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Comparison of Antiglycation and Antioxidant Potentials and Total Phenolic Contents of Decoctions from Antidiabetic Plants

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Abstract

Non enzymatic protein glycation and oxidative stress are the key molecular basis for the macro and micro vascular complications observed in chronic diabetics. Decoctions prepared with medicinal plants with antiglycation and antioxidant potentials therefore have therapeutic potential in preventing diabetic complications. Decoctions of five antidiabetic plants (parts) namely, *Cassia auriculata* flowers, *Osbeckia octandra* leaves, *Syzygium cumini* bark, *Phyllanthus emblica* fruits and *Scoparia dulcis* whole plant were analyzed for their DPPH and ABTS antioxidant potentials, antiglycation potentials and total phenolic contents. Decoctions of *S. cumini*, *O. octandra* and *P. emblica* had significantly high ($p < 0.05$) antiglycation potential ranges of 16.8–35.2, 23.0–28.5 and 37.4–82.3 µg/ml with correspondingly high antioxidant potentials and total phenolic contents of 851, 658 and 625 mg GAE/g respectively.

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Keywords: decoction; medicinal plants; antiglycation activity; antioxidant potential

1.Introduction

Diabetes mellitus [DM] has become one of the most challenging health problems in the 21st century with 9% of the world adult population suffering from this disease[1]. Long term diabetes leads to macro and micro vascular

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complications such as cardio-vascular complications, neuropathy, retinopathy, nephropathy and cerebro-vascular diseases. Glycation is the key molecular basis of these complications. Advanced Glycation End products (AGEs) developed in the late stage of the glycation process, due to hyperglycaemia accumulate on long lived proteins compromising their physiological functions leading to above complications[2]. The rate of the formation of free radicals is accelerated in DM and also during the formation of AGEs. Reactive oxygen species (ROS) in the presence of trace levels of catalytic redox-active transition metal ions also contribute to the formation of AGEs. Natural products of plant origin evaluated as inhibitors of glycation have been found to possess antioxidant potentials[3]. The plant parts of *Cassia auriculata* [flowers][*CA*], *Osbeckia octandra* [leaves][*OO*], *Syzygium cumini* [bark][*SC*], *Phyllanthus emblica* [fruits][*PE*] and *Scoparia dulcis* [whole plant][*SD*] that are being used in many herbal formulations in indigenous medicine for the treatment of DM were analyzed for their antiglycation and antioxidant potentials and total phenol content. The prevalence of high antioxidant and antiglycation potentials of herbal plants and high phenolic contents will prove the efficacy of using these in the treatment of DM.

Nomenclature

ABTS	2,2'–Azinobis (3–ethyl benzothiazoline-6-sulphonic acid) diammonium salt
AGEs	Advanced Glycation End products
BSA	Bovine Serum Albumin
CA	*Cassia auriculata*
DM	diabetes mellitus
DPPH	2, 2 diphenyl-2-picryl hydrazyl hydrate
OO	*Osbeckia octandra*
PE	*Phyllanthus emblica*
ROS	Reactive oxygen species
SC	*Syzygium cumini*
SD	*Scoparia dulcis*

2.Materials and Methods

2.1.Selection and collection of plant materials for the study

The commercially available dried samples of the selected plant materials were purchased from the traditional herbal market and three fresh samples of each plant were collected from three different areas of the country. Identities of the specimens of plant materials collected were authenticated by the Botanist at Bandaranaike Memorial Ayurvedha Research Institute, Nawinna, Sri Lanka.

2.2.Preparation of plant materials and decoctions

Fresh samples and commercially available dried samples were air dried for 24 hours at room temperature. Fresh samples were further dried in a dehydrator (Leader) at 55° C for 24 hrs, powdered (National, Japan) and packed in polyethylene bags and stored in air tight containers at -4 °C in a freezer until analysis. Water extracts powdered samples were prepared according to the traditional method practiced in Ayurvedha to prepare '*Kasaya*' (decoctions). The powdered sample having a similar weight of 12 '*kalan*' (60 g) was boiled under low heat with 960 ml of water (4 *patha*) to obtain a concentrate of 240 ml (1 *patha*)[4]. The above water extract was filtered through a thin silk cloth (500µm). The filtrate was freeze-dried (Feyela, FDU-1200) and samples were kept at -4°C in a cold room in air tight containers.

2.3.Antiglycation activity

Antiglycation activity was determined using the Bovine Serum Albumin (BSA) assay[5] and quantified for the relative amount of glycated BSA based on fluoresce intensity (Spectra Max Gemini EM). The excitation and

emission wavelengths used were 370 nm and 440 nm respectively. Each sample was analyzed in five different concentrations and each in triplicate. Percentage inhibition was calculated using the formula given below and the sample concentration required for 50% inhibition was calculated using Minitab 14.

% inhibition = OD [blank] – (OD[sample] – OD [sample negative]) / OD [blank] X100

OD – Optical density

2.4.Antioxidant potential by DPPH assay

Assay was carried out with DPPH (2, 2 diphenyl-2-picryl hydrazyl hydrate) (Sigma, USA) using a spectrophotometric method[6]. The percentage of DPPH radical scavenging activity was determined in five concentrations using the equation mentioned below. Butyl hydroxy toluene was used as the reference standard. The sample concentration which gives 50% scavenging activity was estimated as IC_{50} value.

% scavenging activity = A0 – As/A0 X 100

As – Absorbance of the DPPH solution of the control sample

A0 – Absorbance of the DPPH solution in the presence of plant extract

2.5.Antioxidant activity by ABTS assay

2,2'–Azinobis (3–ethyl benzothiazoline-6-sulphonic acid) diammonium (ABTS) salt (Sigma-Aldrich, USA) radical cation decolorization assay was used to measure the antioxidant activity[7]. Trolox (6–Hydroxy-2,5,7,8–tetramethylchroman-2-carboxylic acid) was used as the standard and data are reported as mean ± SD of the three replicates as Trolox equivalents using the equation obtained from the calibration curve of Trolox.

2.6.Total phenolic content

The total phenolic content of each extract was determined spectrophotometrically using the Folin-Ciocalteu reagent[8]. Total phenolic content was expressed as mg Gallic acid equivalent /g using the equation obtained from the calibration curve for Gallic acid. Data are expressed as mean± SD of three replicates.

3.Results and discussion

Figure 1 indicates the DPPH antioxidant potential which is inversely proportional to the sample concentration. Among the decoctions the highest DPPH potentials were shown by dry and fresh samples of *P. emblica*, (27.1- 49.5 µg/ml) followed by *S. cumini* (30.3- 68.7 µg/ml) and *O. octandra* (55.7- 98.4 µg/ml). The samples of *C. auriculata* (237-309 µg/ml) and *S. dulcis* (437–540 µg/ml) contain significantly ($p < 0.05$) lower DPPH antioxidant potentials compared to the other samples. The commercial samples of *PE* and *SC* collected from the traditional herbal market had the highest DPPH antioxidant potential. Dried plant material of *O. octandra* was not commercially available as it is used as a fresh ingredient in herbal formulations.

Among the decoctions, *PE* dried sample also had the highest ABTS antioxidant potential (2764 TEAC mmol/g) followed by *SC, OO, CA* and *SD* with the lowest (615 TEAC mmol/g) activity (Figure 2). ABTS potential of all four of *SC* bark decoctions were significantly high ($p < 0.05$) compared to the other plants except *PE* and in the range of 1544-1897 TEAC mmol/g. Strong potential for inhibiting ROS production and free radical scavenging activity of water extract of *P. emblica* fruit prepared according to the Thai Herbal Pharmacopeia is reported[9] and are attributed to hydrolysable tannins such as emblicanin A, emblicanin B and flavonoid rutin[10]. Herbal drugs 'Cogent db' and 'D-400' that contain *SC* bark as the major ingredient are popular traditional medicines for diabetes[11]. Compared to CA and *SD*, *OO* decoction expressed significantly high radical scavenging activity. When comparing the antioxidant potentials of decoctions as per both methods the *PE* had the highest antioxidant potential followed by *SC*. *SD* had the lowest potential with *CA* and *OO* having potentials in increasing order.

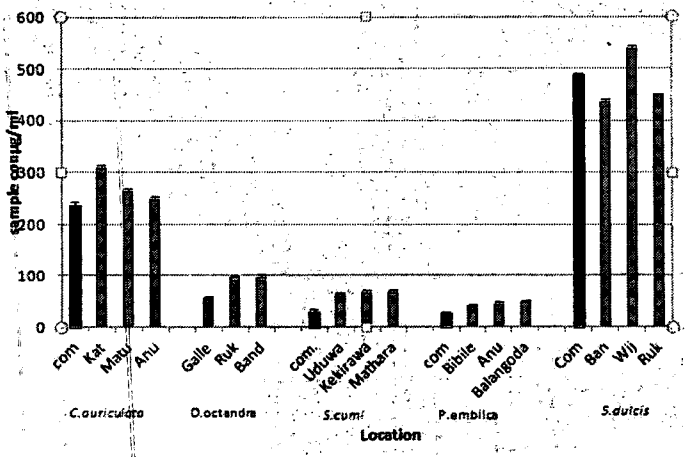

Figure 1 DPPH antioxidant potentials (mean ± SD) of plant decoctions (black – commercial [com]; red – fresh collected from different areas)

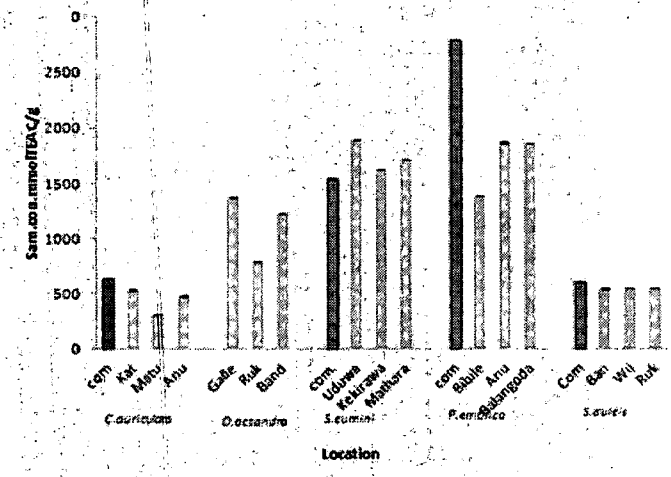

Figure 2 ABTS antioxidant potentials (mean ± SD) of plant decoctions (black – commercial [com]; blue – fresh collected from different areas)

The DPPH and ABTS antioxidant potentials of the samples of *CA* commercial and laboratory dried samples were significantly different ($p < 0.05$) and could be due to the different conditions used in the drying process. In addition the variations in the decoctions prepared from the same plant material collected from different areas may be due to geographical variations.

The antiglycation potential (Figure 3) is inversely proportional to the sample concentration and the highest potentials were found in *SC* decoctions followed by *PE, OO, CA* and *SD* respectively. Decoction prepared with commercial sample of *SC* bark had the highest antiglycation activity (16.8 µg/ml) followed by laboratory dried

samples (31.7 – 35.18 μg/ml) when compared to other medicinal plants used for the study. All parts of *S. cumini* had been used widely in India as an alternative medicine for DM and as a front line antidiabetic medicine in Europe[12]. The results of the present study scientifically proved its efficacy as an antidiabetic herbal treatment.

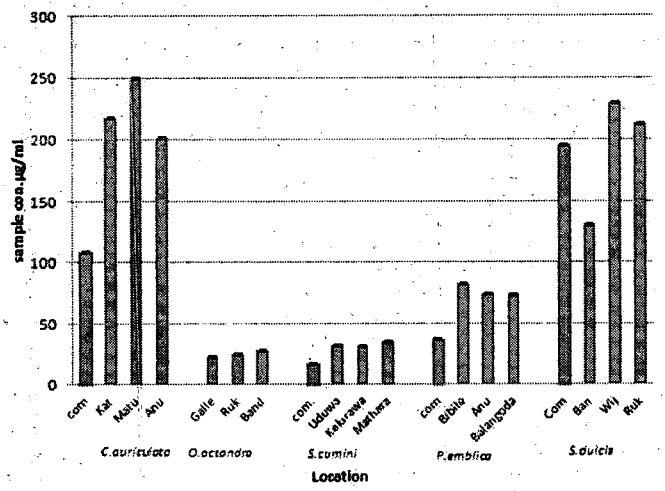

Figure 3 Antiglycation potentials (mean ± SD) of plant decoctions (red – commercial [com]; blue – fresh collected from different areas)

The decoctions of *OO* leaves collected from different areas also showed significantly high antiglycation potential than the positive control arbutin (65.0μg/ml). The results prove that the antidiabetic activity of *SC* and *OO* are based on the inhibitory effect of protein glycation and retardation of oxidative stress. Decoction of the commercial *PE* fruit was significantly high (37.47μg/ml) than the laboratory dried samples (74.1–82.3 μg/ml) as was with *CA* commercial sample (110 μg/ml) and laboratory dried samples (202–250 μg/ml). Antiglycation potential of *SD* was in the range of 13 –213μg/ml which was the lowest among the decoctions studied.

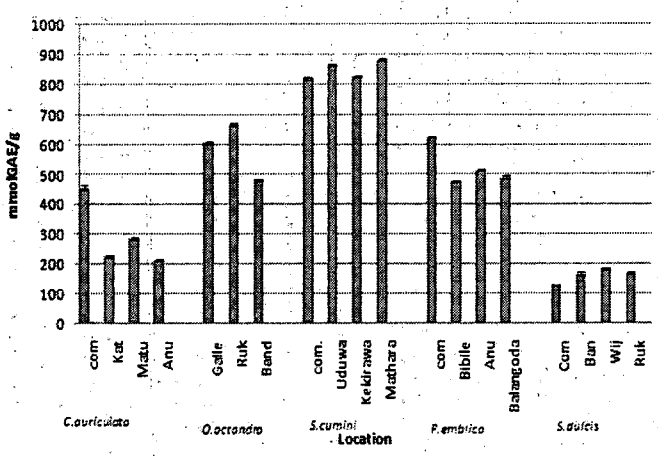

Figure 4 Total phenolic content (mean ± SD) of plant decoctions (red – commercial [com]; blue– fresh collected from different areas)

All *SC* samples contained significantly high total phenolic content (819 -883 mg GAE/g) followed by *OO*, *PE*, *CA* and *SD*. Total phenolic content of the decoctions of *PE* and *CA* were also significantly high in the commercial sample (625 mg GAE /g and 459 mg/GAE/g). Similar findings were reported for the water extracts of *PE* fruit prepared according to the Thai Pharmacopeia[9]. According to literature many phenolic compounds, including flavones, flavanones, and flavonols have strong antiglycation activity[13].

4.Conclusions

The *Syzygium cumini* decoction had the highest phenol content, highest antioxidant and antiglycation potential among the plants studied proving the scientific rationale in using the de