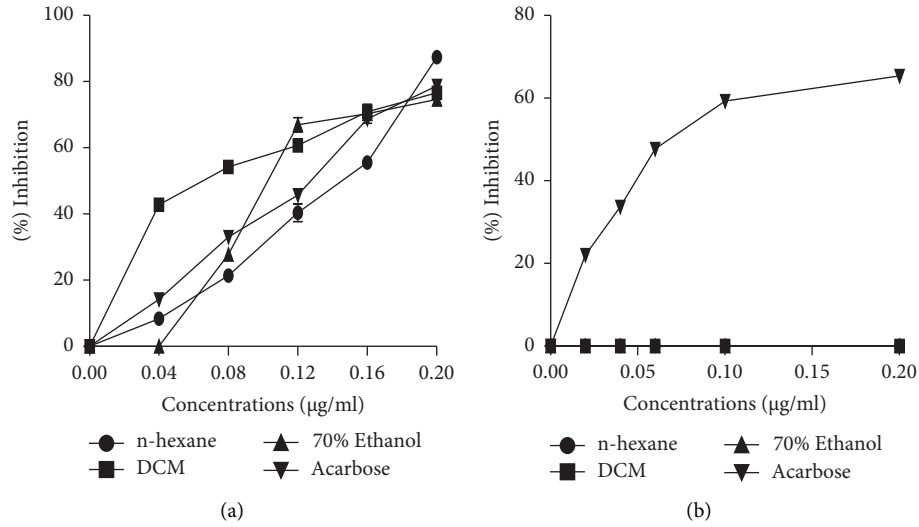

Figure 3: The graph showing (a) α-amylase and α-glucosidase (b) inhibitory activities of honeybush extracts. Data are expressed as the mean ± standard deviation. ($n = 3$). Statistical significance at $p < 0.05$.

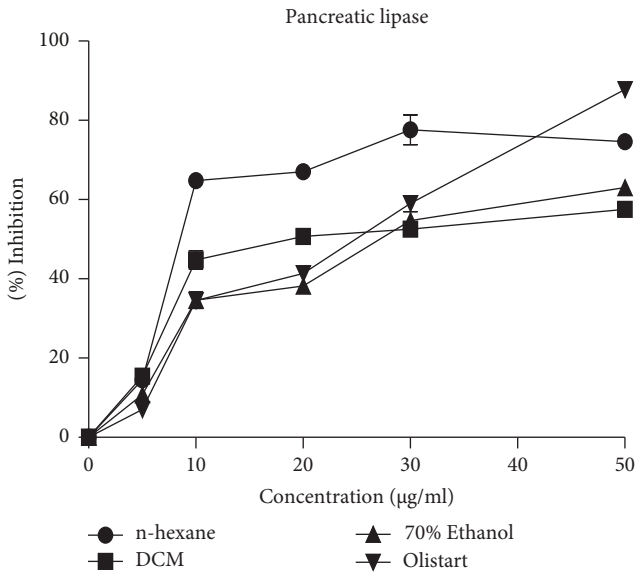

Figure 4: The graph showing pancreatic lipase inhibitory activity. Data are expressed as the mean ± standard deviation. ($n = 3$). Statistical significance at $p < 0.05$.

tea were investigated as well as some *in vitro* antidiabetic and antioxidant properties.

Inhibiting the activity of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, has been highlighted as a basic therapeutic and management strategy for the control of hyperglycaemia, the inhibition of these enzymes' delays glucose absorption from the small intestinal tract [30, 31]. While salivary α-amylase catalyses the hydrolyses of the linear α-(1.4)-glycosidic bonds of large insoluble carbohydrates into oligosaccharides and disaccharides, α-glucosidase further catabolises starch and oligosaccharides, which are then absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels [32]. In our study, all the extracts of honeybush demonstrated remarkable inhibition potential against the α-amylase enzyme activity (Figure 3) with efficacy similar to that of the used standard, acarbose. Hence, as expected, there was no effect observed against the α-glucosidase activity. The ability of honeybush to inhibit amylase activity proves its antidiabetic potential, which therefore suggests that extracts of *Cyclopia genistoides* could be an attractive source for alternative treatment. Xiao et al. [12] also reported significant inhibitory effects of *Cyclopia genistoides* on the activities of α-glucosidase and pancreatic lipase. In our findings, the extracts did not show any inhibitory effect on α-glucosidase activity (Figure 3(b)), which contradicts Xiao's findings, and this may be the result of several factors including the seasons of harvesting the plant material and the geographic area where it was harvested [12]. Moreover, our results are also consistent with the *in vitro* studies by Chipiti et al. [31] and Elbashir et al. [33] who reported α-amylase and α-glucosidase inhibitory effects.

Besides hyperglycemia, diabetes mellitus is highly characterized by high levels of triglycerides which are associated with modern-day lifestyles and increased consumption of high-fat diets [34]. Pancreatic lipase (pancreatic triacylglycerol lipase), a water-soluble enzyme secreted from the pancreas, hydrolyses dietary triglycerides into monoglycerides and fatty acids in order to simplify dietary fat absorption. [35]. Reduction in free fatty acids through inhibition of pancreatic lipase activity has been shown to reduce hyperlipidaemia associated with diabetes mellitus [34, 36]. In the present study, all extracts from honeybush demonstrated a significant inhibitory effect on pancreatic lipase activity in a concentration-dependent manner, with efficacy similar to that of the standard lipase inhibitor, orlistat, which further proves its therapeutic potential (Figure 4). The antihyperlipidemic effects of *Moringa stenopetala* through the inhibition of pancreatic lipase activity have been reported [36]. Secondary metabolites in

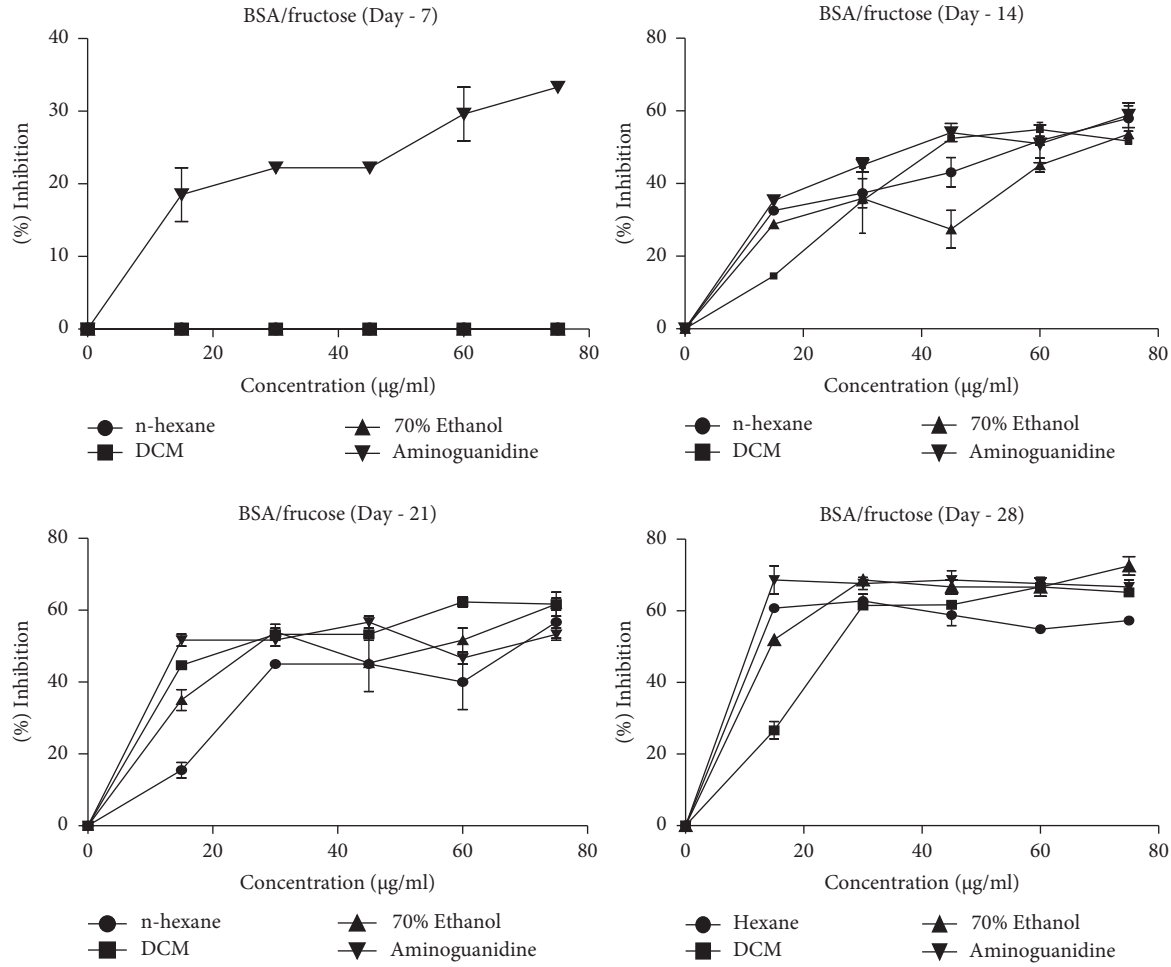

Figure 5: Antiglycation activity of *Cyclopia genistoides* extracts at day 7 to 28. Values were presented as the mean ± standard deviation. ($n = 3$). Statistical significance at $p < 0.05$.

plants, including phenolics, flavonoids, glycosides, and saponins, previously exhibited the activity inhibition of α-amylase and pancreatic lipase [37]. The observed inhibitory effect of these extracts on α-amylase and pancreatic lipase activity could be attributed to the total phenolics and flavonoids present in the extracts (Figure 1). Flavonoids are a heterogeneous group of ubiquitous plant polyphenols, which exhibit a variety of pharmacological activities such as antiatherogenic, antioxidant properties, lipoprotein oxidation, blood platelet aggregation, vascular reactivity, and antihyperglycemic effects [38, 39].

In the current study, the glycation of BSA was attenuated in the presence of extracts (Figure 5). Thus, this indicated that all extracts exhibited inhibitory effects on the formation of AGEs in the late stage of the glycation process. The observed antiprotein glycation activity of the extracts was further supported by the ability of both extracts to attenuate the formation of late-stage Amadori products "fructosamine" during the glycation period (Table 3). Our results are in agreement with those of Meng et al. [40] who showed the extracts of *Melissa officinalis* to exhibit a high inhibitory effect on the AGE formation in the late stage of the glycation process, which prevents diabetic related complication.

Furthermore, chronic hyperglycemia triggers the overproduction of superoxide anions and the increased generation of hydroxyl radicals, which results in the peroxidation of membrane lipids and protein glycation [41]. A number of plants with antidiabetic effects are strongly associated with their antioxidant properties [42]. Findings of this study (Table 2) show that all extracts from honeybush (*Cyclopia*) exhibit variable antioxidant scavenging activity in a concentration-dependent manner, an activity that could be linked to the presence of vital secondary metabolites in the extract (Table 1). It is implicated that the potent antioxidant properties observed in this study could be due to the undecane, dodecane, phytol, 9,12-octadecadienoic acid, 6-octadecenoic acid, heptadecanoic acid, and 9-methyl-, methyl ester 6-octadecenoic acid presence (Table 1) as previously reported by many investigators [43–46]. In addition, the reductive potential of compounds has successfully been used as an indicator of their potential antioxidant capacity [29, 47]. The observed reduction potential displayed by honeybush extracts further supports its antioxidant property and may be a useful tool in the fight against oxidative stress-induced tissue damage (Figure 2).

In the current study, hepatocyte cells (C3A) were used to evaluate the toxicity of *Cyclopia genistoides* extracts

Table 3: Effect of *Cyclopia genistoides* extracts on fructosamine content at days 7 to 28.

Experimental groups	Fructosamine content (μg/ml)			
	Day 7	Day 14	Day 21	Day 28
BSA/fructose	$0.30 \pm 0.01^{**}$	$0.34 \pm 0.01^{**}$	$0.38 \pm 0.01^{**}$	$0.45 \pm 0.03^{**}$
BSA/fructose/AG (1 μg/ml)	0.27 ± 0.11	0.33 ± 0.02	0.40 ± 0.02	0.44 ± 0.07
BSA/fructose/AG (5 μg/ml)	0.25 ± 0.00	0.29 ± 0.00	0.36 ± 0.00	0.39 ± 0.00
BSA/fructose/AG (10 μg/ml)	0.23 ± 0.03	0.27 ± 0.01	0.27 ± 0.01	0.30 ± 0.00
BSA/fructose/AG (25 μg/ml)	0.19 ± 0.02	0.27 ± 0.03	0.20 ± 0.03	0.23 ± 0.01
BSA/fructose/AG (100 μg/ml)	$0.18 \pm 0.01^{\#}$	0.20 ± 0.00	0.11 ± 0.00	$0.17 \pm 0.00^{\#}$
BSA/fructose/*n*-hexane (1 μg/ml)	0.33 ± 0.00	0.33 ± 0.00	0.39 ± 0.05	0.33 ± 0.02
BSA/fructose/*n*-hexane (5 μg/ml)	0.30 ± 0.00	0.30 ± 0.00	0.38 ± 0.02	$0.29 \pm 0.00^{*}$
BSA/fructose/*n*-hexane (10 μg/ml)	0.31 ± 0.01	0.31 ± 0.01	0.41 ± 0.00	$0.27 \pm 0.01^{**}$
BSA/fructose/*n*-hexane (25 μg/ml)	0.32 ± 0.00	0.32 ± 0.00	0.36 ± 0.01	$0.27 \pm 0.03^{**}$
BSA/fructose/*n*-hexane (100 μg/ml)	0.31 ± 0.00	$0.31 \pm 0.00^{**}$	0.37 ± 0.01	$0.20 \pm 0.00^{**}$
BSA/fructose/DCM (1 μg/ml)	0.36 ± 0.05	0.38 ± 0.02	0.34 ± 0.12	0.40 ± 0.02
BSA/fructose/DCM (5 μg/ml)	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.03	0.36 ± 0.00
BSA/fructose/DCM (10 μg/ml)	0.37 ± 0.01	0.33 ± 0.01	0.34 ± 0.00	$0.27 \pm 0.01^{**}$
BSA/fructose/DCM (25 μg/ml)	0.40 ± 0.02	0.33 ± 0.00	0.33 ± 0.01	$0.20 \pm 0.03^{**}$
BSA/fructose/DCM (100 μg/ml)	0.39 ± 0.00	0.32 ± 0.00	0.32 ± 0.01	$0.22 \pm 0.00^{**}$
BSA/fructose/70% ethanol (1 μg/ml)	0.34 ± 0.04	0.34 ± 0.04	0.34 ± 0.04	0.38 ± 0.04
BSA/fructose/70% ethanol (5 μg/ml)	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.38 ± 0.01
BSA/fructose/70% ethanol (10 μg/ml)	0.26 ± 0.00	$0.26 \pm 0.00^{*}$	$0.26 \pm 0.00^{**}$	$0.22 \pm 0.00^{**}$
BSA/fructose/70% ethanol (25 μg/ml)	$0.21 \pm 0.01^{**}$	$0.21 \pm 0.01^{**}$	$0.21 \pm 0.01^{**}$	$0.22 \pm 0.01^{**}$
BSA/fructose/70% ethanol (100 μg/ml)	$0.17 \pm 0.01^{**\#}$	$0.10 \pm 0.01^{**}$	$0.17 \pm 0.01^{**}$	$0.11 \pm 0.01^{**\#}$

Data are expressed as the mean ± standard deviation. ($n = 3$). $^{\#}p < 0.05$ when compared to BSA/fructose/AG (100 μg/ml); $^{**}p < 0.05$ when compared to BSA/fructose. (AG: aminoguanidine).

Table 4: LC_{50} values (μg/ml) of honeybush (*Cyclopia genistoides*) against C3A cell lines.

Extracts	C3A cell lines (μg/ml)
n-hexane	72.6 ± 2.02
DCM	66.3 ± 1.22
70% ethanol	88.4 ± 2.42

(Table 4), and our data revealed that the honeybush crude extracts had a comparatively weak toxicity against C3A cells, considering that extracts with an LC_{50} value of less than the 20 μg/ml threshold are regarded as highly toxic [48, 49]. Thus, the observed cytotoxicity levels of honeybush tea further highlight its medicinal potential for future studies.

5. Conclusion

In the present study, different solvent extracts of honeybush tea (*Cyclopia genistoides*), viz., n-hexane, dichloromethane, and 70% ethanol, were subjected to GC-MS analysis. The GC-MS spectrum confirms the presence of various bioactive compounds in all extracts of *Cyclopia genistoides* with different retention times. Thus, it can be concluded that honeybush tea (*Cyclopia genistoides*) extracts exhibited some vital antidiabetic and antioxidant properties which were due to the observed free radical scavenging potential and inhibitory effects on protein glycation, accompanied by inhibitory effects on α-amylase and pancreatic lipase activity. It is also worth noting that, among all the extracts used, DCM extract displayed highest enzyme inhibition against α-amylase and pancreatic lipase activity. Therefore, the extracts of *Cyclopia genistoides* could be a promising therapeutic in the prevention and management of diabetes and its related complications. In addition to their efficacy, the observed potential cytotoxicity on hepatocyte cell (C3A) toxicity of the extracts partially indicates that *Cyclopia genistoides* should be medicinally used with caution. However, as this is only a preliminary study, the *in vivo* antihyperglycemic potential of honeybush tea extracts and the investigation of their effect on glucose uptake as well as the possible identification of their dominant pure bioactive compound(s) still need to be explored. [48, 49].

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

NDC, NEM, SN, and QBM analysed the data. NDC wrote the manuscript. FT, OJP, MSM, and ARO edited the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Table S1: a complete list of the identified compounds from different extracts of honeybush (Cyclopia sessiliflora) tea using GC-MS. GC -MS analysis of the n-hexane extract of honeybush. GC-MS analysis of the dichloromethane extract of honeybush. GC-MS analysis of the ethanolic extract of honeybush. (*Supplementary Materials*)

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