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Inhibitory effects of four antidiabetic medicinal plants on advanced glycation end-products formation: A phytochemical and pharmacological evaluation

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ABSTRACT

Introduction: Advanced glycation end-products (AGEs) are important players in the development of diabetes complications, making their inhibition a key therapeutic target. This study examined the inhibitory effects of extracts from four antidiabetic medicinal plants, *Murraya koenigii* (curry leaf), *Psidium guajava* (guava leaf), *Sclerocarya birrea* (cider tree stembark), and *Cinnamomum cassia* (cinnamon stembark), on the formation of AGEs. Plants were chosen for their hypoglycemic attributes.

Methods: Extracts were incubated at 37 °C for 20 days using bovine serum albumin (BSA) with fructose/glucose models. The generation of AGEs was evaluated using spectrofluorometry and enzyme-linked immunosorbent assays. Qualitative phytochemical analysis of extracts was also performed.

Results: Phytochemical analysis revealed quinones, phenols, alkaloids, cardiac glycosides, flavonoids, steroids, coumarins, saponins, terpenoids, and tannins in the extracts. The findings indicated substantial suppression of fluorescence AGEs (FAGEs), total immunogenic AGEs (TIAGEs), and specific AGEs, including N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL), by the plant extracts. The inhibitory effects of the plant extracts exceeded the efficacy of aminoguanidine, a recognized inhibitor of AGEs ($P<0.05$). Polar extracts demonstrated enhanced inhibitory effects, indicating that phenolic and flavonoid components may be pivotal in anti-glycation.

Conclusion: This work emphasizes the prospect of these medicinal herbs as natural sources of AGEs inhibitors, offering therapeutic possibilities for the management and prevention of diabetes problems. Further research is advised to discover, isolate, and describe specific bioactive chemicals responsible for these effects to aid in the development of innovative medicinal treatments.

Implication for health policy/practice/research/medical education:
This study gives significant perspectives on the anti-glycation prospects of these plants, which may facilitate the creation of novel natural treatments for addressing diabetic problems. It also underscores the necessity for more study to isolate and characterize the chemical molecules responsible for these effects.

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Introduction

Diabetes, a metabolic disorder that inflicts chronic damage on many organs such as the eyes, liver, kidneys, heart, and blood vessels, constitutes a substantial concern (1,2). One mechanism recognized as contributing to the emergence of diabetes complications is the increased production of advanced glycation end-products (AGEs) (3,4). AGEs are diverse compounds created through non-enzymatic interactions between the carbonyl groups of reducing sugars, such as glucose, and the free amino groups present

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on lipoproteins, nucleic acids, and proteins. This non-enzymatic glycosylation transpires by a series of processes, commencing with the reversible formation of the Schiff base (5). The Schiff base can convert into the more stable ketoamine compound known as the Amadori product (when reacting with glucose) or the Heyns product (when reacting with fructose); thereafter, these products participate in further processes to provide irreversible AGEs (6). The process inducing the generation of AGEs occurs over several weeks and predominantly affects proteins with slow turnover, such as elastin and collagen, as well as shorter-lived molecules, such as intracellular growth factors. AGEs establish irreversible cross-links on durable proteins, including collagen, elastin, and laminin, inside the extracellular matrix of the kidneys, heart, blood vessels, and eyes. The cross-linking of proteins by AGEs modifies the structure and functionality of proteins and enzymes (5).

The pile up of AGEs in body systems is known to induce alterations in the functioning and structure of tissue proteins, both directly and indirectly. AGEs cause alterations in low-density lipoprotein (LDL) cholesterol, making it prone to oxidation, which results in its accumulation in blood vessel walls, leading to foam cell formation and ultimately atheroma, thereby promoting the advancement of macrovascular atherosclerotic complications linked to diabetes (7). AGEs interact with many specialized receptors, notably the receptor for AGEs (RAGE), hence eliciting numerous biological consequences (8). The activation of RAGE by AGEs and the subsequent binding of RAGE to AGEs stimulate several signalling pathways. Thus, the modifications induced by AGEs substantially exacerbate vascular disorders and the advancement of vasculopathy seen in aging, diabetes, and uremia (9). Synthetic inhibitors of AGEs, such as aminoguanidine (AG), have been developed. However, their application is constrained by the side effects found in diabetic patients during clinical trials (10). AG functions as a benchmark chemical for the inhibition of AGEs and as a prototype for the creation of new antiglycative pharmaceuticals (10).

A variety of culinary and medicinal plants, supported by scientific data for their blood glucose-lowering actions, are documented to block the development of AGEs. The research conducted by Saraswat et al (11) on 17 herbs employed in diabetes treatment revealed that numerous herbs have properties that inhibit the formation of AGEs. A multitude of supplementary medicinal herbs that reduce blood glucose levels remain unexamined or unvalidated for their capacity to inhibit AGEs generation. Empirical evaluations of alcoholic and aqueous extracts from medicinal plants, such as *Murraya koenigii*, *Psidium guajava, Sclerocarya birrea,* and *Cinnamomum cassia*, have substantiated their ability to lower blood sugar. These were done using animal models of diabetic mellitus (1,12-17).

Murraya koenigii (L.) Speng, popularly referred to as curry leaf in English, is part of the Rutaceae family (18). *P. guajava* Linn, on the other hand, is classified under the Myrtaceae family and is generally known as guava in English (16). *S. birrea* (A. Richard) Hochst subspecies caffra (Sond.) Kokwaro belongs to the Anacardiaceae family and is frequently referred to as the cider tree in English (19,20). *Cinnamomum cassia* Presl, also known as *Cinnamomum aromaticum* or Chinese cinnamon, belongs to the Lauraceae family (21). *C. cassia* is often called cinnamon. The inhibitory effects of these blood glucose-lowering medicinal plant extracts on the development of AGEs have either not been investigated or remain scientifically unsubstantiated. Comprehending the inhibitory effects of these medicinal plants may aid in the identification and extraction of active compounds that impede AGEs, perhaps averting the late onset of diabetes complications. The study sought to evaluate the *in vitro* effects of several crude extracts from medicinal plants that reduce blood glucose on the formation of AGEs. This was achieved by examining the phytochemical composition of medicinal plants and assessing their extracts for the inhibition of AGEs formation.

Materials and Methods

Plant collection and authentication

Packets of *M. koenigii* leaves (listed in Plant List) were obtained from a local retail outlet in South Africa, while the leaves of *P. guajava* were collected from Mentz-Segoreng village in Limpopo Province, South Africa. The stembark of *S. birrea* was acquired from the premises of Sefako Makgatho Health Sciences University (SMU), Pretoria, South Africa. The encapsulated stembark water extract of *C. cassia*, branded as Diabecinn and manufactured by OTC Pharma SA (Pty) Ltd in Cape Town, South Africa, was procured from a dispensary in South Africa. The taxonomic distinctiveness of the collected herbarium specimens for *P. guajava* and *S. birrea* was confirmed in Pretoria, South Africa, by a taxonomist at the South African National Biodiversity Institute (SANBI). Each collected herbarium specimen was prepared and submitted to the National Herbarium in Pretoria, SANBI, where the taxonomic identities of *P. guajava* (Genspec 5559000) and *S. birrea* (Genspec 4558000) were confirmed. The selection of these species was based on ethnobotanical knowledge and empirically substantiated hypoglycemic qualities (12-17).

Sample preparation and extraction

Freshly harvested leaves of *M. koenigii* and *P. guajava*, along with the stembark of *S. birrea*, were washed with water, air-dried, and subsequently pulverized into fine powder with the use of a laboratory mill (Polymix PX-

MFC 9OD, Kinematica, Thermo Fisher Scientific, Sweden). A systematic and comprehensive extraction of each powdered item was performed using solvents of differing polarities: *n*-hexane (non-polar), ethyl aetate (moderately polar), methanol (polar) and water (polar), in accordance with the methodology outlined by Adeniran et al (22). Sequential solvent extraction was utilized, employing the idea of increasing polarity to extract a diverse array of phytochemicals.

Qualitative phytochemical analysis

A qualitative phytochemical study of several extracts from the leaves of *M. koenigii* and *P. guajava*, stembark of *S. birrea,* along with aqueous extract of *C. cassia* stembark was conducted in accordance with established protocols. The metabolites examined in the plant extract comprise phenols (23), flavonoids (23,24), tannins (23), alkaloids (25), saponins (23), quinones (23), cardiac glycosides (23), steroids (23), coumarins (26), and terpenoids (23).

Measurement of AGEs formation inhibition activities

Dimethyl sulfoxide (DMSO) was used to solubilize non-polar *n*-hexane and mildly polar ethyl acetate extracts, whilst water was used to solubilize polar extracts, methanol and water, based on their solubility. One milligram per millilitre concentration of each extract was utilized to assess the inhibitory actions on immunogenic and fluorescent AGEs (FAGEs) for the preliminary screening of the chosen antidiabetic medicinal plants, due to its widespread acceptability for initial screening investigations. The inhibitory effects of different medicinal plant extracts on the formation of AGEs were evaluated using a previously established method (4), with slight changes. An aliquot of 10 mg/mL bovine serum albumin (BSA) solution, which was prepared using 76 mM sodium phosphate buffer (pH 7.4) containing 0.02% sodium azide, was added to an aliquot of fructose or glucose solution (50 mg/mL). The resultant solution of BSA and sugar was incubated at a physiological temperature of 37 °C for 20 days, with and without plant extracts. The solution of BSA and fructose or glucose was the negative control (100% AGEs). A comparable amount of AG was employed for reference control (positive). The negative control utilized was 100% AGEs-BSA. The final dose utilized to examine the inhibition of AGEs formation activity by the test samples and AG was 1 mg/mL.

Fluorescent AGEs assay

Following the incubation period, the levels of FAGEs were measured according to published techniques (27). Fluorescent measurements were performed at room temperature in triplicate, with an excitation wavelength of 370 nm and an emission wavelength of 440 nm, using the GloMax multi-detection plate reader, product of Promega Corporation, Wisconsin, USA.

Immunogenic AGEs assay

Quantifications of total immunogenic AGEs (TIAGEs), N^{ϵ}-(carboxymethyl)lysine (CML), and N^{ϵ}-(carboxyethyl) lysine (CEL) were performed utilizing enzyme-linked immunosorbent assay (ELISA) kits (STA-317, STA-316, and STA-300 Oxiselect™), manufactured by Cell Biolabs Inc., San Diego, USA, as outlined by Adeniran and Mogale (4). The investigation was carried out in compliance with the manufacturer's specifications.

The outcomes obtained were shown in arbitrary units as the percentage (%) inhibition of FAGEs, TIAGEs, CML, and CEL. The % inhibition indicated the inhibitory activity of extracts and AG on the formation of AGEs. The formula articulated by Adeniran and Mogale (4) is detailed below.

$$\%\ Inhibition = \left(\frac{[AGEs]_{control} - [AGEs]_{test\ sample}}{[AGEs]_{control}} \right) \times 100$$

Where $[AGEs]_{control}$ is the absorbance of 100% AGEs-BSA, while $[AGEs]_{test\ samples}$ are the absorbance of the test sample/standard inhibitor + fructose/glucose + BSA.

Statistical analysis

Phytochemicals being studied were indicated by a plus sign (+) for presence and a minus sign (-) for absence. IBM SPSS® Statistical program (Version 24) was used to analyze the data for the study. The mean of three samples and the standard deviation (SD) were computed. Data on fluorescent and immunogenic AGEs levels obtained for each of the extracts and standard control were analyzed using analysis of variance (ANOVA) for continuous variables. A *P* value under 0.05 was considered statistically significant for group disparities. Results were displayed in the form of tables.

Results

Qualitative phytochemical composition of the extracts

Qualitative phytochemical screening utilizing standard chemical assays identified the presence of cardiac glycosides, coumarins, quinones, phenols, alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins in one or more extracts of *M. koenigii* and *P. guajava* leaves, as well as *S. birrea* stembark extracts and *C. cassia* stembark water extract. Polar extracts (methanol and water) exhibited elevated concentrations of flavonoids and phenols relative to non-polar extracts (Table 1).

Inhibition activities of *Murraya koenigii* leaf extracts on the formation of AGEs

Results of inhibitory activity of *M. koenigii* leaf extracts on the development of BSA-fructose and BSA-glucose generated FAGES showed that the intermediate polar (ethyl acetate) and polar (methanol and water) leaf extracts of *M. koenigii* displayed significant inhibitory activities (61.6-89.5%) against their formation. Polar extracts exhibited

Table 1. Phytochemical composition of selected antidiabetic medicinal plants' crude extracts

Phytochemicals	*Murraya koenigii* leaf				*Psidium guajava* leaf				*Sclerocarya birrea* stembark				CCS
	nH	eT	mE	wA	nH	eT	mE	wA	nH	eT	mE	wA	wA
Phenols	+	+	+	-	+	+	+	+	-	+	+	+	+
Flavonoids	-	-	+	+	+	-	+	+	-	+	+	+	+
Alkaloids	-	-	+	-	+	-	+	-	-	-	+	+	-
Quinones	+	-	-	+	+	+	+	+	-	-	+	+	+
Cardiac glycosides	+	-	+	-	+	+	+	-	+	+	+	-	+
Saponins	-	-	+	+	-	+	+	-	-	+	+	+	+
Steroids	-	-	-	+	+	-	-	+	+	+	-	+	+
Coumarins	+	+	+	+	+	+	+	+	-	-	-	+	+
Terpenoids	-	+	+	+	+	-	-	+	-	-	+	+	+
Tannins	ND	ND	ND	ND	+	+	+	ND	+	+	+	+	ND

CCS: *Cinnamomum cassia* stembark; nH: *n*-hexane; eT: Ethyl acetate; mE: Methanol; wA: Water; ND: Not determined; "+": Present; "-": Absent.

the greatest suppression of FAGEs (Table 2). The extracts of *M. koenigii* leaf exhibited percentage anti-glycation effects on BSA-fructose and BSA-glucose generated TIAGEs, demonstrating inhibition rates of 102.4-110.5%, which exceeded the efficacy of AG, the positive control ($P < 0.001$) (Table 2).

The inhibition activity of the results of *M. koenigii* leaf extracts on the development of CML obtained from both BSA-fructose and BSA-glucose indicated substantial inhibition of CML formation by every single one of the *M. koenigii* leaf extracts (Table 3). The results of the polar extracts (methanol and water) from *M. koenigii* leaves were analogous to those of the positive control, AG. Evaluation of *M. koenigii* leaf extracts on CEL growth from both BSA-fructose and BSA-glucose indicated that all extracts displayed substantial inhibitory effects on CEL production. *n*-Hexane, ethyl acetate, and methanol extracts of *M. koenigii* exhibited markedly more inhibitory effects than AG on the synthesis of BSA-glucose produced CEL ($P < 0.01$). The *n*-hexane extract of *M. koenigii* leaves significantly prevented the development of BSA-fructose-derived CEL more than AG, the standard control ($P < 0.05$).

Inhibition activities of *Psidium guajava* leaf extracts on AGEs formation

The evaluation of the inhibitory effects of *P. guajava* leaf extracts on the development of FAGEs from BSA-fructose

Table 2. Inhibition effects of the extracts of *Murraya koenigii* leaf on the formation of fluorescent and total immunogenic advanced glycation end-products

Extracts and standard	Fluorescent AGEs (%)		Total immunogenic AGEs (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	0	$24.9^{d} \pm 4.54$	$104.58^{a} \pm 0.06$	$102.48^{a} \pm 0.07$
Ethyl acetate	$23.5^{d} \pm 2.5$	$73.6^{c} \pm 0.81$	$108.57^{a} \pm 1.43$	$102.58^{a} \pm 0.41$
Methanol	$61.6^{c} \pm 2.21$	$75.1^{c} \pm 1.75$	$110.42^{a} \pm 0.3$	$102.62^{a} \pm 0.04$
Water	$82.8^{b} \pm 4.71$	$89.5^{b} \pm 4.02$	$110.54^{a} \pm 0.18$	$102.38^{a} \pm 0.03$
Aminoguanidine	$89.5^{a} \pm 0.49$	$99.5^{a} \pm 0.02$	$98.9^{b} \pm 1.43$	$99^{b} \pm 0.27$

AGEs: Advanced glycation end-products. Values that have non-identical letters within identical columns are significantly contrasting ($P < 0.05$), while values that have identical letters within identical columns are not significantly contrasting ($P > 0.05$).

Table 3. Inhibition effects of the extracts of *Murraya koenigii* leaf on the formation of specific advanced glycation end-products, N^{ε}-(carboxymethyl)lysine, and N^{ε}-(carboxyethyl)lysine

Extracts and standard control	N^{ε}-(carboxymethyl)lysine (%)		N^{ε}-(carboxyethyl)lysine (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	$82.9^{e} \pm 0.06$	$69.1^{d} \pm 4.12$	$100^{a} \pm 1.77$	$88.2^{a} \pm 3.15$
Ethyl acetate	$95.6^{d} \pm 1.94$	$83.1^{c} \pm 3.28$	$100.89^{a} \pm 2.66$	$74.8^{e} \pm 2.36$
Methanol	$100^{a} \pm 0.15$	$99.7^{b} \pm 0.02$	$100^{a} \pm 0$	$81.9^{d} \pm 4.72$
Water	$98.2^{c} \pm 0.1$	$100^{a} \pm 0.03$	$93.8^{c} \pm 0.89$	$85.8^{c} \pm 0.79$
Aminoguanidine	$99.8^{b} \pm 0.19$	$99.1^{b} \pm 0.15$	$96.5^{b} \pm 5.31$	$86.6^{b} \pm 1.58$

Values that have different letters within the same column are significantly different ($p < 0.05$), while values that have identical letters within the same column are not significantly different ($P > 0.05$).

and BSA-glucose revealed that all extracts displayed substantial inhibitory action, producing analogous results for both BSA-glucose and BSA-fructose-generated FAGEs (Table 4). The polar extracts showed markedly superior inhibitory effects (97.3-130.9%) relative to the less polar extracts, such as *n*-hexane and ethyl acetate ($P < 0.001$). Also, the inhibitory percentages of several extracts of *P. guajava* leaf on the formation of TIAGEs generated from BSA-glucose and BSA-fructose were evaluated. AG acted as the positive control for comparison (Table 4). The findings revealed a higher percentage of inhibitory effectiveness against both BSA-fructose and BSA-glucose-generated TIAGEs, noted with the *n*-hexane, ethyl acetate, and methanol extracts of *P. guajava* leaf, exceeding that of the positive control, AG ($P < 0.05$).

The inhibition effects of *P. guajava* leaf extracts on the production of CML produced from BSA-fructose and BSA-glucose, as presented in Table 5, revealed that all extracts exhibited significant inhibition of CML formation from both substrates. The outcomes obtained from the polar extracts were analogous to the positive control, AG. Table 5 demonstrates that the extracts significantly inhibited CEL generation from both BSA-fructose and BSA-glucose, akin to the positive control, AG ($P < 0.05$).

Inhibition activities of *Sclerocarya birrea* stembark extracts on advanced glycation end-products formation

The percentage inhibition activities of several extracts of *S. birrea* stembark on the production of BSA-fructose and BSA-glucose generated FAGEs, with AG as the positive control, demonstrated that the ethyl acetate and methanol extracts inhibited FAGEs by 68-77.1% (Table 6). Substantial inhibitory effects on FAGEs generation were observed in the ethyl acetate, methanol, and aqueous extracts of *S. birrea* stembark. Similar results were obtained for both BSA-fructose and BSA-glucose-generated FAGEs utilizing *S. birrea* stembark extracts. The outcomes of the percentage activities of several extracts of *S. birrea* stembark, evaluated for their impact on the synthesis of TIAGEs derived from BSA-glucose and BSA-fructose, with AG serving as a positive control, are displayed in Table 6. The findings indicated that the *n*-hexane extract of *S. birrea* stembark exhibited complete inhibition of TIAGEs formation. The results obtained with the *n*-hexane extract for BSA-glucose-induced TIAGEs were much better than those of the positive control ($P < 0.01$).

The impact of the extracts of *S. birrea* stembark was evaluated for CML development, with findings displayed in Table 7. The results demonstrate that the polar extracts (methanol and water) of *S. birrea* stembark significantly inhibited CML growth, comparable to the positive control, AG. The evaluation of the percentage inhibitory activities of *S. birrea* stembark on CEL production revealed that all extracts significantly inhibited CEL development. The observed inhibitory effects were significantly larger than those demonstrated by the positive control ($P < 0.01$).

Inhibition activities of *Cinnamomum cassia* stembark water extract on AGEs formation

The inhibitory impacts of *C. cassia* stembark water extract

Table 4. Inhibition effects of the extracts of *Psidium guajava* leaf on the formation of fluorescent and total immunogenic advanced glycation end-products

Extracts and standard	Fluorescent AGEs (%)		Total immunogenic AGEs (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	$48.9^{e} \pm 3.06$	$44.5^{e} \pm 1.16$	$101.5^{a} \pm 0.06$	$102.56^{a} \pm 0.04$
Ethyl acetate	$51.4^{d} \pm 3.13$	$56.3^{d} \pm 3.25$	$103.7^{ab} \pm 0.60$	$102.66^{a} \pm 0.03$
Methanol	$98.9^{ab} \pm 3.0$	$97.3^{c} \pm 0.14$	$100.8^{a} \pm 0.48$	$100.26^{b} \pm 0.37$
Water	$130.9^{a} \pm 1.48$	$121.37^{a} \pm 1.78$	$18.3^{d} \pm 0.24$	$98.2^{d} \pm 1.19$
Aminoguanidine	$89.5^{c} \pm 0.19$	$99.5^{b} \pm 0.02$	$98.9^{c} \pm 1.43$	$99^{c} \pm 0.27$

AGEs: Advanced glycation end-products. Values that have non-identical letters within identical columns are significantly contrasting ($P < 0.05$), while values that have identical letters within identical columns are not significantly contrasting ($P > 0.05$).

Table 5. Inhibition effects of the extracts of *Psidium guajava* leaf on the formation of specific advanced glycation end-products, N^{ε}-(carboxymethyl)lysine, and N^{ε}-(carboxyethyl)lysine

Extracts and standard control	N^{ε}-(carboxymethyl)lysine (%)		N^{ε}-(carboxyethyl)lysine (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	$86.9^{b} \pm 0.44$	$77^{c} \pm 1.41$	$88.7^{c} \pm 2.42$	$87.4^{b} \pm 0.79$
Ethyl acetate	$79.9^{c} \pm 2.72$	$93.1^{b} \pm 1.59$	$92.7^{a} \pm 3.23$	$89.8^{a} \pm 1.58$
Methanol	$99.6^{a} \pm 0.19$	$99.9^{a} \pm 0.15$	$87.1^{d} \pm 0.81$	$89.7^{a} \pm 3.15$
Water	$30.1^{d} \pm 4.67$	$99.8^{a} \pm 0.09$	$93.6^{a} \pm 0.81$	$90.5^{a} \pm 2.36$
Aminoguanidine	$99.8^{a} \pm 0.19$	$99.1^{a} \pm 0.15$	$89.5^{b} \pm 4.84$	$86.6^{c} \pm 1.58$

Values that have non-identical letters within identical columns are significantly contrasting ($P < 0.05$), while values that have identical letters within identical columns are not significantly contrasting ($P > 0.05$).

Table 6. Inhibition effects of the extracts of *Sclerocarya birrea* stembark on the formation of fluorescent and total immunogenic advanced glycation end-products

Extracts and standard	Fluorescent AGEs (%)		Total immunogenic AGEs (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	0	0	$100^{a} \pm 0.6$	$87.7^{b} \pm 0.37$
Ethyl acetate	$75.1^{b} \pm 0.93$	$75.1^{c} \pm 0.28$	$3.6^{e} \pm 1.37$	$50.8^{d} \pm 1.13$
Methanol	$68^{d} \pm 2.99$	$77.1^{b} \pm 3.14$	$56.4^{d} \pm 3.57$	$75^{c} \pm 0.17$
Water	$71.2^{c} \pm 4.67$	$74.6^{d} \pm 4.18$	$93.2^{c} \pm 0.06$	$86.9^{b} \pm 1.62$
Aminoguanidine	$89.5^{a} \pm 0.49$	$99.5^{a} \pm 0.02$	$98.9^{b} \pm 1.43$	$99^{a} \pm 0.27$

AGEs: Advanced glycation end-products. Values that have non-identical letters within identical columns are significantly contrasting ($P < 0.05$), while values that have identical letters within identical columns are not significantly contrasting ($P > 0.05$).

Table 7. Inhibition effects of the extracts of *Sclerocarya birrea* stembark on the formation of specific advanced glycation end-products, N^{ε}-(carboxymethyl) lysine, and N^{ε}-(carboxyethyl)lysine

Extracts and standard control	N^{ϵ}-(carboxymethyl)lysine (%)		N^{ϵ}-(carboxyethyl)lysine (%)	
	Glucose	Fructose	Glucose	Fructose
n-Hexane	$56.1^{d} \pm 3.94$	$2.1^{d} \pm 0.21$	$99.2^{a} \pm 1.61$	$100^{a} \pm 3.15$
Ethyl acetate	0	$36.8^{c} \pm 0.33$	$97.6^{b} \pm 1.61$	$97.6^{b} \pm 0$
Methanol	$86.1^{c} \pm 1.26$	$97.9^{b} \pm 0.03$	$96^{c} \pm 1.61$	$92.9^{c} \pm 3.15$
Water	$95.2^{b} \pm 2.04$	$99.0^{a} \pm 0.02$	$94.4^{d} \pm 0.1$	$89.8^{d} \pm 1.58$
Aminoguanidine	$99.8^{a} \pm 0.19$	$99.1^{a} \pm 0.15$	$93.6^{e} \pm 0.81$	$86.6^{e} \pm 1.58$

Values that have non-identical letters within identical columns are significantly contrasting ($P < 0.05$), while values that have identical letters within identical columns are not significantly contrasting ($P > 0.05$).

on the formation of BSA-fructose and BSA-glucose-generated FAGEs, TIAGEs, and specific AGEs, namely CML and CEL, were evaluated, utilizing AG as a positive control. Findings are displayed in Table 8. The findings indicated that the water extract of *C. cassia* stembark markedly suppressed the formation of FAGEs from both BSA-glucose and BSA-fructose. The results indicated a significantly higher percentage inhibition of BSA-glucose TIAGEs synthesis (107.4%) in comparison to AG, the positive control ($P < 0.001$). The results also exhibited similar inhibitory effects to AG against BSA-fructose-generated TIAGEs. Results indicated that the aqueous extract of *C. cassia* stembark demonstrated a considerable inhibition of CML development (99.1%), comparable to the positive control, AG. The findings indicated that the aqueous extract of *C. cassia* stembark significantly inhibited CEL formation. Inhibitory effects seen against both BSA-fructose and BSA-glucose produced CEL were analogous to those of the positive control.

Discussion

Diabetes mellitus is a metabolic disorder which impacts the regulation of blood glucose levels (28). AGEs are connected to numerous complications arising from diabetes mellitus and the intrinsic activity of growing older (3,7). Assessing the inhibitory activities of medicinal plants on the formation of AGEs is essential, given the absence of clinically approved agents with minimal side effects for AGE therapy (29). This research work aimed to assess the inhibitory outcome of selected antidiabetic medicinal plant parts, specifically *M. koenigii* leaves, *P. guajava* leaves, *S. birrea* stembark, and *C. cassia* stembark, regarding the generation of AGEs. The literature and various studies have extensively documented the antidiabetic properties of the chosen plants and their components (1,12-17). The extracts' capacity to impede the generation of AGEs was

Table 8. Inhibition effects of the extracts of *Cinnamomum cassia* stembark on the formation of different types of advanced glycation end-products

Extracts and standard control	*Cinnamomum cassia*		Aminoguanidine	
	Glucose	Fructose	Glucose	Fructose
FAGEs (%)	61.3 ± 4.88	46 ± 4.5	89.5 ± 0.49	99.5 ± 0.02
TIAGEs (%)	107.4 ± 0.24*	98.3 ± 0.21	98.9 ± 1.43	99 ± 0.27
CML (%)	99.1 ± 0.58	97.8 ± 0.03	98.8 ± 0.19	99.1 ± 0.15
CEL (%)	79.8 ± 3.23	93.7 ± 0.79	93.6 ± 0.81	86.6 ± 1.58

FAGEs: Fluorescent advanced glycation end-products; TIAGEs: Total immunogenic advanced glycation end-products; CML: NƐ-(carboxymethyl)lysine; CEL: NƐ-(carboxyethyl)lysine. * Significant at $P < 0.001$ in comparison to aminoguanidine.

evaluated. Bioactive compounds were extracted from selected medicinal plants utilizing solvents of different polarities to enhance the extraction efficiency. The solvents *n*-hexane, ethyl acetate, methanol, and water were chosen based on the principle of increasing polarity and their differing polarities. Ethyl acetate effectively extracts intermediate or semi-polar phytochemicals that are insoluble in the non-polar solvent *n*-hexane, whereas *n*-hexane is capable of extracting non-polar components from plant materials. Polar and semi-polar compounds were extracted using methanol. Compounds insoluble in organic solvents were extracted using water. Plant extracts underwent phytochemical analysis.

Glucose-mediated glycation occurs during the protein turnover period *in vivo*, generally lasting from 1 to 4 weeks (30). The samples were incubated for a minimum of 20 days at physiological temperature (37 °C) in this study. The study utilized glucose and fructose to evaluate the inhibitory effects of the tested extracts on AGEs originating from both sugar sources. A documented distinction exists between the glycating potentials of glucose and fructose (3).

Standard chemical screening assays were utilized to perform the phytochemical profiling of plant extracts for the determination of various phytochemicals in them. The phytochemical screening results demonstrated that the extracts of all studied plants contained notable compounds, including alkaloids, phenols, steroids, flavonoids, saponins, coumarins, cardiac glycosides, quinones, and terpenoids. Olivier et al (31) reported that the identified phytochemicals were documented in the literature as being responsible for various biological activities across different plants. The study's findings verified the effective extraction of notable secondary metabolites from the plants utilizing solvents with different polarities, a process well-documented in the literature on medicinal plants (32). The study's results support previous findings regarding the phytochemical composition of the four medicinal plants examined (*M. koenigii*, *P. guajava*, *S. birrea*, and *C. cassia*).

Murraya koenigii (L.) Spreng (Curry leaf) is recognized for its efficacy in treating various diseases, including rheumatism, diabetes, traumatic injuries, and snake bites, within the Indian Ayurvedic system (33). According to Rajendran et al (18), the leaves and roots of *M. koenigii* are commonly used to mitigate heat, dehydration, itching, and inflammation, in addition to treating piles. The leaves of *M. koenigii* contain a variety of simple phenolic acids, including tannic, chlorogenic, gallic, ferulic, cinnamic, and vanillic acids (34). Flavanols, their polymers, and proanthocyanidins are identified as components of *M. koenigii*. Carbazole alkaloids are prevalent in the leaves of *M. koenigii* (18,33). Vinodh et al (35) identified specific aromatic hydrocarbons, monoamine alkaloids, and organic compounds as bioactive constituents in fractions from water extracts of *M. koenigii* leaves. This was ascertained using gas chromatography mass spectrometry (GC-MS). Additional isolated phyto-constituents of *M. koenigii* leaf include coumarins, glycosides, calcium, phosphorus, iron, vitamin C, carotene, riboflavin, niacin, thiamine, and oxalic acid (18). Tembhurne and Sakarkar (36) demonstrated in their study that the antioxidant properties of *M. koenigii* leaf might be linked to its antidiabetic effects. They attributed this to biologically active constituents including carbazole alkaloids, glycosides, triterpenoids, and phenolic compounds, which are capable of scavenging free radicals.

Guava leaves, scientifically referred to as *P. guajava* Linn, are employed in the management and treatment of conditions such as diabetes in various regions globally (37,38). *P. guajava* exhibits anticancer, antioxidant, hepatoprotective, and anti-inflammatory activities (16,38). The leaves of *P. guajava* primarily consist of phenolic acids, such as gallic, protocatechuic, ellagic, caffeic, tannic, chlorogenic, and ferulic acids (37,38). Epicatechin and tannins are present in the foliage as well. Quercetin and its derivatives are prevalent in the leaves of *P. guajava*. The hypoglycaemic and hypotensive effects of *P. guajava* leaves are attributed to their high content of tannins and polyphenols (16). *Sclerocarya birrea* (A. Richard) Hochst, commonly referred to as the cider tree, is utilized for the management of conditions including diabetes (20). The stembark of *S. birrea* comprises epicatechin, epicatechin-3-O-galate (ECG), gallolepicatechin-epigallocatechin-3-O-gallate, and catechin in its phytochemical composition (19). Adeniran et al (22) identified alcohols and fatty acids in a study aimed at determining the phytochemical compositions of *S. birrea* stembark through GC-MS analysis. Several alcohols, including stigmasterol, gamma-sitosterol, campesterol, and 1-heptatricontanol, were identified through GC-MS analysis. L-(+)-ascorbic acid, 2,6-dihexadecanoate, and 6-octadecanoate are examples of fatty acids detected in the stembark of *S. birrea* (22). *S. birrea* contains a significant amount of high molecular weight tannins and residues of alkaloid (19). Gallic acids and phenolic acids have been identified in the stembark of *S. birrea* and other associated parts. The biological activities of *S. birrea* stembark encompass antioxidant, hypoglycaemic, anti-inflammatory, antibacterial, antifungal, astringent, and anticonvulsant properties (19,20). The properties of *S. birrea* stembark are attributed to its phenolic content. Campsterol is noted for its antioxidant and hypocholesterolemic effects, whereas stigmasterol is acknowledged for its hypoglycaemic, hypolipidemic, and antioxidant properties (22). L-(+)-ascorbic acid, 2,6-dihexadecanoate, demonstrates antioxidant and anti-inflammatory properties. 1-Heptatricontanol is a compound documented to induce antidiabetic effects, potentially contributing to the

antidiabetic properties of *S. birrea* (22,39).

Cinnamomum cassia Presl (cinnamon) is employed as a traditional Chinese remedy to alleviate gastritis, inflammatory diseases, diabetes, and peptic disorders (21). Procyanidins, phenylpropanoids, and mucilage are prevalent in the bark. It also includes tannins, coumarins, diterpenes, and proanthocyanidins (40). Aqueous preparations of *C. cassia* contain high levels of aromatics, diterpenes, and polyphenols, such as anthocyanins, flavonoids, and tannins (21,40). Catechin, epicatechin, and procyanidin B2 are identified as constituents of *C. cassia* (40). Luo et al (41) reported the isolation of phenolic glycosides from *C. cassia*. The biological activities of *C. cassia* stembark include the reduction of cardiovascular disease and the lowering of cholesterol and lipids. *C. cassia* stembark contains compounds reported to possess therapeutic properties for disorders including Parkinson's and Alzheimer's diseases (15,42). *C. cassia* is associated with various biological activities, including antioxidant, antimicrobial, hypotensive, antiulcer, and antiglycative effects (42).

The phytochemical analysis assessed the inhibitory activities of four medicinal plants on the generation of AGEs. The inhibition activities were assessed for different forms of AGEs, including TIAGEs, FAGEs, CEL, and CML. The research utilized two sugar models for the formation of AGEs. These are BSA-glucose and BSA-fructose. The two sugar types were used because of the difference in reactivity between the two sugar varieties. Reports indicate that fructose exhibits reactivity that is ten times greater than that of glucose (6,7). This preliminary study screened the antiglycative potential of selected antidiabetic medicinal plants at a fixed concentration of 1 mg/mL, a standard practice in initial screening experiments.

The polar extracts of *M. koenigii*, *P. guajava*, and *S. birrea* demonstrated the highest activity in inhibiting the production of FAGEs. The BSA-fructose and BSA-glucose generated AGEs demonstrated consistent activity when subjected to polar extracts of medicinal plants, specifically in terms of their impacts on the formation of FAGEs. The medicinal plants *M. koenigii*, *P. guajava*, and *S. birrea*, along with the aqueous extract of *C. cassia* stembark, demonstrated inhibitory activity against the formation of TIAGEs. The formation of TIAGEs was predominantly inhibited in non-polar extracts, particularly in the leaves of *P. guajava* and the stembark of *S. birrea*. All extracts of *M. koenigii* demonstrated a significant inhibition of TIAGEs formation. The water extract was the only component evaluated for *C. cassia* stembark capsules. The chosen plants demonstrated increased inhibitory activities in the polar extracts when assessed for their inhibitory effects on specific immunogenic AGEs, CML, and CEL. The results also consistently demonstrated that the medicinal plant extracts exhibited greater effect against the production of FAGEs, TIAGEs, CML, and CEL over AG. The results, therefore, indicate that the phytochemical components of *M. koenigii* leaf, *P. guajava* leaf, *S. birrea* stembark and water extract of *C. cassia* stembark possess a reasonable level of AGEs formation inhibition.

Our research also verified the existence of numerous secondary metabolites important in the biological realm. Numerous phytochemicals, including amino acids, phenolic acids with their derivatives, terpenoids, flavonoids plus their derivatives, as well as polysaccharides, are recognized as possessing anti-glycation properties (10,43). Polar extracts, particularly those that contain flavonoids and phenols, are frequently cited as more efficacious anti-glycating agents in the literature (10,44). Antiglycation activities are primarily mediated by phenolic compounds (10). Khangholi et al (44) have demonstrated that phenolic acids, such as chlorogenic acids, prevent the generation of AGEs by modulating how the antioxidant enzyme genes express themselves or by exhibiting metal chelating activities. The extracts of *M. koenigii* (34) and *P. guajava* (43), which contain flavanols such as quercetin, have been documented. Several sources have documented the antiglycative effect of quercetin (10,45). Odjakova et al (43) reported that quercetin had an inhibitory effect on the formation of AGEs to the extent of over 80 %. Rutin, a glycoside of quercetin, is a well-established antioxidant and is a popular dietary flavonoid. Rutin is a strong inhibitor of CML and FAGEs generation. *In vitro* experiments carried out using collagen type 1 also showed the ability of rutin to prevent the production of glycation products that are initiated by glucose (43).

Kaempferol, an extensively studied flavanol, has been shown to inhibit the generation of CML and other AGEs in BSA-fructose and BSA-glucose systems, as well as α-glucosidase (43). Reports by Kim et al (46) demonstrated that the build-up of AGEs and RAGE expression was influenced by the brief feeding of kaempferol to rodents of advanced age. Additionally, *in vitro* antiglycation activity is mediated by numerous other flavonoid derivatives (10). *In vitro* experiments have also demonstrated that the derivatives of cinnamic acid, such as caffeic acids, and cinnamic acid itself can prevent the production of CML and fluorescent AGEs (47). Ferulic acid is a cinnamic acid that has been reported to act as an antioxidant and is able to prevent the formation of CML and FAGEs (43).

The antiglycation potential of catechins (flavanol) and proanthocyanidins (polymers of catechin) is a result of their antioxidant activities (10). This is associated with their capacity to capture reactive carbonyl species, including methyl glyoxal (MGO). Epigallocatechin gallate, a proanthocyanidin, was discovered to reduce the DNA-binding activity of NF-κB, AGEs-stimulated gene expression, and AGEs-mediated activation (10,43). Tannins are recognized for their ability to scavenge free radicals, the antioxidant characteristics they possess, and their capacity to prevent the production of AGEs

(10,43,48). Antioxidant, anti-inflammatory, and antiglycative properties have been observed in certain coumarins (49). Cardiovascular diseases are managed through the utilization of cardiac glycosides (50).

The formation of AGEs has been significantly inhibited by certain terpenoids. For example, diterpene labdadiene, which was purified from the rhizomes of *Alpinia zerumbet,* demonstrated inhibitory actions against the production of α-carbonyl compounds and fructose adducts (51). In an *in vitro* study, it was determined that the antiglycation effect of a triterpenoid (pentacyclic triterpene carboxylic acid) was more potent than that of AG (47).

In general, the majority of compounds in mixtures and extracts function in a mutually stimulating manner to exert the various therapeutic effects they are known for (52). Consequently, it is believed that the metabolites, including cardiac glycosides, saponins, quinones, and terpenoids, that were identified in the extracts of the selected antidiabetic medicinal plants may be responsible for the general AGEs inhibition outcome noted with the crude extracts.

Certain identified compounds may function in conjunction with others that have not yet been identified to improve the inhibition activities against the diverse AGEs that have been tested. Consequently, these medicinal plants can be used to isolate and identify novel phytochemicals that can be directly used in the regulation of complications associated with impaired glucose regulation. The potential for drug discovery is present in the results of this study, particularly in the development of therapies that target metabolic syndromes. It is recommended that future research be conducted to assess the relevance of these findings in clinical trials. Furthermore, *in vivo* explorations are essential to further throw light on the therapeutic effects of the isolated compounds and extracts. Additionally, mechanistic investigations into the reactions between AGEs and RAGE are encouraged. It will also be crucial to conduct a more thorough investigation of their mechanisms of action in order to gain a comprehensive understanding of their pharmacological characteristics.

Limitations and recommendations

The medicinal plants' capacity to inhibit AGEs formation was primarily demonstrated with unrefined extracts. Therefore, it is yet to be determined the extent to which the compounds of the selected medicinal plants will inhibit AGEs formation with respect to their purest form. Thus, whether the compounds exhibit synergistic or antagonistic interactions is still undetermined. Additionally, the selected plants were collected from a single location and during a single season. Consequently, it is yet to be determined whether the inhibition properties of the selected medicinal plants in inhibiting the formation of AGEs are influenced by environmental growing conditions. Consequently, it is advised to conduct a comprehensive purification/isolation of the biologically active ingredients that are naturally occurring in the plant parts of the selected medicinal plants in order to compare the potency of their AGEs formation inhibition activity with that of the crude extracts. Also, the mechanisms of action of these plants are still speculative, even though multiple studies have investigated and documented the antiglycation effects of plant and herb extracts (11,53). AGEs-RAGE interaction and *in vivo* studies involving the extracts and pure compounds are also required. It is therefore imperative to conduct additional research to detail the processes by which the plant extracts and their isolates exert their AGE-inhibiting activities. Additionally, it is advised to contemplate the possible impact of environmental growing conditions on the observed inhibition activity strengths of AGEs formation in the selected plants.

Conclusions

Murraya koenigii leaves, *P. guajava* leaves, *S. birrea* stembark, and *C. cassia* stembark were the subjects of a phytochemical investigation. The formation of AGEs was observed to be inhibited by the tested medicinal plants, which exhibited a wide variety of bioactive compounds, such as phenolics, flavonoids, and terpenoids. These findings underscore the potential of these plants as sources of innovative antiglycation agents for the management of diabetes-related complications. Future research should include detailed *in vitro* studies, concentrate on the isolation of purified compounds, the account of their modes of action, such as the interactions between AGEs and RAGE, and assessment of their efficacy in clinical and *in vivo* models.

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