

## Antiglycation and Antioxidant Activities of a Ready to Serve Herbal Drink of *Syzygium Cumini* Bark Extract

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### Abstract

Diabetes mellitus is a major public health issue in most countries around the world. About 60% of the world's population use herbal medicines as a remedy to prevent complications which arise due to glycation of proteins, enhanced by free radical formation. A previous study has proven the antiglycation and antioxidant potentials of *Syzygium cumini* decoction. The present study attempted to produce a ready to serve herbal drink using a water extract of *Syzygium cumini* bark. The consumer acceptable formulation selected by a sensory evaluation was investigated for the physical characteristics, microbial stability and antiglycation and antioxidant potentials up to a three months storage period. Antiglycation and antioxidant potentials were determined by Bovian Serum Albumin assay, ABTS and DPPH assay respectively.

The overall acceptability of herbal formulation of 20 ml of decoction and 80 ml of 0.01% sucralose solution was optimum. Antiglycation activity of the freshly prepared drink was 35.8 µg/ml and did not decrease significantly during storage of 45 (39.2 µg/ml) and 90 days (41.1 µg/ml). The antioxidant potentials of fresh drink, during storage on day 45 and 90 days were 1314, 1200 and 1095 mmol/g TEAC and 82.3, 84.8 and 87.0 µg/ml for ABTS<sup>+</sup> and DPPH<sup>•</sup> assays respectively. The herbal drink was microbiologically safe up to three months under refrigerated conditions and physical characteristics did not change significantly. It is concluded that *S. cumini* decoction could be used as a valuable ingredient for the production of an herbal drink with many health benefits.

**Keywords:** Antiglycation activity; Antioxidant activity; *Syzygium cumini* herbal drink

### Introduction

Diabetes mellitus is a major public health issue in most countries around the world. This is the main threat to human health in the 21<sup>st</sup> century and is the 5<sup>th</sup> leading cause of death in most developed countries [1]. However, by year 2015 more than 75% of individuals with diabetes would reside in the developing countries [2] which will be a major health problem to these countries.

Diabetes can affect nearly every organ system in the body. It can cause blindness, end stage renal disease, lower extremity amputation, increase the risk of stroke, ischemic heart diseases, peripheral vascular disease and neuropathy [3]. Hyperglycemia is one of the most important causative factors for secondary complications in diabetes. Sustained hyperglycemia leads to glycation of the proteins, preferably at amino group of lysine residue, which adversely affects their function. Reducing sugars, such as glucose reacts non-enzymatically with the free amino groups of proteins to initiate advanced glycation, resulting in formation of a diverse group of moieties [4]. This reaction initiates formation of reversible Schiff bases, which by intermolecular rearrangement are converted into stable, covalently-bonded Amadori products [5]. When large amounts of Amadori products are accumulated, they undergo further rearrangement and cross-link to form a heterogeneous group of protein-bound moieties called 'advanced glycated end products' (AGEs). Structural proteins such as collagen and elastin undergo continual non-enzymatic cross-linking during aging and in diabetic individuals. AGE-derived protein cross-linking of structural proteins contributes to the complications of long-term diabetes [6]. Various studies have shown that diabetes mellitus is associated with an increased production of free radicals leading to oxidative stress. Oxidation plays an important role in the formation of AGEs and the plant derived agents with antiglycation and antioxidant activities are highly important in preventing diabetic complications.

About 60% of the world's population use herbal medicines as a remedy to prevent complications which arise due to glycation of proteins, enhanced by free radical formation. *Syzygium cumini* decoction is being used as a treatment in diabetes mellitus in Ayurvedha medicine. A previous study proved the *Syzygium cumini* decoction to have high antiglycation and antioxidant activities. The present study attempted to produce and investigate the antiglycation and antioxidant potentials of a ready to serve herbal drink prepared with the water extract of *Syzygium cumini* bark targeting the diabetic population as this may have a better therapeutic potential.

### Materials and Methods

#### Collection of the plant material

Commercially available dried bark of *Syzygium cumini* was purchased from the traditional herbal market in Colombo, Sri Lanka and identification was carried out by a Botanist at Bandaranayke Memorial Ayurvedha Research Institute at Nawinna, Sri Lanka.

#### Preparation of plant material

Dried plant parts were ground to a fine powder with a grinder

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(National, Japan) at room temperature and stored in air tight containers in a refrigerator (4°C).

### Preparation of the decoction

Water extracts of dried and milled powdered bark of *Syzygium cumini* were prepared according to the traditional method practiced in Ayurvedha medicine to prepare 'Kasaya' (herbal decoction). The powdered sample (60 g) having a similar weight of 12 'kalan' was boiled under low heat with 960 ml of water (4 patha) until a concentrate of 240 ml (1 patha) was obtained. This water extract was filtered through a fine silk cloth and used to prepare the herbal drink.

### Preparation of herbal drink

Four combinations of ready to serve drink samples were prepared with respect to two factor factorial experimental design using two variables at two levels, low and high using the *S.cumini* decoction and sucralose solution (0.01g/100ml) (Table 1). Hot filling was carried out in to sterilized bottles and capped, pasteurized at 80°C for 20 minutes and stored at 4°C.

### Sensory evaluation

Sensory evaluation was carried out using Cruskal-Wallis non parametric ANOVA method using five point unipolar hedonic scale using 30 numbers of untrained panel to measure the sensory properties of color, sweetness, astringency, after taste and overall acceptability to find the best combination from the four samples.

### Total soluble solids

Total Soluble Solids (TSS) of the drink was analyzed by a Digital Refractometer (Master-alpha). A drop of the drink was placed on a refractometer prism and the total soluble solids were recorded as °Brix [7].

### pH of the drink

pH value was measured using a digital pH meter (HANNA H<sub>1</sub> 98129) standardized with buffers of pH 4.0 and 7.0 (AOAC 2005). The electrode of the pH meter was dipped in 20 ml of the sample measured in to a glass beaker and was allowed to stabilize for sometime after which the reading was taken.

### Total viable count of the herbal drink

Total viable count was carried out using the pour plate method [8] up to twelve weeks at fifteen days intervals (Table 4). Nutrient agar was used for enumeration of bacteria. A well homogenized sample was serially diluted with sterile distilled water up to 10<sup>-6</sup>. One ml aliquot from a 10<sup>-2</sup> to 10<sup>-5</sup> was transferred aseptically into sterile petri dishes. To each about 15 ml of sterile melted and cooled nutrient agar was added. The inoculum was evenly mixed with media by rotating the plates and allowed to solidify. The inverted plate was incubated at 37°C for 48 hours in an incubator. The total viable count was determined using a colony counter (Table 5).

### Antiglycation activity

Antiglycation activity was determined using the Bovian Serum Albumin (BSA) assay [9] with slight modification. In all experiments the final reaction volume was 1.0 ml. Bovian serum albumin 500 µl (1 mg/ml) was incubated with glucose 400 µl (500 mM) and 100 µl sample, 100 µl phosphate buffer saline instead of the sample(sample control) and 100 µl Arbutin as the reference standard. A negative

Sample code	Volume of <i>S.cumini</i> decoction	Volume of 0.01% sucralose solution
376	20	80
352	20	60
390	40	80
325	40	60

Table 1: Sample codes and designation.

Treatment	Colour	Sweetness	Astringency	After taste	Overall acceptability
376	52.4	56.8	62.4	62.4	58.9
352	60.4	49.9	41.9	41.9	51.7
390	40.6	50.5	52.5	52.5	58.0
325	48.6	44.8	45.1	45.1	33.4

Table 2: Average ranks of ranking charts of sensory analysis for the developed formulas of the herbal drink.

Parameter	0 days	45 days	90 days
pH value	6.4	6.3	6.0
Brix	1	1	1
Colour	Light brown	Light brown	Light brown

Table 3: Physical characteristics of the herbal drink (376).

Parameter	0 days	15 days	30 days	45 days	90 days
Total colony count	1.02×10 <sup>-1</sup>	1.05×10 <sup>-1</sup>	1.03×10 <sup>-2</sup>	1.02×10 <sup>-2</sup>	1.05×10 <sup>-2</sup>

Table 4: Microbial load of the herbal drink (376).

Parameter	0 days	45 days	90 days
Herbal drink	35.88 ± 1.1 µg/ml	39.2 ± 1.3µg/ml	41.1 ± 0.9 µg/ml
Arbutin	65.31.5 ± µg/ml	63.5 ± 0.9 µg/ml	65.8 ± 1.1 µg/ml

Table 5: Antiglycation activity of herbal drink (376).

control was carried out at the same time with BSA 500 µl (1 mg/ml), 400 µl phosphate buffer saline and 100 µl sample incubated under the same conditions. The reaction was allowed to proceed at 60°C for 24 hours and terminated, by adding 10 µl of 100% (W/V) trichloroacetic acid (TCA). The TCA added mixture was kept at 4°C for 10 minutes and centrifuged (4 minutes) at 13000 rpm. The precipitate was redissolved with alkaline PBS (pH 10) and quantified for the relative amount of glycated BSA based on fluoresce intensity by Fluorescent Microplate Reader (Spectra Max Gemini EM). The excitation and emission wavelengths used were 370 nm and 440 nm respectively. Each sample was analyzed in five concentrations and each in triplicate. Percentage of inhibition was calculated using the formula given below and the sample concentration required for 50% inhibition was calculated using Minitab 14.

$$\% \text{ of inhibition} = \frac{\text{OD blank} - (\text{OD sample} - \text{OD sample negative}) / \text{OD blank}}{\text{OD} - \text{Optical Density}} \times 100$$

### Antioxidant activity by DPPH assay

Assay was carried out with DPPH (2,2 diphenyl-2-picryl hydrazyl hydrate) (Sigma-Aldrich, USA) using a spectrophotometric method [10]. Freshly prepared DPPH solution was used for each experiment. Reaction mixture was prepared using 2.5 ml of 6.5×10<sup>-5</sup> M DPPH solution and 0.5 ml of sample dissolved in methanol and a control sample with 2.5 ml of 6.5×10<sup>-5</sup> M DPPH solution and 0.5 ml of methanol. Samples were tested in five concentrations and each sample in triplicate. All samples were incubated at room temperature for 30 minutes in dark and absorbance measured at 540 nm using UV-Vis spectrophotometer (SHIMADZU UV mini 1240). The percentage of DPPH radical scavenging activity was determined in five concentrations

using the equation mentioned below. Butyl Hydroxy Toluene (BHT) was used as the reference standard. The sample concentration which gives 50% scavenging activity was estimated as IC<sub>50</sub> value.

$$\% \text{ scavenging activity} = A_0 - A_s / A_0 \times 100$$

A<sub>s</sub> = Absorbance of the DPPH solution of the control sample

A<sub>0</sub> = Absorbance of the DPPH solution in the presence of plant extract

### Free radical scavenging activity using ABTS assay

2,2'-azinobis (3-ethyl benzothiazoline-6-sulphonic acid) diammonium (ABTS<sup>+</sup>) salt (Sigma-Aldrich, USA) radical cation decolorization assay was used to measure the antioxidant activity [11] with slight modifications. The stock solutions of 7 mM ABTS<sup>+</sup> solution and 2.4 mM potassium per sulphate solution were prepared and the working solution was prepared by mixing equal parts from each and allowing the mixture to stand in the dark at room temperature for 12–16 hrs before use. The solution was then diluted by mixing 1.0 ml ABTS<sup>+</sup> solution with 16.0 ml phosphate buffer to obtain an initial absorbance of 0.700 ± 0.01. ABTS<sup>+</sup> solution was freshly prepared for each assay. Freeze dried water extract of each sample was prepared in 3 concentrations by dissolving in phosphate buffer solution and the reaction mixture was prepared mixing 100µl of plant extract with 3.0 ml ABTS<sup>+</sup> solution. ABTS<sup>+</sup> radical scavenging activity was determined after measuring the fall of absorbance exactly after 15 min. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and the blank sample was prepared adding 100 µl phosphate buffer to 3.0 ml ABTS<sup>+</sup> solution. Data are reported as mean ± SD of the three replicates as Trolox equivalents using the equation obtained from the calibration curve of Trolox.

### Results

The four herbal drinks (Table 1) were subjected to sensory evaluation and the scores were analyzed using Minitab 14 version (Table 2). According to the results sample code 376 was significantly different in astringency and after taste (p > 0.05) from all other samples. Color, sweetness and overall acceptability of the herbal drinks were not significantly different. The selected sample with the highest overall acceptability (376) contained 20 ml of *S.cumini* decoction and 0.01% sucralose solution and was used for further analyses. The dosage of *S. cumini* used in the herbal drink is below the dosage used in Ayurvedha medicine for the treatment of diabetes mellitus (Ayurveda Pharmacopoeia).

The total soluble solids of the fresh drink was 1 °Brix in formulated herbal drink and showed a negligible change throughout three months of storage period under refrigerated conditions. The pH of the freshly prepared herbal drink was 6.4 and there was a slight increase up to 6.0 at the end of the three months storage period. Colour of the product was examined visually and did not change during the storage period (Table 3).

The formulated herbal drink had a pH of 6.0. However, the total viable count ranged between 1.02×10<sup>-1</sup> to 1.05×10<sup>-2</sup> (Table 4). The total viable count was within the acceptable limit of Sri Lanka standard for drinks, juices and beverages (SLS 729). According to the SLS standards pH of a ready to serve drink should be lower than 4 in ready to serve (RTS) drinks to prevent the microbial growth [12]. As the total soluble solids were low (1 °Brix) and the decoction used to prepare the drink contains glycosides, tannins, flavonoids, saponins and phenols like

Parameter	0 days	45 days	90 days
Herbal drink (DPPH)	82.3 ± 1.2 µg/ml	84.8 ± 1.5 µg/ml	87.0 ± 1.5 µg/ml
BHT	18.2 ± 0.8 g/ml	17.6 ± 1.0 µg/ml	18.9 ± 0.5 µg/ml
Herbal drink (ABTS)	1314 ± 4.5 TEAC mmol/g	1200 ± 5.1 TEACmmol/g.	1095 ± 4.8 TEAC mmol/g

Table 6: Antioxidant activity (DPPH and ABTS assays).

secondary metabolites [13] which have antimicrobial activity, these may prevent the microbial development despite a pH of 6. The heat treatment followed by refrigeration is therefore sufficient to extend the shelf life of the herbal drink to three months and safe for human consumption.

Antiglycation activity is inversely proportional to the sample concentration and the herbal drink had a higher antiglycation activity (1.7 times) than the reference compound arbutin. A significant decline in antiglycation activity was not observed during storage.

DPPH antioxidant activity is also inversely proportional to the sample concentration and compared to the reference sample BHT, the herbal drink showed five times less activity. However, only a low dose 82-87 µg/ml of the herbal drink was required for scavenging 50% of the DPPH free radicals showing a higher antioxidant potential. Herbal drink showed a high ABTS potential which did not decrease significantly during the storage of three months (Table 6).

A significant decrease in antioxidant and antiglycation potentials was not observed during the storage period of 90 days under refrigerated conditions. Previous studies by the authors on screening medicinal plants used in the treatment of diabetes mellitus has proven the high antioxidant potential (DPPH; 30.0 ± 3.1 µg/ml and ABTS; 1544 ± 5.6 TEAC mmol/g), high antiglycation activity (16.8 µg/ml) and phenolic content (819 ± 4.01) in the decoction of *S. cumini* commercial sample. Phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions [14]. Several studies have demonstrated that the antiglycation activity correlates significantly with the phenolic content of the tested plant extracts [7]. Polyphenols are the most abundant dietary antioxidants.

### Conclusions

It can be concluded that a consumer acceptable ready to serve herbal drink with high antiglycation and antioxidant potentials could be prepared using the decoction of *S. cumini* with a dose below the recommended level in Ayurvedha medicine with the non-caloric sweetener sucralose. The product's therapeutic efficacy in diabetics is being evaluated.

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