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Original Research Article (Experimental)

The effect of *Salacia reticulata, Syzygium cumini, Artocarpus heterophyllus*, and *Cassia auriculata* on controlling the rapid formation of advanced glycation end-products

G.C. Thilakarathna [a, d, *], S.B. Navaratne [a], I. Wickramasinghe [a], P. Ranasinghe [b], S.R. Samarkoon [c], J.K.R.R. Samarasekera [b]

[a] *Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka*
[b] *Herbal Technology Section (HTS), Industrial Technology Institute (ITI), 363, Bauddhaloka Mawatha, Colombo, 07, Sri Lanka*
[c] *Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, Cumarathunga Munidasa Mawatha, 90, Colombo, 3, Sri Lanka*
[d] *Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka*

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ABSTRACT

Background: The excessive formation of Advanced Glycation End-products (AGEs) by non-enzymatic glycation mediates many health complications in the human body and the formation of AGEs largely accelerated under the hyperglycaemic condition.
Objective: The prospect of the study to assess the strength of inhibiting the rapid AGE formations in four Ayurvedic medicinal plants, namely; *Salacia reticulata* (stems), *Syzygium cumini* (barks), *Artocarpus heterophyllus* (mature leaves) and, *Cassia auriculata* (flowers).
Materials and methods: Herbal decoctions of four medicinal plant materials were prepared by simmering with hot water as prescribed by the Ayurvedic medicine. The effectiveness of the decoctions was analyzed *in vitro* based on their Anti-AGE formation activity, glycation reversing, and anti-oxidant potentials.
Results: According to the results, the decoctions of *S. reticulata*, *A. heterophyllus* and *C. auriculata* indicated the strong Anti-AGE forming (IC_{50}: 23.01 ± 2.70, 32.01 ± 2.09, 43.66 ± 2.11 mg/mL, respectively), glycation reversing (EC_{50}: 183.15 ± 7.67, 91.85 ± 1.93, 252.35 ± 4.03 mg/mL, respectively) and antioxidant potentials in terms of total polyphenol content (TPC), total flavonoid content (TFC), ferric ion reducing power (FRAP), ABTS and DPPH radical scavenging activities. However, the decoction of *S. cumini* reported the significantly high ($p < 0.05$) Anti-AGE forming, (IC_{50}: 9.75 ± 0.32 mg/mL), glycation reversing (EC_{50}: 66.45 ± 4.51 mg/mL), and antioxidant potentials against the decoctions of the other three plant materials.
Conclusion: S. cumini bark extract was identified as the best source in controlling the formation of AGEs excessively. Further, the other three plant extracts can also be effectively used as potential therapeutic agents to control the pathological conditions associated with AGEs-mediated health complications.

1. Introduction

Rapid formation of Advanced Glycation End-products (AGEs) occurrs under the prolonged exposure to hyperglycaemic conditions which are badly contributing to the development of secondary chronic diabetic complications such as aging, retinopathy, nephropathy, neuropathy, and some cardiovascular diseases [20,34]. Elevated blood glucose levels at hyperglycaemia increase the process of auto-oxidation of glucose and lead to the formation of free radicals excessively, beyond the ability to control through the natural antioxidant defence system, causing oxidative stress [27]. Therefore, oxidation reactions play a major role in accelerating the rate of producing AGEs due to the detrimental effect of glucose toxicity facilitated by chronic hyperglycaemia [35]. AGE formation is known as glycation that involves a series of non-enzymatic reactions between the carbonyl group on reducing sugars and the amino group on proteins [12]. Initially glycation responsible for

* Corresponding author.
E-mail: gayanthilakarathna@gmail.com
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generating reactive oxygen species and reactive carbonyl species such as methylglyoxal and glyoxal through oxidative and non-oxidative pathways [45]. Thus, both glycation and oxidative stress are referred to as glycoxidation which has been closely linked and synergistic [18], accountable for the formation of highly reactive chemical species and AGEs by altering the structures and functions of long-lived proteins, phospholipids, and DNA [29,39]. AGEs further promote the development and progression of diabetic complications through direct and receptor-dependent pathways [17,53]. However, the formation of AGEs can be restricted by preventing further oxidation of metal-catalyzed glucose oxidation via constituents empowered with Anti-AGE forming and antioxidant activities [12].

Although synthetic drugs are invented for controlling AGE related pathologies, still the effectiveness and side effects of these therapeutic agents are questionable [16,37]. For an instance, Aminoguanidine (nucleophilic hydrazine) which has been recognized as a source of inhibiting AGE formation (*in vivo*) were found with harmful side effects such as congestive heart failure, atrial fibrillation, myocardial infarction, anemia, and gastrointestinal disturbance, in diabetes patients [16]. Meanwhile, many traditional medicinal plants possessed Anti-AGE forming and antioxidant activities and are found to be useful to combat diabetes and associated complications by controlling the excessive formation of AGEs [14,40]. Usage of herbal medicines is a common practice of forefathers in many Asian countries, was considered as a safe and cheap therapeutic strategy for various diseases [33]. Plant species such as *S. reticulata, S.cumini, A. heterophyllus*, and *C. auriculata* are recognized as herbal plants, generally utilized in Ayurvedic medicine to treat *Diabetes mellitus* for centuries of times in the form of decoctions [25,24,40].

However, still, the effectiveness of these plant materials as potential sources of having Ant-AGE forming activities and the effectiveness of their decoctions prepared by following traditional ayurvedic medicinal practices are required to be assessed scientifically. Therefore, the purpose of this research work is to determine the Anti-AGE forming activity, glycation reversing and antioxidant potentials (*in vitro*) of the decoctions prepared from the aforementioned four herbal materials. Further, these findings are important to identify the potentials of manufacturing pharmacologically important therapeutic agents in the future with a view to preventing or delaying the onset of health complications caused by the undue formation of AGEs.

2. Materials and methods

2.1. Chemicals and equipment

Bovine serum albumin (BSA, Fraction V), D-Glucose, Sodium azide, Trolox, Quercetin, Aluminum chloride, Dimethyl sulfoxide (DMSO), Iron (III) chloride, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, 1,1-diphenyl-2-picrylhydrazine (DPPH), 2,2'- Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium salt (ABTS), 2,4,6-Tripyridyl-s-triazine (TPTZ), Potassium persulfate, Gallic acid, and Folin-ciocalteu phenol reagent in the analytical grade were purchased from Sigma–Aldrich. Other required chemicals and reagents used in the study were also in analytical grade.

2.2. Collection and authentication of plant specimens

Fresh herbal plants specimens such as stems of *S. reticulata,* barks of *S. cumini,* flowers of *C. auriculata,* and matured fallen leaves of *A. heterophyllus* were collected from Anuradhapura, and gall districts in Sri Lanka and all samples were authenticated at the National Herbarium, Royal Botanical Gardens (voucher specimen no. KT-1, MB, RW, JL respectively), Peradeniya, Sri Lanka.

2.3. Preparation of herbal decoctions

Herbal decoctions were prepared according to the method described by Ref. [40]. Initially, plant materials were washed with distilled water and shade drying for 24 h at room temperature (27 ± 2 C°) and hot air oven-dried (Memmert-ULE500) at 55 °C for another 24 h under laboratory condition. Dried materials were ground and passed through a sieve (200 μm aperture size) to get a fine powder. Powdered plant materials were used to prepare the decoctions to extract bioactive compounds by following traditional Ayurvedic methods.

Sixty grams (60 g) of powdered plant material were taken and boiled with 960 ml of distilled water until the volume becomes 240 ml. The extracts were filtered through a fine silk cloth and centrifuged at 5000 rpm at 25 min. Supernatants were filtered with Whatman no. 1 filter papers and freeze-dried (using Laboratory Freeze dryer: Telstar-Lyobeta 4 PS, SPAIN) for 48 h to get a lyophilized powder. The yields obtained from the herbal decoction after the freeze-drying process were 5.86%, 6.75%, 5.76%, and 8.69% (w/w) for *S. reticulata, S. cumini, A. heterophyllus*, and C. *auriculate* respectively. Thereafter, stock solutions with known concentrations were prepared using lyophilized powder; which was dissolved in dimethyl sulfoxide (DMSO) and used for *in vitro* analysis.

2.4. Anti-Advanced Glycation End-product (AGE) formation activity (in vitro) of herbal decoctions

The Anti-AGE formation activity of herbal decoctions was determined based on the method described by Refs. [32] with a few modifications. Briefly, 0.8 mg/ml of BSA was incubated with 400 mM D-glucose (containing 0.02% sodium azide), 80 μl of herbal extract, and 50 mM phosphate buffer (pH 7.4), in a total volume of 1 mL, at 60 °C for 40 h. The reactions were stopped by adding 200 μLl of 50% (w/v) TCA to the reaction mixture and it was centrifuged at 14,000 rpm for 5 min at 4 °C. The resulting precipitate was re-dissolved in alkaline phosphate buffer saline (pH 10). The fluorescence intensity of glycated products was quantified with Fluorescent Microplate Reader (Spectra Max Gemini EM, Molecular Devices, USA) at the excitation and emission wavelength of 370 nm and 440 nm respectively. Inhibition concentration fifty (IC_{50}) of herbal samples (n = 6) and dose-response relationships were determined for a concentration series at 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/mL respectively. Rutin was used as a positive control and inhibition (%) of AGE formation of the samples was calculated using the following equation.

$$\text{AGE Inhibition (\%)} = [(Fc\text{-}Fb)\text{-}(Fs\text{-}Fsb) / (Fc\text{-}Fb)] \times 100$$

Where, Fc = Fluorescence of incubated BSA, glucose and DMSO (control), Fb = Fluorescence of incubated BSA alone (blank), Fs = Fluorescence of incubated BSA, glucose and sample extracts, and Fsb = Fluorescence of incubated BSA with sample extracts.

2.5. Glycation reversing potential (in vitro) of herbal decoctions

Glycation reversing potentials of the samples were determined according to the method described [43]. Initially, 0.8 mg/mL of BSA and 400 mM D-glucose (containing 0.02% Sodium azide) was incubated with 50 mM phosphate buffer (pH 7.4) at 60 °C for 40 h. After the reactions were stopped by adding 200 μL of 50% (w/v) TCA to the total reaction volume (1 mL), the total mixture was centrifuged at 14,000 rpm for 5 min at the temperature of 4 °C. The

resulting precipitate was re-dissolved in phosphate buffer (pH 7.4). Thereafter, the second reaction mixture was prepared by adding 80 μL herbal extracts to the 0.8 mg/mL of BSA and 400 mM D-glucose (containing 0.02% Sodium azide) and 50 mM phosphate buffer (pH 7.4). The reaction mixture was again incubated at 60 °C for 40 h and thereafter 200 μl of 50% (w/v) TCA was added to terminate the reaction. The resulting AGEs-BSA precipitates were dissolved in 1 mL of phosphate buffer saline (pH 10) and fluorescence intensity was measured under the excitation and emission wavelengths of 370 nm and 440 nm respectively, using the fluorescent microplate reader. Glycation reversing potential (%) of samples was calculated using the following equation.

$$\text{Glycation reversing potential (\%)} = [(\text{Fc-Fb})-(\text{Fs-Fsb}) / (\text{Fc-Fb})] \times 100$$

Where, Fc = Fluorescence of incubated BSA, glucose and DMSO (control), Fb = Fluorescence of incubated BSA alone (blank), Fs = Fluorescence of incubated BSA, glucose and sample extracts, and Fsb = Fluorescence of incubated BSA with sample extracts.

2.6. Antioxidant activity (in vitro) of herbal decoctions

2.6.1. Total polyphenol content (TPC)

The TPCs of herbal extracts were quantified using Folin-Ciocalteu's colorimetric method [52]. Twenty microliters of sample extract and 110 μL of freshly prepared Folin-Ciocalteu's phenol reagent (diluted 10 times) were mixed with 70 μL of 7.5% Sodium carbonate. Absorbance was recorded at 765 nm, after incubating the reaction mixture at room temperature (25 ± 1 °C) for 30 min using a 96-well microplate reader (Spectra Max Plus384, Molecular Devices, USA). Gallic acid was used as the reference standard for constructing the calibration curve. Results were expressed as milligrams of Gallic acid equivalents per gram of sample (mg GAE/g).

2.6.2. Total flavonoid content (TFC)

The TFC assay was performed according to the method stated by Ref. [51]. One hundred microliters of 2% Aluminium chloride in methanol solution were mixed with 100 μL of herbal extract, diluted with Methanol to a known concentration. The mixture was incubated at room temperature (25 ± 1 °C) for 10 min and the absorbance was measured at 415 nm using 96-well microplate reader. The calibration curve was constructed by using Quercetin as the standard and results were expressed as mg quercetin equivalents per gram of sample (mg QE/g).

2.6.3. ABTS+ radical scavenging activity

The ABTS (2,2' Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging assay was conducted based on the method of [48]. Initially, the ABTS stock solution was prepared by reacting 7.8 mM of ABTS in Potassium per-sulfate at 37 °C for 16 h. Thereafter, 40 μl of seven times diluted ABTS stock solution, 50 μl of diluted sample extract, and 110 μL of phosphate buffer saline at pH 7.4 were mixed and incubated at (25 ± 1 °C) for 10 min. Afterward, absorbance was determined at 734 nm with 96-well microplate reader. The standard curve was prepared by using Trolox as the standard and results were expressed as mg Trolox equivalents per gram of sample (mg, TE/g).

2.6.4. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of samples was determined according to the method of [4] with few modifications. Initially, FRAP reagent was prepared freshly by mixing 300 mM of acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mMHCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 and incubated at 37 °C for 10min. Thereafter, 150 μLl of FRAP reagent, 30 μl of acetate buffer, and 20 μl of the diluted sample extract were added and incubated for 8 min at room temperature (25 ± 1 °C). Absorbance was measured under the wavelength of 600 nm using 96 well plate readers. Trolox was used as the standard for the calibration curve and results were expressed as mg Trolox equivalent/g of sample (mg, TE/g).

2.6.5. DPPH radical scavenging activity

The DPPH assay was performed according to the method of [7]. Total reaction volumes of 200 μL were prepared by adding 60 μL of 125 mM of DPPH radical and 50 μL of sample extracts in methanol. Samples were incubated at room temperature (25 ± 1 °C) for 10 min and the absorbance was measured at 517 nm using 96-well plate reader. Trolox was used as the reference standard material to construct the standard curve and final results were expressed as mg Trolox equivalent/g of sample (mg, TE/g).

2.7. Statistical analysis

Results were presented as mean ± SD of sample replicates. Data obtained from each experiment were statistically analysed using SPSS statistics 20 software. Analysis of variance (One way -ANOVA) and Tukey HSD test was used to assess the differences among the means. Correlation analysis was performed using Pearson's correlation coefficient. For all analysis, $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Anti-AGE formation activity (in vitro) of herbal decoctions

The percentage inhibitions of AGE formation pertaining to the dose-response relationships of four herbal decoctions are illustrated in Fig. 1.

According to the graphs given in Fig. 1, all herbal decoctions, at the concentration of 200 μg/ml were shown their highest activities which were more than 90% inhibitions. The highest AGE inhibition of 96.77 ± 2.19% was reported by *S. cumini* and the inhibitions of *S. reticulata, A. heterophyllus, C. auriculata,* and Rutin were 94.63 ± 2.95%, 93.28 ± 1.15%, 92.93 ± 5.46%, and 90.40 ± 4.00% respectively. Although the Anti-AGE formation activity was reduced along with the reduction of concentration, a remarkable drop of AGE inhibition was observed after passing a certain concentration by each decoction. For an instance, the great decline of

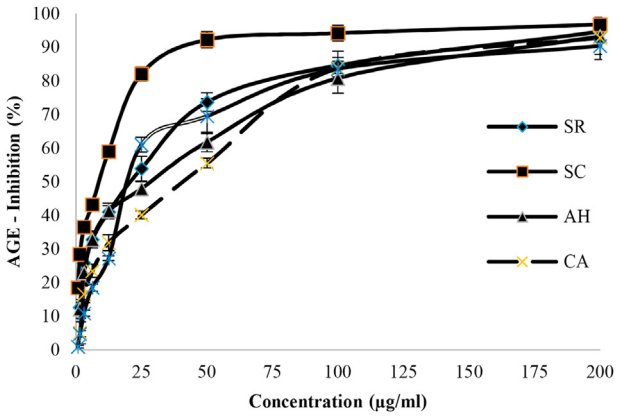

Fig. 1. The dose-response relationships of Anti-AGE formation activities in herbal decoctions prepared by *S. reticulata* (SR), *S. cumini* (SC), *A. heterophyllus* (AH), *C. auriculata* (CA) with positive control of Rutin (values are mean ± SD of replicates (n = 6); Mean separation, ANOVA, Tukey HSD test at $p < 0.05$).

Anti-AGE formation activity in *S. cumini* and *S. reticulata* was given after the dose concentration of 50 μg/ml. In the case of *A. heterophyllus* and *C. auriculata*, it had after 100 μg/mL. The control treatment Rutin also exhibits a remarkable decline when the dose concentration drops down from the point of 25 μg/mL. All the decoctions except *S. cumini* and Rutin did not show any Anti-AGE formation activities beyond the point of dose concentration of 1.56 μg/mL, however, *S. cumini* and Rutin demonstrated their activities until the concentration reach up to 0.78 μg/mL. The minimum AGE inhibition values at the critical point (from which no Anti-AGE formation activity occur) were 12.72 ± 1.38%, 5.14 ± 2.79%, 12.19 ± 3.30%, 18.55 ± 1.70%, and 0.96 ± 0.41% for *S. reticulata, C. auriculata, A. heterophyllus, S. cumini,* and Rutin respectively. In general, Anti-AGE formation activities of herbal decoctions were significantly varied ($p < 0.05$) in a dose-depending manner. The precise strength of Anti-AGE formation activities of the decoctions of selected plant materials and Rutin (positive control) in terms of IC_{50} values are illustrated in Fig. 2.

As illustrated in Fig. 2, IC_{50} values obtained for Anti-AGE formation activity of different herbal decoctions were significantly different to each other ($p < 0.05$). However, significantly low IC_{50} value (9.75 ± 0.32 μg/mL, $p < 0.05$) was reported by *S. cumini* compared to the positive control Rutin (20.79 ± 0.63 μg/mL). While the IC_{50} values of *S. reticulata* and Rutin were not significantly different ($p > 0.05$) to each other, the highest IC_{50} value was given by *C. auriculata* which was 43.66 ± 2.11 μg/mL. Thus, the order of the strength in Anti-AGE formation activity of decoctions prepared with plant material with respect to the IC_{50} values can be lined up as in ascending manner as *S. cumini* > *S. reticulata* > *A. heterophyllus* > *C. auriculata*.

3.2. Glycation reversing potential (in vitro) of herbal decoctions

Despite of inhibiting AGE formation, another significant approach in AGE inhibition is breaking of cross-links and/or reversing of already formed AGEs [37,49]. The reversing potentials of selected herbal plant decoctions with respect to the dose-response relationship are given in Fig. 3.

According to Fig. 3, the highest glycation reversing abilities of herbal decoctions were given at the decoction concentration of 400 μg/ml. Among them, *S. cumini* showed significantly higher glycation reversing potential ($p < 0.05$) and it was 91.86 ± 2.11%. However, glycation reversing potentials of *S. reticulata* and *C. auriculata* were significantly lower ($p < 0.05$) that of other two decoctions at the same concentration and they were 68.05 ± 2.96% and 69.20 ± 0.51% respectively. While the decoction prepared from *A. heterophyllus* leaves remarkably having higher glycation reversing potential (85.43 ± 1.09%) in comparison to *S. reticulata* and *C. auriculata* ($p < 0.05$). Although *S. reticulata* decoction had a better strength in Anti-AGE formation, it was not shown strong glycation reversing potential. The pattern of glycation reversing ability was almost similar to the pattern of the dose-response related to the Anti-AGE formation activity. Generally, the glycation reversing potentials were gradually as well as significantly reduced ($p < 0.05$) in parallel to the reduction of dose concentrations of herbal decoctions. The lowest glycation reversing potentials of herbal decoction were given at different concentrations beyond which no reversing activity occurs. *S. cumini* showed its lowest reversing ability of 4.50 ± 1.43% at the concentration of 3.125 μg/mL. *S. reticulata* and *A. heterophyllus* imparted their lowest reversing potentials at 6.25 μg/mL, which were 9.27 ± 0.71% and 8.76 ± 1.11% correspondingly. *C. auriculata* reported the lowest value (1.96 ± 0.69%) at the concentration of 12.5 μg/mL. The exact vigour of glycation reversing potentials of the decoctions of selected plant materials in terms of EC_{50} values are given in Fig. 4.

According to Fig. 4, the EC_{50} values pertaining to the glycation reversing potentials for four herbal decoctions were significantly different from each other ($p < 0.05$). While the lowest EC_{50} value of 66.45 ± 4.51 μg/mL was reported by *S. cumini* highest value of it (252.35 ± 4.03 μg/ml) was given by *C. auriculata.* Interestingly, *A. heterophyllus* displayed higher glycation reversing potential against the decoction prepared from *S. reticulata*. This finding has confirmed that matured *A. heterophyllus* leaves can be used as a

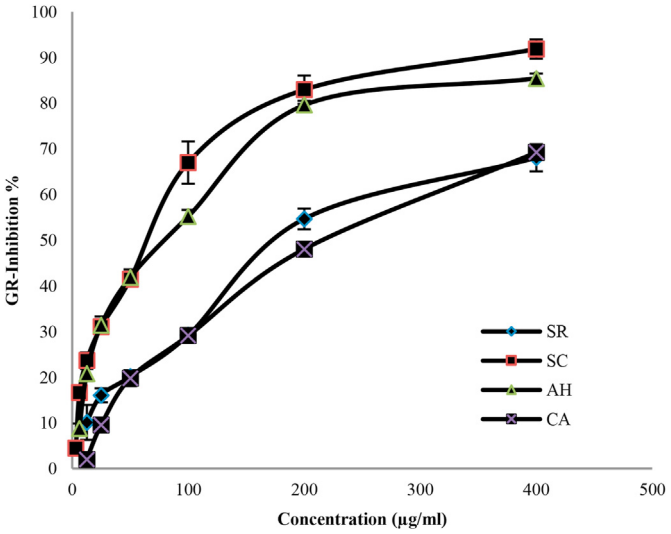

Fig. 3. Dose-response relationships of glycation reversing potentials in herbal decoctions prepared by *S. reticulata* (SR), *S. cumini* (SC), *A. heterophyllus* (AH), *C.auriculata* (CA), (Values are mean ± SD of replicates (n = 6); Mean separation, ANOVA, Tukey HSD test at $p < 0.05$).

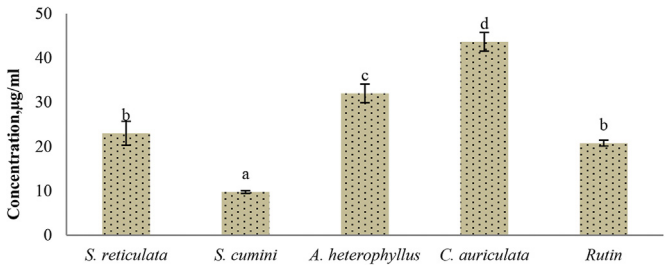

Fig. 2. IC_{50} values of herbals relevant to the AGE formation activity (Bar values are mean ± SD of replicates (n = 6) and superscripted by different letters indicated mean separations with ANOVA, Tukey HSD tests at ($p < 0.05$); IC_{50} values (μg/ml) are: *S. reticulata*-23.01 ± 2.70, *S. cumini*- 9.75 ± 0.32, *A. heterophyllus*-32.01 ± 2.09, *C. auriculata*- 43.66 ± 2.11 and Rutin-20.79 ± 0.63).

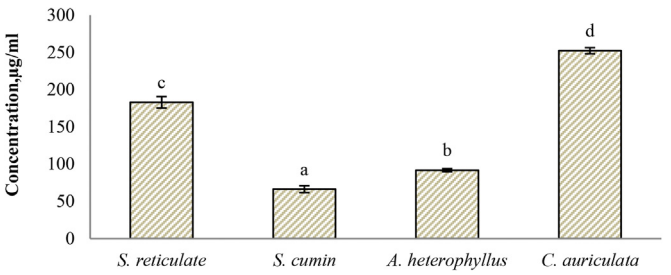

Fig. 4. EC_{50} values for herbals pertaining to the glycation reversing potential (Bar values are mean ± SD of replicates (n = 6) and superscripted by different letters indicated mean separations with ANOVA, Tukey HSD tests at ($p < 0.05$); EC_{50} values (μg/mL) are: *S. reticulata*-183.15 ± 7.67, *S. cumini*-66.46 ± 4.51, *A. heterophyllus*-91.85 ± 1.93 and *C. auriculata*-252.35 ± 4.03).

better glycation reversing source for diabetic complications. Based on the EC_{50} values, the strength of the glycation reversing potentials of herbal decoctions were varied and it can be categorized in descending manner as *S. cumini* > *A. heterophyllus* > *S. reticulata* > *C. auriculata*.

3.3. Antioxidant activity (in vitro) of herbal decoctions

The antioxidant activities in terms of TPC, TFC and ABTS radical scavenging activity, FRAP, and DPPH radical scavenging activity of freeze-dried herbal decoctions, are given in Table 1.

Data given in Table 1 indicate that *S. cumini* having a significantly high ($p < 0.05$) TPC (243.68 ± 27.33 mg GAE/g), TFC (5.26 ± 0.50 mg QE/g) and FRAP (456.021 ± 25.87 mg TE/g) values than the other herbal decoctions. However, TPCs, TFCs, and FRAP values of *S. reticulata, C. auriculata,* and *A. heterophyllus* were not significantly different ($p > 0.05$) to each other. The highest ABTS radical scavenging activity was reported by *S. cumini* and it was 150.41 ± 3.31 mg TE/g. ABTS radical scavenging activities of *C. auriculata* and *A. heterophyllus* were 19.65 ± 0.25 and 20.16 ± 0.26 mg TE/g respectively and these values were the lowest ($p > 0.05$) comparatively other two decoctions. DPPH radical scavenging activities of herbal decoctions were significantly different ($p < 0.05$) among the samples. While the lowest DPPH radical scavenging activity was given by *C. auriculata* (8.56 ± 0.053 mg TE/g) the highest value (70.29 ± 0.21 mg TE/g) was reported by *S. cumini*. The IC_{50} values of herbal decoctions pertaining to the ABTS and DPPH, radical scavenging activities are given in Table 2.

According to Table 2, the IC_{50} values obtained for herbal decoctions pertaining to the ABTS radical scavenging activity and DPPH radical scavenging activity were significantly different ($p < 0.05$) from each other. The lowest IC_{50} value for ABTS and DPPH activities were demonstrated by *S. cumini*, which were 2.85 ± 0.06 and 8.33 ± 0.03 μg/mL respectively and these values even lower than the reference standard values of Trolox, 6.36 ± 0.05 and 8.68 ± 0.07 μg/mL correspondingly. However, the IC_{50} value relevant to the DPPH assay of *S. cumini* (8.33 ± 0.03 μg/mL) was not significantly different ($p > 0.05$) to the same value of Trolox (8.68 ± 0.07 μg/mL). Based on the IC_{50} values obtained for ABTS and DPPH assays, the order of the strength in antioxidant activities of decoctions can be arranged in a descending manner as *S. cumini* > *S. reticulata* > *A. heterophyllus* > *C. auriculata.* The important observation of this study was the order of strength of herbal decoctions pertaining to the antioxidant activities were almost similar to the order of the strength found in Anti-AGE formation activity. AGEs and their precursors altering the function of intracellular proteins, altering matrices by disintegrating the binding/interacting other essential matrix components. Modification of proteins (plasmatic) can bind with the AGE receptor (RAGE) in cells and production of free radicals such as reactive oxygen and reactive nitrogen species [8].

According to Pearson's correlation analysis for the data of antioxidant activities, significant and strong positive correlations ($p < 0.05$) were found between TPC & FRAP ($r = 0.976$), TPC & ABTS ($r = 0.992$) and TPC & DPPH ($r = 0.980$). Moreover, significant and strong positive correlations ($p < 0.05$) were also shown between TFC & FRAP ($r = 0.989$), TFC & ABTS ($r = 0.990$) and TFC & DPPH ($r = 0.972$). While the Pearson's correlation analysis of the IC_{50} values, significant negative correlations ($p < 0.05$) were reported between Anti-AGE formation activity & TPC ($r = -0.791$), Anti-AGE formation activity & TFC ($r = -0.768$), Anti-AGE formation activity & FRAP ($r = -0.786$), Anti-AGE formation activity & ABTS ($r = -0.850$) and Anti-AGE formation activity & DPPH ($r = -0.911$) of herbal decoctions. Likewise, EC_{50} values were also having significant negative correlations ($p < 0.05$) between glycation reversing potential (GRP) & TPC ($r = -0.637$), GRP & TFC ($r = -0.658$), GRP & FRAP ($r = -0.657$), GRP & ABTS ($r = -0.645$) and GRP & DPPH ($r = -0.728$). Moreover, these findings imparted, that if higher the antioxidant potentials in herbal decoctions, lower the concentrations required to 50% inhibition or else, IC_{50}/EC_{50} values were inversely proportional to the antioxidant activities. Therefore, the present findings strongly confirmed the robust involvement of phytochemicals of plant origin to reduce the formation of AGEs under the oxidative stress conditions.

4. Discussion

Based on the results of four selected herbal materials, namely; *S. reticulata* (stems), *S. cumini* (barks), *A. heterophyllus* (mature leaves), and *C. auriculata* (flowers) had very strong Anti-AGE formation activities. Therefore, using herbal decoctions prepared with so-called herbal materials, in order to control AGE, is a very important and productive option against synthetic drugs in the

Table 1
Total polyphenol content (TPC), total flavonoid content (TFC), ferric ion reducing power (FRAP), ABTS and DPPH radical scavenging activities of herbal decoctions.

Type of Herbal	TPC (mg, GAE/g)	TFC (mg,QE./g)	FRAP (mg, TE./g)	ABTS (mg, TE./g)	DPPH (mg, TE./g)
S. reticulata	81.31 ± 2.41[a]	1.35 ± 0.16[a]	110.62 ± 3.70[a]	24.71 ± 0.54[a]	19.75 ± 0.46[a]
S. cumini	243.68 ± 27.33[b]	5.26 ± 0.50[b]	456.021 ± 25.87[b]	150.41 ± 3.31[b]	70.29 ± 0.21[b]
C. auriculata	82.53 ± 0.62[a]	1.69 ± 0.15[a]	130.15 ± 9.12[a]	20.16 ± 0.26[c]	8.56 ± 0.053[d]
A. heterophyllus	80.17 ± 1.39[a]	1.54 ± 0.09[a]	118.81 ± 7.64[a]	19.65 ± 0.25[c]	17.68 ± 0.47[c]

Values are presented as mean ± SD of replicates ($n = 6$) for TPC, TFC, FRAP and $n = 3$ for ABTS and DPPH. Mean values within each column superscripted by different letters are significantly different at $p < 0.05$ according to ANOVA, Tukey HSD test.

Table 2
The IC_{50} values of herbal decoctions and reference standard pertaining to the ABTS and DPPH radical scavenging activities.

Herbal decoction/Reference standard	The IC_{50} value for ABTS radical scavenging activity (μg/ml)	The IC_{50} value for DPPH radical scavenging activity (μg/ml)
S. reticulata	15.07 ± 0.33[c]	25.32 ± 0.59[b]
S. cumini	2.85 ± 0.06[a]	8.33 ± 0.03[a]
C. auriculata	28.12 ± 0.36[e]	28.78 ± 0.76[d]
A. heterophyllus	18.16 ± 023[d]	88.13 ± 0.54[c]
Trolox (Reference standard)	6.36 ± 0.05[b]	8.68 ± 0.07[a]

Values are presented as mean ± SD of replicates ($n = 3$) Mean values within each column superscripted by different letters are significantly different at $p < 0.05$ according to ANOVA, Tukey HSD test.

future because these drugs may impede the adverse consequences for the user if he or she prolong exposed to them. For an instance, at the higher concentrations of Aminoguanidine which is a synthetic drug used to prevent the formation of AGEs can react with vitamin B6 and lead to a vitamin B6 deficiency in the body [49]. The reason for this aftermath is the dosage of Aminoguanidine which needs to be taken at a fairly high concentration (1 g/L of water) in order to get the proper activity, since its half-life in plasma is very short -about 1 h [37].

Moreover, decoctions prepared from these herbals can be used as potential sources to break the AGE cross-links instead of therapeutic agents. Main synthetic therapeutic agents currently use for the AGE cross-links breaking are 3-Thiazolium derivatives (N-phenyl-1,3-thiazolium bromide (PTB) and N-phenacyl-4,5-dimethyl-1,3-thiazolium chloride (alagebrium chloride)) [42]. However, relevant to the *in vivo* studies, these compounds have limited reversing or cross-link breaking capabilities [37,49]. Enzymatic system of GLO (Glyoxalase) and enzymes known as Fructosyl-Amine Oxidase (FAOXs) and Fructosamine-3-Kinase (FN3K), have also been recognized as an AGE cross-link breaker [19]. However, fewer side effects may be associated with these systems [36,57,58]. On the other hand, FAOXs and FN3K found in bacteria, yeast, and fungi, not in mammals, therefore, their usage in humans remains to be scrutinized and enzyme isolation and related other manufacturing steps may also be complex and high cost involved process [54]. Hence, there is a huge prospect to develop natural therapeutic agent**s** possessed with glycation reversing or cross-links breaking capabilities while protecting inhibitory effect on other vital steps of the glycation process, to prevent AGEs related pathologies.

Herbals consisted of both inhibiting and reversing abilities of the glycation process. Thus, they can impart a greater value as they can mitigate the AGE risks in either way. Furthermore, these herbals can be used to develop therapeutic agents commercially. However, the effective concentration**s** required to perform the glycation reversing potential of herbals were significantly higher ($p < 0.05$) compared to the concentration required to inhibiting the AGE formations of the same herbal. Although different studies relevant to the Anti-AGE forming and anti-oxidant activities have been conducted by several researchers including [31,38,40]; there was no clear evidence regarding the investigations on the herbal decoctions prepared from these materials with respect to Anti-AGE forming activities or glycation reversing potentials including IC_{50}/EC_{50} values. Because, extracting of active compounds as well as Anti-AGE forming activity and glycation reversing potentials, are largely depending on the extracting medium and the method. Therefore, this study may be the first investigation that has been carried out to determine the Anti-AGE formation activity and glycation reversing potential of decoctions prepared from *A. heterophyllus* leaves and glycation reversing potentials of the decoctions prepared from *S. cumin* bark and *C. auriculata* flowers.

Herbal decoctions prepared from plant materials possessed strong antioxidant activities due to the presence of phytochemical substances, especially polyphenols, and flavonoids [23]. It was also reported that anti-oxidative and pharmacological properties possessed by plant extracts mainly due to the presence of phenolic compounds being secondary plant metabolites; because phenolics and flavonoids are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron and chelating metal ions in aqueous solutions [41]. The Anti-AGE forming activity and glycation reversing potential that existed in plant decoctions were strongly governed by the antioxidant activities and phytochemical substances present in the plant particularly polyphenols and flavonoids. This was further confirmed according to the correlations found pertaining to Anti-AGE formation activities and glycation reversing potentials (IC_{50} and EC_{50} values) against the antioxidant activities.

Previous studies also confirmed that the strong correlation between phenolic contents and Anti-AGE formation activities in natural products [22]. The prevention of further oxidation of Amadori product and metal-catalyzed glucose oxidation are the main mechanisms involved with retardation of AGE synthesis by the natural anti-oxidative agents [46]. Further, the strong chelating activity of polyphenols inhibits the formation of AGE cross-links via blocking AGE precursors generate during the glycoxidation reactions by contributing to the reversing of glycation [44]. Hence, herbal plant's compounds consisted of Anti-AGE forming and antioxidant activities synergistically are more effective as a therapeutic agent for alleviating diabetes complications [30].

Phytochemicals such as phenols and flavonoids in plant extracts are the main compounds possessing Anti-AGE formation activities [1,56]. *S. cumini* plant is also a potential source rich in flavonoids such as oleanolic acid, triterpenoids, ellagic acid, isoquercetin, catechin, quercetin, kaempferol, and myricetin, etc. [3,47]. Other than those, β-sitosterol, gallic acid [5], epi-friedelanol, betulinic acid, friedelin, eugenin [50], and tannins [6] also contained in this plant extract which is also responsible for Anti-AGE formation and antioxidant activities. Likewise [13,2], reported that mangiferin, salacinol, kotalanol as the major polyphenols and triterpenes in *S. reticulata* with anti-diabetic properties. In addition, epi-catechin, 1,3-diketones, leucopelargonidin, dulcitol, phlobatannin, hydroxyferruginol, lambertic acid, kotalagenin 16-acetate, 26-hydroxy-1,3-friedelanedione, and tannings have also been found in *S. reticulata* [2].

Further, *C. auriculata* flowers contain a higher amount of phenolic acid, tannins, and anthocyanins [21], and flavonoids including quercetin, catechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, gallocatechin gallate and catechin gallate [26]. In addition, glycosides, alkaloids, saponins, anthroquinones, and β-sitosterol were also available [28]. [10] revealed that mature leaves of *A. heterophyllus* were shown in higher hypoglycemic activity due to the presence of higher flavonoid concentrations in matured fallen leaves. Moreover, the hypoglycemic effect of the flavonoid fraction of the mature leaves of *A. heterophyllus* was higher than that of tolbutamide, a sulphonylurea drug commonly used for the treatment of hyperglycemia [11]. Instead of that, many *in vivo* studies have also confirmed the existence of anti-diabetic properties of mature leaves of *A. heterophyllus* [9,38,15]. [55] were also found that there was a remarkable inhibitory action of certain flavonoids (i.e. quercetin) compared to the synthetic drugs (e.g.: Aminoguanidine) on AGEs. Nevertheless, flavonoids (catechin, epicatechin, epicatechin gallate, epigallocatechin-3-gallate, kaempferol, and quercetin, etc.) are the most effective group of compounds endowed with higher Anti-AGE formation activities [55]. The synergistic action of multiple types of polyphenols will be much more effective as a therapeutic agent to reduce AGE formation and promoting health, due to its minimum side effects and toxicities compared to the synthetic drugs [55,49,33].

5. Conclusion

The herbal decoctions prepared with *S. reticulata* (stems), *S. cumini* (barks), *A. heterophyllus* (mature leaves), and *C. auriculata* (flowers) displayed strong Anti-AGE formation activities together with glycation reversing and antioxidant potentials. Out of those, the decoction of *S. cumini* (barks) has been identified as an outstanding source for Anti-AGE forming, glycation reversing, and antioxidant properties. However, all of these herbal materials can effectively be used as therapeutics to inhibit the excess AGE

formation under hyperglycaemic conditions and also to mitigate the risk associated with the long-term consequences related to diabetes complications.

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Conflict of interest

None.

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