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

by

Pathirage Rupika Damayanthi Perera

Ph.D 2014
Antiglycation and antioxidant activities of some selected medicinal plants and selective value addition to *Syzygium cumini* (Madan) decoction

by Pathirage Rupika Damayanthi Perera

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 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.
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 Syzygium cumini 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 S. cumini 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 *S. cumini* extracts ................................................................. 71
4.4.1 Total solids and moisture content of the *S. cumini* 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 *S. cumini* decoction ................................. 76
4.6.1 Antioxidant potentials and antiglycation activity of the fractions of *S. cumini*
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