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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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Thesis submitted to the University of Sri Jayewardenepura for the 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.

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Prof. K. K. D. S. Ranaweera

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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.

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Prof. K. K. D. S. Ranaweera

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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
$O_2^{\cdot-}$	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°)) 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.