



**Antiglycation and antioxidant activities of some
selected medicinal plants and selective value addition
to *Syzygium cumini* (Madan) decoction**

by

Pathirage Rupika Damayanthi Perera

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award of the Degree of Doctor of Philosophy in Food Science on 2014**

Certification of supervisors

We certify that the candidate has incorporated all corrections additions and amendments recommended by the examiners.



Prof. K. K. D. S. Ranaweera



Prof. (Mrs) Sagarika Ekanayake

DECLARATION

The work described in this thesis was carried out by me under the supervision of Professor K.K.D.S.Ranaweera, Director, Bandaranaike Memorial Ayurvedha Research Institute and Senior Lecturer, Department of Food Science and Technology, University of Sri Jayewardenepura and Professor Sagarika Ekanayake, Head, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura. The report on this has not been submitted in whole or in part to any University for another Degree/Diploma.

P. R. D. Perera

17.2.2015.

Pathirage Rupika Damayanthi Perera

Date

We certify that the above statement made by the candidate is true and that thesis is suitable for submission to the University for the purpose of evaluation.



Prof. K. K. D. S. Ranaweera



Prof. (Mrs) Sagarika Ekanayake

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LIST OF ABBREVIATIONS

| | |
|-----------------------------|--|
| ABTS | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| TEAC | Trolox Equivalent Antioxidant Capacity |
| GAE | Gallic Acid Equivalent |
| TLC | Thin Layer Chromatography |
| R _f | Refractive Index |
| IGT | Impaired Glucose Tolerance |
| AGEs | Advanced Glycated End Products |
| IFG | Impaired Fasting Glycemia |
| DKA | Diabetic Keto Acidosis |
| HNC | Hyperosmolar Non-ketonicoma |
| LA | Lactic Acidosis |
| CML | Carboxy methyl lysine |
| CEL | Carboxy ethyl lysine |
| MOLD | Methylglyoxal induced lysine dimer |
| GOLD | Glyoxal derived lysine dimer |
| O ₂ ⁻ | Superoxide anion |
| ·OH | Hydroxyl radical |
| ROS | Reactive oxygen species |
| RNS | Reactive nitrogen species |
| STZ | Streptozotocin |
| PBS | Phosphate buffered saline |
| FC | Folin Ciocaltue |
| BHT | Butylated Hydroxy Toluene |

| | |
|------------|---|
| ANOVA | Analysis of variance |
| HAT | Hydrogen Atom Transfer |
| SET | Single Electron Transfer |
| TSS | Total Soluble Solid |
| HPLC | High Performance Liquid Chromatography |
| LC UV- Vis | Liquid Chromatographic Ultra Violet – Visible |

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ABSTRACT

According to the current statistics of Diabetes Atlas of International Diabetes Federation, 285 million among the world population suffer from Diabetes mellitus. Oxidative stress due to the rapid formation of free radicals and protein glycation are the key molecular basis of macro and micro complications of diabetes mellitus. There is a growing tendency to use herbal treatments in Diabetes mellitus due to the minimal adverse effects, safety and low cost. More than 500 traditional antidiabetic plants have been recorded in traditional medicine, but very few scientific investigations have been carried out to prove the efficacy of using these herbal plants in the treatment of Diabetes mellitus. Five medicinal plants commonly used in the treatment of diabetes mellitus were selected for the study by gathering information from the traditional and Ayurvedha medical practitioners. The selected herbal plant parts are *Cassia auriculata* flowers, *Osbakia octandra* leaves, *Syzygium cumini* bark, *Phyllanthus emblica* fruits and *Scoparia dulcis* whole plant. These are administrated as decoctions of poly herbal formulations and as individual plants, prepared according to the Ayurvedha pharmacopeia.

Decoctions of the five plants, prepared using the commercial samples available in the traditional herbal market and three fresh samples of each, collected from three different

regions of Sri Lanka and dried under laboratory conditions were analyzed for the antiglycation potentials using the Bovian serum albumin assay, antioxidant potentials by ABTS and DPPH methods and total phenolic contents using Folin Ciocaltue method.

Decoctions of *S. cumini* bark, *O. octandra* and *P. emblica* showed significantly high antiglycation potentials in the range of 16.8–35.18, 23.0–28.5, 37.4–82.28 µg/ml while *C. auriculata* and *S. dulcis* showed moderate antiglycation potentials as 109–250 µg/ml and 131–213 µg/ml. The DPPH potentials were also significantly high in *S. cumini*, *O. octandra* and *P. emblica* and were in the range of 30.3–69, 55.5–98.4, 27.1–49.5 µg/ml respectively. *C. auriculata* and *S. dulcis* showed moderate DPPH potentials as 237–309 and 437–540 µg/ml. The highest ABTS potential was reported in *P. emblica* decoction of commercial sample as 2764 TEAC mmol/g, other laboratory dried samples showed 1393–1871 TEAC mmol/g and *S. cumini*, *O. octandra* also contained significantly high ABTS potentials in the ranges of 1544–1897, 794–1375 TEAC mmol/g respectively. Moderate ABTS potentials were showed by *C. auriculata* and *S. dulcis* (313–648, 549–615 TEAC mmol/g). The total phenolic contents were significantly high in *S. cumini*, *O. octandra* and *P. emblica* as 819–867, 483–666, 491–625 mg GAE/g and moderate values were given by *C. auriculata* and *S. dulcis* as 215–459, 131–186 mg GAE/g.

S. cumini commercial sample with the highest antiglycation potential, significantly high DPPH and ABTS potentials and phenolic contents was further analyzed for the availability of phytochemical constituents and the decoction contained glycosides, tannins, flavonoids, saponins and phenols.

A ready to serve herbal drink was developed using the decoction of *S. cumini* commercial sample, by selecting the best consumer acceptable formula among four

formulations developed based on the two factor factorial designing and analyzing data obtained using 30 numbers of untrained sensory panelists. The herbal drink contained 20 ml of the *S. cumini* decoction and the dosage was below the recommended level in Ayurvedha Pharmacopeia. Sucralose (0.01%) was used to mask the bitter and astringent taste of the drink and was one tenth of the recommend level. Storage studies of the herbal drink were conducted for three months under refrigerated conditions. Its physical characteristics (colour, pH value and total soluble solids (Brix^o)) and antiglycation and antioxidant potentials were measured at 45 days intervals. Microbiological assays for viable colony counts for bacteria and fungi were conducted at 15 days intervals. No significant difference was found in physical characteristics and the drink was microbiologically safe during the storage period. Antiglycation potentials were in the range of 35.8–41.1 µg/ml and ABTS and DPPH potentials were in the range of 82.3–87.0 µg/ml, 1314–1095 TEAC mmol/g and no significant decrease in the potentials during the storage period were detected.

Activity guided fractionation of the decoction of the *S. cumini* commercial sample was carried out by sequential extraction of organic solvents and hexane, ethyl acetate and water fraction and were tested for antiglycation, ABTS and DPPH antioxidant potentials. No DPPH activity was found in hexane fraction but ABTS and antiglycation potentials were 320 TEAC mmol/g, 119 µg/ml respectively. Ethyl acetate fraction showed the highest DPPH potential as 1.39 µg/ml and ABTS and antiglycation potentials were as 3151 TEAC mmol/g and 5.2 µg/ml respectively. The highest ABTS potential was reported in the water fraction (5739 TEAC mmol/g) while DPPH and antiglycation potentials were 6.76 and 3.6 µg/ml.

Compound isolation of the ethyl acetate and water fraction was carried out by Thin Layer Chromatographic method (TLC), High Performance Liquid Chromatographic method and UV- Visible spectrophotometric method.

The presence of gallic acid, ellagic acid and umbelliferone were confirmed by the TLC method with similar R_f values with standards and gallic acid and ellagic acid were further confirmed applying the co spotting technique.

The findings of the present investigation support in proving the antidiabetic properties of the above herbal plants on the basis of their efficacy in preventing the protein glycation and oxidative stress. This data prove the efficacy of using these plants in the treatment of diabetes mellitus for many years and might be useful in the herbal drug development industry.