

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

by

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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.



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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.

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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



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Table of Contents

LIST OF TABLES	iv
LIST OF FIGURES	vi
LIST OF PLATES	ix
LIST OF ABBREVIATIONS	x
ACKNOWLEDGEMENT	xii
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	5
LITERATURE REVIEW.....	5
2.1 Literature survey.....	5
2.1.1 Diabetes mellitus.....	5
2.1.2 History of diabetes mellitus	6
2.1.3 Complications of diabetes mellitus.....	6
2.1.4 Formation of Advanced Glycation End Products in diabetes mellitus.....	8
2.1.5 Chemistry of advanced Glycated End Products	10
2.1.6 Formation of Free radicals in diabetes mellitus.....	12
2.1.7 Chemistry of antioxidant compounds	13
2.1.8 Mechanisms of action of antiglycation compounds	15
2.1.9 Mechanisms of action of antioxidant compounds	15
2.2 Medicinal plants used in the treatment of diabetes mellitus	16
2.2.1 Selected medicinal plants used in the study.....	20
2.3 Plant derived antidiabetic compounds.....	31
CHAPTER 3	40
MATERIALS AND METHODS.....	40
3.1. Materials	40
3.1.1 Water.....	40
3.1.2 Chemicals.....	40
3.2 Methods	41

3.2.1 Selection of the plant materials for the study	41
3.2.2 Collection of plant materials.....	41
3.2.3 Identification of plant materials	43
3.3 Preparation of samples	44
3.3.1 Preparation of plant materials	44
3.3.2 Preparation of herbal decoctions.....	44
3.3.3 Antiglycation activity by Bovine Serum Albumin assay.....	44
3.3.4 Antioxidant potential by DPPH assay	45
3.3.5 Antioxidant activity by ABTS assay	46
3.3.6 Total phenolic content	48
3.4 Preliminary phytochemical analysis of <i>Syzygium cumini</i> water extract and ethanolic extract	49
3.4.1 Preparation of extracts for phytochemical screening.....	49
3.6 Preparation of ready to serve drink	54
3.6.1 Formulations of ready to serve herbal drink prepared with <i>S. cumini</i> decoction	56
3.6.2 Physical chemical and microbiological analysis of the ready to serve herbal drink.....	57
3.7 Statistical analysis	59
3.8 Extraction, Isolation and characterization of bioactive compounds from plant extracts.....	59
3.8.1 Activity guided fractionation	59
 CHAPTER 4	 63
RESULTS	63
4.1 Results of antioxidant activity	65
4.1.1. DPPH antioxidant activity	65
4.1.2. ABTS antioxidant activity	66
4.1.3 Calibration curve of Trolox for determination of ABTS antioxidant assay ...	67
4.2 Antiglycation activity of the plants	68
4.3 Total phenolic content of the plants	69
4.3.1 Calibration curve of Gallic acid for the determination of total phenolic content.....	70

4.4 Phytochemical analysis of <i>S.cumini</i> extracts.....	71
4.4.1 Total solids and moisture content of the <i>S. cumini</i> bark decoction	72
4.5 Results of ready to serve herbal drink	72
4.4.1 Results of sensory analysis of herbal drink	72
4.5.2 Physical characteristics of the herbal drink	73
4.5.3 Antiglycation activity of herbal drink.....	74
4.5.4 Antioxidant activity (DPPH assay).....	75
4.5.5 Antioxidant activity (ABTS assay).....	75
4.6 Results of activity guided fractionation of <i>S. cumini</i> decoction.....	76
4.6.1 Antioxidant potentials and antiglycation activity of the fractions of <i>S. cumini</i> decoction.....	77
4.6.2 Results of the compound isolation and identification.....	77
 CHAPTER 5	 90
DISCUSSION	90
 CHAPTER 6	 108
CONCLUSION.....	108
 REFERENCES.....	 111
 APPENDICES	 i
Appendix 1- List of Publications and Communications arising from the present work.i	
Appendix 11- Statistical calculations of antioxidant activity.....	iii

LIST OF TABLES

Table 2.1	Medicinal plants commonly used in the treatment of diabetes mellitus	17
Table 3.1	Treatment combination of the herbal drink	57
Table 4.1.1	<i>In vitro</i> DPPH antioxidant activity of plant materials	66
Table 4.1.2	<i>In vitro</i> ABTS antioxidant activity of plant materials	67
Table 4.2.1	<i>In vitro</i> Antiglycation activity of plant materials	69
Table 4.3	Total phenolic content of plant materials	70
Table 4.4	Preliminary phytochemical Analysis of <i>Syzygium cumini</i> water extract and ethanolic extract	72
Table 4.5.1.1	Average ranks of Sensory score for the developed formulas of the herbal drink	73
Table 4.5.1.2	Statistical outcome for the response of the respondents for sensory stimuli	74
Table 4.5.2	Physical characteristics of the herbal drink	74
Table 4.5.3	Antiglycation activity of herbal drink	75
Table 4.5.4	Antioxidant activity of herbal drink (DPPH assay)	76

Table 4.5.5	Antioxidant activity of herbal drink (ABTS assay)	76
Table 4.5.6.1	The total viable count of the herbal drink	77
Table 4.6.1	DPPH and ABTS activities and antiglycation activity of the fractions	78
Table 4.6.2	R _f values of standards and phenolic compounds in <i>S.</i> <i>cumini</i> ethyl acetate fraction	81
Table 4.6.3	R _f values of phenolic compounds in <i>S. cumini</i> decoction and RTS	85

LIST OF FIGURES

Figure 2.1	Simplified reaction pathway involved in the formation of Advanced Glycated End Products	09
Figure 2.2	Non Fluorescent/ Non cross linked Advanced Glycated End Products.	11
Figure 2.3	Fluorescent/ Cross linked Advanced Glycated End Products	12
Figure 2.4	Phytochemical constituents isolated from <i>S. cumini</i> (L.)	26
Figure 2.4.1	Basic flavonoids skeleton	32
Figure 2.4.2	Structure of Arbutin	32
Figure 2.4.3	Structure of Luteolin	33
Figure 2.4.4	Structure of Flavanones	34
Figure 2.4.5	Structure of Flavonols	35
Figure 2.4.6	Structure of Cyanidine	36
Figure 2.4.7	Structure of Genistin	37
Figure 2.4.8	Structures of Phenolic acids	37
Figure 2.4.9	Structure of Mitragynine	39
Figure 3.1	Locations of plants	43
Figure 3.2	Flow diagram of the preparation of ready to serve herbal drink using <i>S. cumini</i> bark decoction	56
Figure 4.1	TLC of ethyl acetate fraction of <i>S. cumini</i> under UV-Vis light (254nm)	79

Figure 4.2	TLC of ethyl acetate fraction of <i>S. cumini</i> under UV light (366 nm)	80
Figure 4.3	TLC of ethyl acetate fraction of <i>S. cumini</i> cospotting technique	83
Figure 4.4	TLC of Ethyl acetate fraction of <i>S. cumini</i> cospotting technique	83
Figure 4.5	Ethyl acetate fraction of <i>S. cumini</i> decoction and RTS	85
Figure 4.6	HPLC chromatogram of ready to serve herbal drink made of <i>S.cumini</i> decoction	86
Figure 4.7	Expanded HPLC Chromatogram of ready to serve herbal drink	87
Figure 4.8	Spectrums before and after spiking the sample with Gallic acid	88
Figure 4.9	Spectrums before and after spiking the sample with Ellagic acid	88
Figure 4.9.1	LC-UV spectrum of Ellagic acid obtained from RTS	89
Figure 4.9.2	LC-UV spectrum of Gallic acid obtained from RTS	90
Figure 5.0	Summary of the results of DPPH antioxidant potentials of the decoctions	92
Figure 5.1	Summary of the results of ABTS antioxidant potentials of the decoctions.	93
Figure 5.2	Summary of the results of antiglycation potentials of the decoctions	95
Figure 5.3	Summary of the results of total phenolic contents of the decoctions	98
Figure 5.4	Antiglycation and DPPH potentials of ready to serve herbal drink	103
Figure 5.5	ABTS potential of ready to serve herbal drink	104

Figure 5.6.1	Structure of Gallic acid	106
Figure 5.6.2	Structure of Umbelliferon	106
Figure 5.6.3	Structure of Ellagic acid	106

LIST OF PLATES

Plate 2.1	<i>Cassia auriculata</i>	20
Plate 2.2	<i>Osbeckia octandra</i>	22
Plate 2.3	<i>Syzygium cumini</i>	24
Plate 2.4	<i>Phyllanthus emblica</i>	27
Plate 2.5	<i>Scoparia dulcis</i>	29

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 administered 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 Ciocalteu 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.