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In vitro α-glucosidase inhibitory activity of medicinal plants used traditionally for treating diabetes in Vhembe District, South Africa

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ABSTRACT

Introduction: α-Glucosidase is the major enzyme implicated in intestinal glucose absorption, and its inhibition is a target for the management of diabetes mellitus. This study investigated the *in vitro* α-glucosidase inhibitory activity of extracts from different parts of 20 selected medicinal plants and the potential for plant-part substitution and plant species combinations used by traditional healers to treat diabetes.

Methods: Acetone and petroleum ether extracts from different parts of 20 plant species traditionally used to treat diabetes were individually evaluated *in vitro* using an α-glucosidase assay. The potential for plant-part substitution was investigated by including leaf extracts where non-renewable parts are used traditionally. The extracts of plant species were combined and investigated as used traditionally.

Results: *Anthocleista grandiflora* stem bark acetone, *Artabotrys brachypetalus* leaf petroleum ether, and *Dichrostachys cinerea* root petroleum ether extracts exhibited remarkable α-glucosidase inhibitory activities with IC_{50} values of 9, 14, and 12 µg/mL, respectively. The α-glucosidase inhibitory activities of *A. grandiflora*, *A. brachypetalus*, *Asparagus virgatus*, *Brackenridgea zanguebarica*, *Maerua edulis*, *Pterocarpus angolensis,* and *Tabernaemontana elegans* were documented for the first time, suggesting their antidiabetic potential. The leaf acetone extracts of *Brackenridgea zanguebarica* and *Terminalia sericea* had similar α-glucosidase inhibitory activities when compared to their stem bark and root, respectively. The combination of *Dichrostachys cinerea* leaf with *Elephantorrhiza elephantina* root, extracted with petroleum ether, resulted in a synergistic inhibitory effect.

Conclusion: The valorization of these newly documented species holds potential for the discovery of more effective and perhaps novel antidiabetic remedies or drug principles.

Implication for health policy/practice/research/medical education:
This study demonstrated the potential antidiabetic effects of *Anthocleista grandiflora, Artabotrys brachypetalus, Asparagus virgatus, Brackenridgea zanguebarica, Maerua edulis, Pterocarpus angolensis,* and *Tabernaemontana elegans* extracts, providing scientific evidence for their use in traditional medicine to treat diabetes.

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Introduction

Diabetes mellitus is characterized by increased blood glucose due to pancreatic inability to secret insulin, its action deficit, or a combination of both (1). Long-term hyperglycemia can develop neuropathy, nephropathy, and retinopathy (2,3). Globally, 537 million adults (aged 20-79 years) are living with diabetes, with a projection of this number rising to 643 million adults by 2030

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and 784 million adults (46% increase) by 2045 (4). In 2021, diabetes caused 6.7 million deaths worldwide and health expenditure of USD 966 billion (4). Recently, the prevalence of diabetes has increased, especially in developing countries (5), with 81% of people with diabetes living in low-income and middle-income countries (4). While the prediction of global diabetes by 2045 is a 46% increase, the Africa region is predicted to experience an alarming 134% increase in cases of diabetes by 2045 (4). In Africa, South Africa currently has the highest proportion of adults (aged 20-79 years) living with diabetes; one in nine adults, as opposed to one in 10 adults globally (4).

Diabetes can affect persons of any age, lessening the person's quality of life. Since diabetes can cause huge personal financial challenges owing to the cost of medications, it also presents a significant economic burden for every country and its health systems. According to the International Diabetes Federation, diabetes economic burden is due to the long-term support required to reduce diabetes and its cognitive complications, increased utilization of health services, and loss of productivity (6). The diabetes disease burden globally is expected to increase from USD 966 billion in 2021 to USD 1028 billion by 2030 (4). A large number of those within the workforce category suffering from diabetes places huge economic and social burdens on countries and their national health systems. In terms of the socioeconomic impact, diabetes affliction leads to losses in productivity and economic growth due to lost work days, restricted activities, reduced work productivity, increased health cost, mortality burden, and permanent disability resulting from diabetes (7). Therefore, the need for readily-available, cost-effective management or solutions to decrease the prevalence cannot be over-emphasized. Herbal medicines are usually more accessible and cost-effective than synthetic drugs. The adverse effects and high cost of synthetic antidiabetic drugs necessitate the search for new and efficient remedies to manage diabetes (8).

Medicinal plants have been used for centuries in traditional medicine worldwide for the treatment and management of several diseases, including diabetes. To date, medicinal plants are still used by many who believe strongly in the efficacy of natural products, irrespective of the presence of conventional antidiabetic therapies. We recently documented medicinal plants used by traditional healers in Vhembe District, Limpopo Province, in South Africa, for treating diabetes (9). As with many medicinal plants and their products used traditionally for diabetes treatment, there is a dearth of information on the scientific efficacy of these plants to valorize their use in traditional medicine.

α-Glucosidase, a major enzyme in carbohydrate digestion, catalyzes the hydrolytic cleavage of α-glucosidic linkages in oligosaccharides and disaccharides into monosaccharides, leading to an increased blood glucose level through intestinal absorption (10). Unlike the products of α-amylase activity during carbohydrate metabolism, monosaccharides produced through the action of α-glucosidase are more quickly absorbed (11). The inhibition of α-glucosidase remains a therapeutic target for post-prandial hyperglycaemia modulation (12). Thus, α-glucosidase inhibition assay is often used to evaluate *in vitro* antidiabetic activity. Given the conservation concerns that beset the use of non-renewable plant parts in traditional medicine, the use of renewable plant parts such as the leaves was evaluated to determine the potential for plant part substitution in the case of species whose non-renewable parts are mainly used traditionally for diabetes treatment. In evaluating the antidiabetic potential of crude extracts from the selected species, we also evaluated the α-glucosidase inhibitory activity of plant species combinations used by the traditional healers (9).

Material and Methods

Chemicals and reagents

α-Glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), acarbose, potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (South Africa). Sodium carbonate, petroleum ether, and acetone were purchased from Merck (South Africa). All solvents and chemicals were of analytical grade.

Plant selection and collection

From a total of 63 medicinal plants mentioned by traditional healers as remedies for diabetes in four local municipalities of the Vhembe district in South Africa (9), 20 medicinal plants (Table 1) were selected based on the number of citations by the participating traditional healers and documented research activities on the species. Plant materials identified by the traditional healers were authenticated by Prof Milingoni Tshisikhawe and Dr. Ndivhaleni Masevhe, Department of Botany, University of Venda, South Africa. The collection of the selected plants and their voucher specimen preparation and deposition in the University of Venda herbarium in South Africa were done as previously described (9).

Preparation of plant extracts

Different parts of each selected plant species were collected, rinsed in clean water, chopped or fragmented into small pieces, and oven-dried inside a brown paper bag at 50°C. Dried plant materials were milled using Fritsh Pulverisette 14 (Idar-Oberstein, Germany) into fine powders (1 mm sieve size), transferred into airtight containers, and properly labelled. The finely ground plant parts were weighed and extracted using two solvents of different polarities; acetone and petroleum ether. Powdered plant materials were extracted in a ratio of 1:10 (w/v), whereby 50 g of plant materials were extracted with

Table 1. Selected plant species traditionally used for treating diabetes in Vhembe District (South Africa), their voucher numbers, and α-glucosidase inhibitory activities of extracts from different parts

Plant species	Family	Voucher number	Plant part	α-Glucosidase inhibition (IC_{50}, µg/mL)	
				Acetone extract	PE extract
Anthocleista grandiflora Gilg.	Gentianaceae	TEU049	Root	55 ± 2^{abc}	1109 ± 336^{i}
			Stem bark	9 ± 1^{a}	239 ± 18^{bcdef}
			Leaf	933 ± 58^{i}	105 ± 16^{abcde}
Artabotrys brachypetalus Benth.	Annonaceae	TEU019	Root	68 ± 2^{abc}	30 ± 2^{ab}
			Leaf	313 ± 16^{f}	14 ± 2^{a}
Asparagus virgatus Baker.	Asparagaceae	TEU047	Whole plant	917 ± 31^{i}	67 ± 9^{abcd}
Brackenridgea zanguebarica Oliv.	Ochnaceae	TEU037	Stem bark	145 ± 15^{abcde}	125 ± 4^{abcde}
			Leaf	119 ± 2^{abcd}	41 ± 4^{abc}
Capparis tomentosa Lam.	Capparaceae	TEU050	Root	115 ± 8^{abcd}	43 ± 1^{abc}
			Stem	85 ± 3^{abcd}	508 ± 5^{gh}
			Leaf	1780 ± 45^{k}	202 ± 27^{abcdef}
Cassia abbreviata Oliv.	Fabaceae	TEU039	Stem bark	606 ± 53^{gh}	50 ± 4^{abcd}
			Leaf	ND	308 ± 30^{ef}
Dichrostachys cinerea (L) Wight and Arn.	Fabaceae	TEU040	Root	167 ± 2^{cde}	12 ± 0^{a}
			Leaf	587 ± 32^{gh}	86 ± 8^{abcd}
Elaeodendron transvaalense (Burtt Davy) R.H.Archer.	Celastraceae	TEU003	Root bark	264 ± 13^{ef}	53 ± 4^{abcd}
			Stem bark	109 ± 3^{abcd}	54 ± 3^{abcd}
			Leaf	657 ± 18^{h}	348 ± 28^{fg}
Elephantorrhiza elephantina (Burch.) Skeels	Fabaceae	TEU001	Root	49 ± 10^{abc}	110 ± 3^{abcde}
Heteromorpha arborescens (Spreng.) Cham. and Schltdl.	Apiaceae	TEU030	Root	ND	684 ± 78^{h}
			Leaf	1272 ± 60^{j}	1655 ± 123^{j}
Hypoxis hemerocallidea Fisch., C.A.Mey. and Ave-Lall	Hypoxidaceae	TEU053	Corm	ND	139 ± 2^{abcde}
Maerua edulis (Gilg and Gilg-Ben.) DeWolf.	Capparaceae	TEU004	Root	62 ± 6^{abc}	142 ± 13^{abcde}
			Leaf	849 ± 31^{i}	257 ± 10^{def}
Moringa oleifera Lam.	Moringaceae	TEU017	Stem bark	1230 ± 73^{j}	18 ± 1^{a}
			Leaf	ND	1033 ± 21^{i}
Pterocarpus angolensis DC.	Fabaceae	TEU022	Root bark	24 ± 2^{ab}	24 ± 4^{a}
			Stem bark	31 ± 7^{abc}	244 ± 4^{cdef}
			Leaf	1179 ± 57^{j}	44 ± 4^{abc}
Sclerocarya birrea (A.Rich.) Hochst.	Anacardiaceae	TEU010	Root bark	41 ± 1^{abc}	31 ± 1^{ab}
			Stem bark	34 ± 4^{abc}	191 ± 20^{abcdef}
			Leaf	156 ± 12^{bcde}	309 ± 31^{ef}
Securidaca longepedunculata Fresen.	Polygalaceae	TEU028	Root	59 ± 2^{abc}	26 ± 1^{a}
			Leaf	255 ± 31^{ef}	1721 ± 59^{j}
Senna petersiana (Bolle) Lock.	Fabaceae	TEU014	Root	212 ± 10^{def}	43 ± 1^{abc}
			Leaf	513 ± 57^{g}	567 ± 31^{h}
Strychnos henningsii Gilg.	Loganiaceae	TEU032	Stem bark	82 ± 7^{abcd}	46 ± 3^{abc}
			Leaf	1184 ± 175^{j}	ND
Tabernaemontana elegans Stapf.	Apocynaceae	TEU045	Leaf	99 ± 2^{abcd}	ND
Terminalia sericea Burch. ex DC.	Combretaceae	TEU018	Root	97 ± 3^{abcd}	245 ± 23^{cdef}
			Leaf	39 ± 1^{abc}	123 ± 13^{abcde}

In each column, values (mean ± standard error of mean) followed by different superscript letters indicate statistically significant ($P \leq 0.05$) differences. PE = Petroleum ether, ND = not determined. IC_{50} = Half maximal inhibitory concentration. Acarbose (positive control) IC_{50} = 980 µg/ml.

500 mL of solvent with sonication (Branson, 5510E-MT, Lasec, South Africa) for one hour. The mixtures were filtered using a Buchner Filtration kit (Buchner flask + filter funnel + filter paper + rubber hose + vacuum pump). The filtered extracts were condensed *in vacuo* using a rotary evaporator (Stuart, RE300DB, Lasec, Johannesburg, South Africa). The resultant extracts were transferred into glass vials and air-dried. After drying, the samples were kept in a refrigerator at 4°C for α-glucosidase inhibition assay. For combination studies, plant materials were combined 1:1 (*w*/*w*) and extracted as described above. The selection of the combined plant materials was based on the ethnobotanical combinations of the plant materials as practiced by the interviewed traditional healers in the four local municipalities of the Vhembe district in South Africa (9).

α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was done according to the method described by Li et al (13) with modifications detailed by Mabotja et al (14). In this assay, α-glucosidase breaks down the substrate *p*NPG into *p*-nitrophenol, which is measured spectrophotometrically using a 96-well microplate reader (FLUOstar Omega, BMG LabTech, Ortenberg, Germany) to determine the inhibitory activities of the samples. Briefly, into each sample well of a 96-well microplate was pipetted 20 µL of the extract dissolved in DMSO, 100 µL of potassium phosphate buffer (0.1M, pH 6.8), and 20 µL of α-glucosidase solution (0.5 unit/mL). The same volume of DMSO was used to replace the sample in the case of the negative control. After incubation of the mixture at 37°C for 5 minutes, 20 µL of the substrate (5mM pNPG) was added, and the plates were incubated at 37°C for 30 minutes. Sodium carbonate (80 µL, 0.2M) was added to terminate the reaction. Each determination was done in triplicates in independent experiments. Acarbose was used as a positive control. The absorbance was read at 405 nm, and α-glucosidase inhibitory activity was calculated using the following equation:

$$\text{a-Glucosidase inhibitory activity (\%)} = \frac{A_{405}\ control - A_{405} sample}{A_{405}\ control} \times 100\%$$

where $A_{405}\ control$ is the absorbance value of negative control and $A_{405}\ sample$ is the absorbance value of the sample or acarbose. Half maximal inhibitory concentration (IC_{50}) determination was done for each sample evaluated at different concentrations.

Fractional inhibitory concentration index (∑FIC) was calculated using the following equation:

$$\sum FIC = \left(\frac{C1}{Cs1}\right) + \left(\frac{C1}{Cs2}\right) + \ldots + \left(\frac{C1}{Csn}\right)$$

where *C*1 is the IC_{50} of the combined sample while *Cs*1, *Cs*2, and *Csn* refer to the IC_{50} of sample 1, sample 2, up to sample *n* in the same combination. Following the classification by van Vuuren and Viljoen (15), ∑FIC ≤ 0.5 is synergistic, 0.5 < ∑FIC ≤ 1.0 = additive, ∑FIC > 1.0 but ≤ 4.0 = indifference, and ∑FIC > 4.0 = antagonistic.

Data analysis

For the determination of IC_{50}, a non-linear regression analysis was done using the GraphPad Prism software (version 4.03). Data were subjected to one-way analysis of variance followed by Duncan's multiple range test as a post-hoc test using SPSS software (version 16.0) in order to separate mean values where significant ($P \leq 0.05$) differences were established. The results were expressed as mean IC_{50} values ± standard errors of the mean.

Results

α-Glucosidase inhibitory activities of plant extracts

The α-glucosidase inhibitory activities of acetone and petroleum ether extracts from different parts of the 20 selected medicinal plants are indicated in Table 1. With the exception of *Capparis tomentosa* leaf, *Heteromorpha arborescens* leaf, *Moringa oleifera* stem bark, and *Pterocarpus angolensis* leaf, as well as *Cassia abbreviata* leaf, *H. arborescens* root, *Hypoxis hemerocallidea* corm, and *Moringa oleifera* leaf, whose IC_{50} values could not be computed because of low activity at the highest concentrations tested; all other acetone extracts had lower IC_{50} values when compared to acarbose (Table 1). Overall, *Anthocleista grandiflora* stem bark acetone extract had the least $IC_{50,}$ making it the most potent acetone extract. Other acetone extracts with very low IC_{50} values (IC_{50} < 100 µg/mL) indicating their strong potency include *Anthocleista grandiflora* root, *Artabotrys brachypetalus* root, *Capparis tomentosa* stem, *Elephantorrhiza elephantina* root, *Maerua edulis* root, *Pterocarpus angolensis* root bark and stem bark, *Sclerocarya birrea* root bark and stem bark, *Securidaca longepedunculata* root, *Strychnos henningsii* stem bark, *Tabernaemontana elegans* leaf, and *Terminalia sericea* root and leaf. There were no significant differences in all acetone extracts with very low IC_{50} values. Only the leaf acetone extracts of *Brackenridgea zanguebarica* and *Terminalia sericea* demonstrated lower IC_{50} values when compared to their respective other parts (stem bark and root, respectively).

All the petroleum ether extracts except those of *Anthocleista grandiflora* root, *Heteromorpha arborescens* leaf, *Moringa oleifera* leaf, and *Securidaca longepedunculata* leaf displayed lower IC_{50} values than that of acarbose. Seventeen petroleum ether extracts particularly demonstrated potent activity with very low IC_{50} values (IC_{50} < 100 µg/mL). These were obtained from *Artabotrys brachypetalus* roots and leaves, *Asparagus virgatus* whole plants, *Brackenridgea zanguebarica* leaves, *Capparis tomentosa* roots, *Cassia abbreviata* stem barks,

Dichrostachys cinerea roots and leaves, *Elaeodendron transvaalense* root bark and stem barks, *Moringa oleifera* stem barks, *Pterocarpus angolensis* root barks and leaves, *Sclerocarya birrea* root barks, *Securidaca longepedunculata* roots, *Senna petersiana* roots, and *Strychnos henningsii* stem barks. In terms of potential plant-part substitution, the leaves of *Anthocleista grandiflora, Artabotrys brachypetalus, Brackenridgea zanguebarica,* and *Terminalia sericea* demonstrated potencies that were at least comparable to their root and/or stem barks.

α-Glucosidase activities of combined plant extracts

Table 2 shows the α-glucosidase inhibitory activities of acetone extract of different plant combinations. All the combinations demonstrated stronger activity than acarbose control. Only two combinations, *Brackenridgea zanguebarica* stem bark with *Securidaca longepedunculata* root (fractional inhibitory concentration index of 0.6), as well as *Elephantorrhiza elephantina* root with *Dichrostachys cinerea* leaf (fractional inhibitory concentration index of 0.9), demonstrated additive α-glucosidase inhibitory effect with very low IC_{50} values (Table 2). The other combinations were either indifferent or antagonistic. For petroleum ether extracts, all the combinations exhibited strong potency with very low IC_{50} values (Table 3). Combining *Elephantorrhiza elephantina* root with *Dichrostachys cinerea* leaf resulted in a synergistic inhibitory effect (fractional inhibitory concentration index of 0.3) (Table 3). Similar to the acetone extract, a combination of *Brackenridgea zanguebarica* stem bark with *Securidaca longepedunculata* root exhibited an additive effect, whereas all other petroleum ether extract combinations were indifferent (Table 3).

Discussion

α-Glucosidase is an enzyme found in the mucosal brush lining of the small intestine, and is responsible for the hydrolysis of disaccharides into monosaccharides, which are more readily absorbed, resulting in an increased blood glucose level (16). α-Glucosidase inhibitors slow down the breakdown of carbohydrates and decrease postprandial hyperglycemia (17). The inhibition of intestinal α-glucosidase has been reported in the successful treatment of patients with diabetes (16). Diabetic patients in developing countries usually cannot afford or have not readily access to synthetic drugs for diabetes management. The undesirable side effects such as lactic acidosis, liver problems, abdominal discomfort, flatulence, and diarrhea (18,19) associated with the current drugs also present another challenge in the management of diabetes. The use of acarbose and metformin has been associated with gastrointestinal problems in some patients (19,20). The presence of such side effects can negatively affect the compliance of patients with the treatment regimen, leading to further diabetic complications (21). Hence, the search for less costly and high potent antidiabetic drugs, especially of natural origin ones with minimal or no adverse effects, is important. The World Health Organization has acknowledged the importance of alternative and complementary medicines in various disease prevention and treatment of diseases, especially for improving the quality of life of people living with

Table 2. α-Glucosidase inhibitory activities of acetone extract combinations

Combination number	Plant species	Plant part	α-Glucosidase inhibition (IC_{50}, µg/ml)	Fractional inhibitory concentration index	Remarks
1	*Elephantorrhiza elephantina* (Burch.) Skeels	Root	170 ± 4[d]	3.8	Indifference
	Dichrostachys cinerea (L) Wight and Arn.	Leaf			
2	*Brackenridgea zanguebarica* Oliv.	Stem bark	24 ± 0[a]	0.6	Additive
	Securidaca longepedunculata Fresen.	Root			
3	*Anthocleista grandiflora* Gilg.	Root	152 ± 7[c]	3.0	Indifference
	Cassia abbreviata Oliv	Stem bark			
4	*Elephantorrhiza elephantina* (Burch.) Skeels	Root	30 ± 3[a]	0.9	Additive
	Dichrostachys cinerea (L) Wight and Arn.	Leaf			
	Elaeodendron transvaalense (Burtt Davy) R.H.Archer	Stem bark			
5	*Brackenridgea zanguebarica* Oliv.	Stem bark	46 ± 2[b]	1.5	Indifference
	Securidaca longepedunculata Fresen.	Root			
	Capparis tomentosa Lam.	Root			
6	*Anthocleista grandiflora* Gilg.	Root	192 ± 2[e]	7.1	Antagonistic
	Cassia abbreviata Oliv.	Stem bark			
	Securidaca longepedunculata Fresen.	Root			

Values (mean ± standard error of mean) followed by different letters in the α-glucosidase column indicate statistically significant ($P \leq 0.05$) differences. IC_{50} = Half maximal inhibitory concentration. Acarbose (positive control) IC_{50} = 980 µg/mL.

Table 3. α-Glucosidase inhibitory activities of petroleum ether extract combinations

Combination number	Plant species	Plant part	α-Glucosidase inhibition (IC_{50}, µg/mL)	Fractional inhibitory concentration index	Remarks
	*Elephantorrhiza elephantin*a (Burch.) Skeels	Root	14 ± 2[a]	0.3	Synergistic
	*Dichrostachys cinere*a (L) Wight and Arn	Leaf			
	Brackenridgea zanguebarica Oliv.	Stem bark	20 ± 2[a]	0.9	Additive
	*Securidaca longepedunculat*a Fresen	Root			
	*Moringa oleifer*a Lam.	Leaf	32 ± 1[b]	1.1	Indifference
	*Sclerocarya birre*a (A.Rich.) Hochst	Root bark			
	*Anthocleista grandiflor*a Gilg.	Root	92 ± 1[d]	1.9	Indifference
	*Cassia abbreviat*a Oliv.	Stem bark			
	Elephantorrhiza elephantina (Burch.) Skeels	Root	30 ± 2[b]	1.2	Indifference
	*Dichrostachys cinere*a (L) Wight and Arn.	Leaf			
	Elaeodendron transvaalense (Burtt Davy) R.H.Archer	Stem bark			
	Brackenridgea zanguebarica Oliv.	Stem bark	54 ± 4[c]	3.8	Indifference
	*Securidaca longepedunculat*a Fresen	Root			
	*Capparis tomentos*a Lam.	Root			
	*Moringa oleifer*a Lam.	Leaf	34 ± 1[b]	1.8	Indifference
	*Sclerocarya birre*a (A.Rich.) Hochst.	Root			
	*Cassia abbreviat*a Oliv.	Stem bark			
	Anthocleista grandiflora Gilg.	Root	31 ± 5[b]	1.8	Indifference
	*Cassia abbreviat*a Oliv.	Stem bark			
	*Securidaca longepedunculat*a Fresen	Root			

Values (mean ± standard error of mean) followed by different letters indicate statistically significant ($P \leq 0.05$) differences. IC_{50} = Half maximal inhibitory concentration. Acarbose (positive control) IC_{50} = 980 µg/mL.

chronic diseases (22). Researchers have thus documented variation in the α-glucosidase inhibitory activity of different plant materials and preparations (16,23). In the current study, 67 extracts obtained from different parts of the selected species exhibited IC_{50} values below that of the positive control, acarbose, including 32 extracts (with IC_{50} values below 100 µg/mL) whose α-glucosidase inhibitory activities were nine times stronger, based on their IC_{50} values, than acarbose activity. This indicates the potential for discovering potential α-glucosidase inhibitors from natural products that are more active than acarbose. *Anthocleista grandiflora* stem bark acetone, *Artabotrys brachypetalus* leaf petroleum ether, and *Dichrostachys cinerea* root petroleum ether extracts exhibited remarkable α-glucosidase inhibitory activities with IC_{50} values of 9, 14, and 12 µg/mL, respectively. The α-glucosidase activities of *Anthocleista grandiflora*, *Artabotrys brachypetalus*, *Asparagus virgatus*, *Brackenridgea zanguebarica*, *Maerua edulis*, *Pterocarpus angolensis*, and *Tabernaemontana elegans* documented for the first time, to the best of our knowledge, suggest their antidiabetic potential.

In evaluating medicinal plant extracts, the choice of extraction solvent is a very important consideration as different solvents extract different compounds based on their polarity and can influence the pharmacological activities of the extracts (24,25). Although water is a common solvent, the use of organic solvents of varying polarities has become a common modern practice in order to exploit phytoconstituents with different solubilities (26). Seventeen petroleum ether extracts had IC_{50} values below 100 µg/mL in comparison to 15 acetone extracts that exhibited a similar response. Acetone extracts of *Anthocleista grandiflora* root and stem bark had stronger inhibitory activities than their respective petroleum ether extracts, whereas the leaf petroleum ether extract showed stronger activity than its acetone extract. Unlike acetone, petroleum ether is known to mainly extract non-polar compounds. In a previous study (27), baruol, 3-deacetylmongolenin, anthocleistone, 6-ketoanthocleistone, scopoletin, lupenone, and (+)-de-*O*-methyllasiodiplodin were isolated from the combined hexane and dichloromethane extracts of *A. grandiflora* stem bark. The antidiabetic activities of lupenone and scopoletin have been established *in vitro* and *in vivo* (28,29). 3-Deacetylmongolenin was isolated from *A. grandiflora* leaf dichloromethane extract while lupenone was isolated from a combined hexane and dichloromethane extract of its root bark (27). Sweroside was obtained from the combined ethyl acetate and methanol extract of the root bark (27).

A similar or stronger inhibitory activity was shown by the leaf extract of *Anthocleista grandiflora* (petroleum ether extract), *Artabotrys brachypetalus* (petroleum ether), *Brackenridgea zanguebarica* (both acetone and petroleum ether), and *Terminalia sericea* (both acetone and petroleum ether) when compared to their root and/or stem bark extracts. Their leaves were included to compare their potencies with other plant parts, mainly the traditionally used non-renewable parts. Based on their IC_{50} values, the leaves of *Brackenridgea zanguebarica* and *Terminalia sericea* can substitute for their stem bark and root, respectively. The use of leaves can ameliorate the biodiversity conservation issues associated with the harvesting and use of underground or stem barks of medicinal plants. Plant part substitution is a recommended strategy for the sustainable use of medicinal plants (30,31), especially for commercialization.

In many instances, medicinal plants are combined in traditional practice for improved efficacy, among other reasons. Combining medicinal plants can increase their therapeutic ability as compared to the single use of plants (32). In line with their use in traditional medicine (9), the combination of *Brackenridgea zanguebarica* stem bark with *Securidaca longepedunculata* root demonstrated an additive α-glucosidase inhibitory effect, irrespective of the extraction solvent. The combination of *Dichrostachys cinerea* leaf with *Elephantorrhiza elephantina* root petroleum ether extract resulted in a synergistic inhibitory effect, whereas the acetone extract of the same combination exhibited an indifferent effect. This again indicates the influence of extraction solvent in the pharmacological screening of medicinal plants. On the other hand, the inclusion of *Elaeodendron transvaalense* stem bark in the acetone extract combination having *Dichrostachys cinerea* leaf and *Elephantorrhiza elephantina* root resulted in an additive effect. Similarly, the additive effect shown by the acetone extract combination of *Brackenridgea zanguebarica* stem bark with *Securidaca longepedunculata* root changed to an indifferent effect when *Capparis tomentosa* root was added to the combination. The improved antidiabetic activity of some extracts of combined medicinal plant materials supports the valuable indigenous knowledge on combining plants for herbal treatment by the traditional healers.

Conclusion

The α-glucosidase inhibitory activities of *Anthocleista grandiflora*, *Artabotrys brachypetalus*, *Asparagus virgatus*, *Brackenridgea zanguebarica*, *Maerua edulis*, *Pterocarpus angolensis,* and *Tabernaemontana elegans* were documented for the first time. On the basis of their IC_{50} values, the leaves of *Brackenridgea zanguebarica* and *Terminalia sericea* can substitute for their stem bark and root, respectively, for their sustainable use. The use of renewable parts such as leaves with proven similar biological potency is a conservation strategy to be further explored, especially for highly utilized plants in traditional medicine. Consistent with their combinational use in traditional medicine, the combination of some plant species exhibited a synergistic or additive α-glucosidase inhibitory effect. However, the efficacy and safety of medicinal plants that showed strong inhibitory activity need to be evaluated in other mechanism-based antidiabetic assays for the development of effective herbal remedies.

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Authors' contributions

SOA conceptualized the study, acquired funding, and analysed the data. TEM carried out the investigation and drafted the manuscript with SOA. JOO and SOA provided the resources, edited the manuscript, and supervised the study. All authors read the final version of the article and confirmed its publication.

Conflicts of interests

The authors declare no conflict of interest.

Ethical considerations

All ethical issues including plagiarism, data fabrication, misconduct, falsification, double publication or redundancy were completely considered by the authors. This study protocol was approved by Sefako Makgatho University Research Ethics Committee (SMUREC/S/296/2020: PG).

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References

1. Gajbhiye RL, Ganapathy A, Jaisankar P. A review of a-glucosidase and a-amylase inhibitors for type 2 diabetes

isolated from some important Indian medicinal plants. Ann Clin Pharmacol Ther. 2018;1(1):1003.
2. Seferovic JP, Bentley-Lewis R, Claggett B, Diaz R, Gerstein HC, Køber LV, et al. Retinopathy, neuropathy, and subsequent cardiovascular events in patients with type 2 diabetes and acute coronary syndrome in the ELIXA: The importance of disease duration. J Diabetes Res. 2018;1631263. doi. 10.1155/2018/1631263.
3. Le Floch JP, Doucet J, Bauduceau B, Verny C, the SFD/SFGG Intergroup. Retinopathy, nephropathy, peripheral neuropathy and geriatric scale scores in elderly people with type 2 diabetes. Diabet Med. 2014;31(1):107-11. doi. 10.1111/dme.12327.
4. International Diabetes Federation. 2021. IDF Diabetes Atlas, 10th edition. Brussels, Belgium: 2021. Available from: https://www.diabetesatlas.org. Accessed December 29, 2021.
5. World Health Organization. 2015. Global Report on Diabetes. Geneva: World Health Organisation. Available from: https://www.who.int/publications/i/item/9789241565257. Accessed December 29, 2021.
6. International Diabetes Federation. 2015. International Diabetes Federation Atlas – 7th edition. Brussels: International Diabetes Federation. Available from: https://www.diabetesatlas.org/upload/resources/previous/files/7/IDF%20Diabetes%20Atlas%207th.pdf. Accessed December 30, 2021.
7. International Diabetes Federation. 2009. International Diabetes Federation Atlas – 4th edition. Brussels: International Diabetes Federation. Available from: https://diabetesatlas.org/atlas/fourth-edition/. Accessed December 31, 2021.
8. Mondal P, Bhuyan N, Das S, Kumar M, Borah S, Mahato K. Herbal medicines useful for the treatment of diabetes in North-East India: A review. Int J Pharm Biol Sci. 2013;3(1):575-89.
9. Mudau TE, Olowoyo JO, Amoo SO. Ethnobotanical assessment of medicinal plants used traditionally for treating diabetes in Vhembe district, Limpopo Province, South Africa. S Afr J Bot. 2022;146:304-24. doi. 10.1016/j.sajb.2021.10.016.
10. Dirir AM, Daou M, Yousef AF, Yousef LF. A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. Phytochem Rev. 2022;21(4):1049-1079. doi: 10.1007/s11101-021-09773-1.
11. Dyer J, Wood IS, Palejwala A, Ellis A, Shirazi-Beechey SP. Expression of monosaccharide transporters in intestine of diabetic humans. Am J Physiol Gastrointest Liver Physiol. 2002;282(2):G241-8. doi: 10.1152/ajpgi.00310.2001.
12. Hinnen DA. Therapeutic options for the management of postprandial glucose in patients with type 2 diabetes on basal insulin. Clin Diabetes. 2015;33(4):175-80. doi: 10.2337/diaclin.33.4.175.
13. Li X, Zhu L-J, Chen J-P, Shi C-Y, Niu L-T, Zhang X. C-Methylated flavanones from the rhizomes of *Matteuccia intermedia* and their α-glucosidase inhibitory activity. Fitoterapia 2019;136:104147. doi. 10.1016/j.fitote.2019.04.002.
14. Mabotja MB, Venter SL, Du Plooy CP, Kudanga T, Amoo SO. Phytochemical content, antioxidant, alpha-glucosidase inhibitory and antibacterial activities of spineless cactus pear cultivars. Plants (Basel). 2021;10(7):1312. doi. 10.3390/plants10071312.
15. van Vuuren S, Viljoen A. Plant-based antimicrobial studies – methods and approaches to study the interaction between natural products. Planta Med. 2011;77(11):1168-82. doi: 10.1055/s-0030-1250736.
16. Deutschländer MS, Van de Venter M, Roux S, Louw J, Lall N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. J Ethnopharmacol. 2009;124(3):619-24. doi: 10.1016/j.jep.2009.04.052.
17. Rengasamy KRR, Aderogba MA, Amoo SO, Stirk WA, Van Staden J. Potential antiradical and alpha-glucosidase inhibitors from *Ecklonia maxima* (Osbeck) Papenfuss. Food Chem. 2013;141(2):1412-5. doi: 10.1016/j.foodchem.2013.04.019.
18. Rajalaksmi M, Eliza J, Cecilia E, Nirmala A, Daisy P. Antidiabetic properties of *Tinospora cordifolia* stem extracts on streptozotocin-induced diabetic rats. Afr J Pharm Pharmacol. 2009;3(5):171-80. doi: 10.5897/AJPP.9000027.
19. Bonnet F, Scheen A. Understanding and overcoming metformin gastrointestinal intolerance. Diabetes Obes Metab. 2017;19(4):473-81. doi: 10.1111/dom.12854.
20. Kast RE. Acarbose related diarrhea: increased butyrate upregulates prostaglandin E. Inflamm Res. 2002;51(3):117-8. doi: 10.1007/pl00012389.
21. Stein SA, Lamos EM, Davis SN. A review of the efficacy and safety of oral antidiabetic drugs. Expert Opin Drug Saf. 2013;12(2):153-75. doi: 10.1517/14740338.2013.752813.
22. World Health Organization. Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A Worldwide Review. World Health Organization, Geneva. Available from: https://apps.who.int/iris/handle/10665/42452. Accessed December 31, 2021.
23. Assefa ST, Yang E-Y, Chae S-Y, Song M, Lee J, Cho M-C, et al. Alpha glucosidase inhibitory activities of plants with focus on common vegetables. Plants (Basel). 2019;9(1):2. doi: 10.3390/plants9010002.
24. Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. Braz J Pharm Sci. 2020;56:e17129. doi: 10.1590/s2175-97902019000417129.
25. Abarca-Vargas R, Peña Malacara CF, Petricevich VL. Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea x buttiana* Holttum and Standl, (var. Rose) extracts. Antioxidants (Basel). 2016;5(4):45. doi: 10.3390/antiox5040045.
26. World Health Organization. 2018. WHO guidelines on good herbal processing practices for herbal medicines. WHO Technical Report Series, No. 1010. Available from: https://www.gmp-compliance.org/files/guidemgr/TRS1010annex1.pdf. Accessed February 21, 2022.
27. Magadula JJ, Mulholland DA, Crouch NR. Triterpenoids from *Anthocleista grandiflora* (Gentianaceae). Nat Prod Commun 2008;3:885-889.
28. Xu F, Huang X, Wu H, Wang X. Beneficial health effects of lupenone triterpene: A review. Biomed Pharmacother. 2018;103:198-203. doi: 10.1016/j.biopha.2018.04.019.
29. Chang WC, Wu SC, Xu KD, Liao BC, Wu JF, Cheng

AS. Scopoletin protects against methylglyoxal-induced hyperglycemia and insulin resistance mediated by suppression of advanced glycation endproducts (AGEs) generation and anti-glycation. Molecules 2015;20(2):2786-801. doi: 10.3390/molecules20022786.

30. Zschocke S, Rabe T, Taylor JL, Jäger AK, van Staden J. Plant part substitution – a way to conserve endangered medicinal plants? J Ethnopharmacol. 2000;71(1-2):281-92. doi: 10.1016/s0378-8741(00)00186-0.
31. Sulaiman CT, Balachandran I. Plant part substitution for medicinal use in *Aegle marmelos* – a phytochemical approach. J Trop Med Plants. 2013;14(1-2):19-22.
32. Moukette BM, Ama Moor VJ, Biapa Nya CP, Nanfack P, Nzufo FT, Kenfack MA, et al. Antioxidant and synergistic antidiabetic activities of a three-plant preparation used in Cameroon folk medicine. Int Sch Res Notices. 2017;9501675. doi: 10.1155/20017/9501675.