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**P.R.D Perera**

Department of Food Science and  
Technology, Faculty of Applied  
Sciences, University of Sri  
Jayewardenepura

**S. Ekanayake**

Department of Biochemistry, Faculty  
of Medical Sciences, University of Sri  
Jayewardenepura

**K.K.D.S. Ranaweera**

Department of Food Science and  
Technology, Faculty of Applied  
Sciences, University of Sri  
Jayewardenepura

**Correspondence:****P.R.D Perera**

Department of Food Science and  
Technology, Faculty of Applied  
Sciences, University of Sri  
Jayewardenepura

Email: [rupika.perera@yahoo.com](mailto:rupika.perera@yahoo.com)

## *In vitro* study on antiglycation activity, antioxidant activity and phenolic content of *Osbeckia octandra* L. leaf decoction

P.R.D Perera, S. Ekanayake, K.K.D.S. Ranaweera

**ABSTRACT**

Non enzymatic glycation is the major cause of spontaneous damage to proteins leading to various complications due to formation of non-reversible Advanced Glycation End Products (AGEs) and oxidative stress. Medicinal plants having both antiglycation and antioxidant activities may have good therapeutic potential in the treatment of diabetic mellitus. The present study was undertaken to investigate the antiglycation effect, antioxidant activity and phenolic content of *Osbeckia octandra* leaf decoction used in the treatment of diabetes mellitus in Ayurvedic medicine. The water extracts of three samples of *Osbeckia octandra* leaves from three different areas had significant anti-glycation activity (23.0 µg/mL, 25.2 µg/mL, 28.5 µg/mL), ABTS antioxidant activity (1375, 794, 1231 TEAC mmol/g) and DPPH activity (55.5 µg/mL, 97.7 µg/mL, and 98.4 µg/mL). Total phenolic content ranged from 483-667 mg/GAE/g.

**Keywords:** Antiglycation activity, antioxidant activity, ABTS antioxidant activity, DPPH activity, Phenolic content.

**1. Introduction**

Glycation is the key molecular basis of several diabetic complications like diabetes retinopathy, nephropathy, neuropathy and some cardiovascular diseases. This is a non enzymatic reaction between amino groups of proteins and carbonyl groups of reducing sugars forming fluorescent, insoluble Advanced Glycation End Products that accumulate on long lived proteins thus compromising the physiological functions<sup>[1]</sup>. The reaction is sub divided into three stages as early, intermediate and late. In early stage unstable Schiff base forms and through acid base catalysis this compound undergoes further rearrangement to a more stable Amadori product<sup>[2]</sup> and via dehydration, oxidation and other chemical reactions degrades to more reactive carbonyl compounds and they act as propagators of the reaction again reacting with more free amino groups of biomolecules. In the late stage Advanced Glycation End Products (AGEs)<sup>[3]</sup> are formed. The formation of AGEs progressively increases with normal aging and is accelerated in diabetes<sup>[4]</sup>. Various studies have shown that diabetes mellitus is associated with an increased production of free radicals leading to oxidative stress. Thus disturbed balance between radical formation and radical neutralization leads to oxidative damage of cell components such as proteins, lipids and nucleic acids<sup>[5]</sup>. Oxidation plays an important role in the formation of Advanced Glycation End Products and the Plants derived agents with the antiglycation and antioxidant activities are highly important in preventing diabetic complications. *Osbeckia octandra* is a plant used in Ayurvedic medicine for the treatment of diabetes mellitus. Several studies have proven its hepatoprotective activity and further the plant is reported to possess antidiabetic activity<sup>[6]</sup>. The water extract of *Osbeckia octandra* significantly lowered the fasting glucose level and markedly improved glucose tolerance in Sprague-Dawley rats and the maximum hypoglycemic activity was observed after 3 hours<sup>[7]</sup>. Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy, safety, minimal adverse effects and low cost.

## 2. Materials and Methods

### 2.1 Collection of samples

Three samples of *Osbeckia octandra* plant were collected from three different areas where they are grown in Sri Lanka (Southern, Western and Uva provinces). Identification of samples were carried out by the Botanist in Bandaranaike Memorial Ayurvedic Research Institute at Nawinna.

### 2.2 Preparation of plant materials

Leaves of plants were washed using distilled water and air dried for 24 hours at room temperature and dried in a dehydrator (Leader) at 55 °C for 24 hrs and powdered using a domestic grinder to obtain fine particles. Powdered samples were packed in polyethylene bags and stored in air tight containers at -4 °C in a cold room until used for analyses.

### 2.3 Preparation of water extracts

Water extracts of dried and milled powdered samples were prepared according to the traditional method practiced in Ayurvedic Medicine to prepare 'Kwatha' with 60 gm of the powdered sample having a similar weight of 12 'kalan' simmer boiled with 960 ml of (4 patha) to obtain the decoction of 240 ml (1 patha) as the final volume. This water extract was filtered through a fine silk cloth. The filtrate was freeze dried to get a dry powder. Samples were kept at -4 °C in a cold room in air tight containers.

### 2.4 Determination of free radical scavenging activity using ABTS assay

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt (Sigma) radical cation decolorization assay was used to measure the antioxidant activity<sup>[8]</sup> with slight modifications. The stock solution of 7 mM ABTS solution and 2.4 mM Potassium per sulphate solution were prepared and the working solution was prepared mixing equal parts from each and allowing the mixture to stand in the dark at room temperature for 12 hrs before use. The solution was then diluted mixing 1.0 ml of ABTS<sup>+</sup> solution with 16.0 ml phosphate buffer to obtain initial absorbance as 0.700±0.02. Additional dilution was needed if the ABTS<sup>+</sup> value measured was over the linear range of the standard. ABTS<sup>+</sup> solution was freshly prepared for each assay. Freeze dried water extract of each sample was prepared in three concentrations dissolving in phosphate buffer solution and the reaction mixture was prepared mixing 100 µl of plant extract with 3.0 ml ABTS<sup>+</sup> solution and analysis carried out in triplicate. ABTS<sup>+</sup> radical scavenging activity was determined after measuring the fall of absorbance exactly after 15 min. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and the blank sample was prepared adding 100 µl phosphate buffer to 3.0 ml ABTS<sup>+</sup> solution. Data are reported as µM Trolox equivalents (TE)/100 µg.

### 2.5 Determination of free radical scavenging activity by DPPH assay

DPPH (2,2 diphenyl-2-picryl hydrazyl hydrate) (Sigma) scavenging activity was determined using a spectrophotometric method<sup>[9]</sup>. Freshly prepared DPPH solution was used for each experiment. Reaction mixture was prepared using 2.5 ml of 6.5x10<sup>-5</sup> M DPPH solution and 0.5 ml of sample dissolved in methanol and control sample with 2.5 ml of 6.5 x 10<sup>-5</sup> M DPPH solution and 0.5 ml of methanol. Samples were tested in five concentrations and

each sample was assayed in triplicate. All samples were incubated at room temperature for 30 minutes in dark and then absorbance was measured at 540 nm using UV-Vis spectrophotometer (SHIMADZU UV mini 1240). The percentage of DPPH radical scavenging activity was determined in five concentrations using the equation mentioned below. BHT (Butyl Hydroxy Toluene) was used as the reference standard.

$$\% \text{ scavenging activity} = A_0 - A_s/A_0 \times 100$$

Where,

A<sub>s</sub> - Absorbance of the DPPH solution of the control sample

A<sub>0</sub> - Absorbance of the DPPH solution in the presence of plant extract.

The sample concentration which gives 50% scavenging activity was estimated as IC<sub>50</sub> value from regression analysis using Minitab 14.

### 2.6 Determination of total phenolic content

The total phenolic content of each extract was determined using Folin-Ciocalteu reagent<sup>[10]</sup>. Freeze dried sample of each plant material was used for the extraction and extracted using 70% methanol. The reaction mixture was prepared using 0.5 ml of extracted sample with 2.5 ml of Folin-Ciocalteu reagent which was diluted 10 times using methanol. 2.0 ml of 7.5% sodium carbonate (w/v) solution was added after 3 minutes to the above mixture and kept at 45 °C for 10 minutes in an incubator (Microsil, India). Then absorbance of each plant extract and prepared blank adding 0.5 ml of methanol instead of the plant extract was measured at 765 nm using the UV-Vis spectrophotometer (SHIMADZU UV mini 1240). 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.

### 2.7 Determination of antiglycation activity

Antiglycation activity was determined using the Bovine Serum Albumin assay<sup>[11]</sup> with slight modification. In all experiments, the final reaction volume was 1.0 ml and carried out in 1.5 ml Eppendorf tubes. Bovine Serum Albumin (Sigma-Aldrich) 500 µl (1 mg/ml concentration) was incubated with glucose 400 µl (500 mM final concentration) and 100 µl sample, 100 µl phosphate buffer saline was used as the sample control and 100 µl Arbutin (Sigma-Aldrich) as the reference standard. A negative control was carried out at the same time with BSA 500 µl (1 mg/ml concentration), 400 µl phosphate buffer saline and 100 µl sample incubated under same conditions. The reaction was allowed to proceed at 60 °C for 24 hours and terminated by adding 10 µl of 100% (W/V) trichloroacetic acid (TCA) (Sigma-Aldrich). The TCA added mixture was kept at 4 °C for 10 minutes and centrifuged 4 minutes at 13000 rpm. The precipitate was redissolved with alkaline phosphate buffer saline (pH 10) and was quantified for the relative amount of glycated BSA based on fluorescence intensity by Fluorescent Microplate Reader (Spectra Max Gemini EM). The excitation and emission wavelength used were at 370 nm and 440 nm respectively. Each sample was analyzed in five concentrations and in triplicate. Percentage of inhibition was calculated using the formula given below and the sample concentration required for the 50% of inhibition was calculated using Minitab 14.

$$\% \text{ of inhibition} = \frac{\text{OD blank} - (\text{OD sample} - \text{OD sample negative})}{\text{OD blank}} \times 100$$

### 3. Statistical Analysis

All experiments were carried out in three replicates and presented as mean  $\pm$  standard error (SE) using Minitab 14. One way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test for any significant difference between the means. The level of statistical significance was set at  $P < 0.05$ .

### 4. Results and Discussion

**Table 1:** DPPH antioxidant activity of *Osbeckia octandra* leaf decoction

Location of sample	DPPH antioxidant activity( $\mu\text{g/mL}$ )
Galle (Southern Province)	55.5 $\pm$ 2.4 <sup>b</sup>
Rukmale (Western Province)	97.7 $\pm$ 3.1 <sup>a</sup>
Bandarawela (Uva Province)	98.4 $\pm$ 4.0 <sup>a</sup>
BHT	19.12 $\pm$ 2.2

Different letters in a column indicate significant differences at 95% confidence interval

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable molecule. The capability of the natural antioxidants to reduce the DPPH free radical is measured by the decrease in absorbance at 517 nm and butylated hydroxyl toluene is used as the positive control. The sample concentration required for half maximal inhibitory concentration ( $\text{IC}_{50}$ ) was compared in the study and the value is inversely proportional to the activity. Substances capable of donating electrons or hydrogen atoms are able to convert DPPH into their non-radical form 1,1-diphenyl-2-picrylhydrazine. The scavenging activity of extracts are shown in Table 1. The highest activity was shown by *Osbeckia octandra* sample collected from Galle (55.5  $\mu\text{g/mL}$ ) followed by samples collected from Rukmale (97.7  $\mu\text{g/mL}$ ) and Bandarawela (98.4  $\mu\text{g/mL}$ ). The phenolic compounds present in the extracts could be responsible for the observed DPPH radical scavenging activity, since they can readily donate hydrogen atoms to the radical<sup>[12]</sup>. According to the results of the study the DPPH activity is higher in the sample collected from Southern province. Variation in the content of plant secondary metabolites is the results of many factors. There may be a genetic component to such variations. Bowers & Stamp<sup>[13]</sup>, and Niknam<sup>[14]</sup> illustrates that the content of phenolics and important group of secondary metabolites, varies among the maturity levels, the plant parts and geographical location of the species.

**Table 2:** ABTS antioxidant activity of *Osbeckia octandra* leaf decoction.

Location of sample	ABTS antioxidant activity(TEAC mmol/g)
Galle (Southern Province)	1375 $\pm$ 6.6 <sup>a</sup>
Rukmale (Western Province)	794 $\pm$ 5.6 <sup>b</sup>
Bandarawela (Uva Province)	1231 $\pm$ 4.2 <sup>a</sup>

Different letters in a column indicate significant differences at 95% confidence interval

Table 2 shows the ABTS radical cation scavenging activity of the extracts. The antioxidant activity of the samples were calculated in terms of Trolox equivalent antioxidant capacity (TEAC). The highest activity was shown by the Galle sample (1375 $\pm$ 6.6 TEAC mmol/g) followed by the samples collected from Bandarawela (1231 $\pm$ 4.2 TEAC mmol/g) and Rukmale (794 $\pm$ 5.6 TEAC mmol/g). Addition of antioxidants to the pre formed radical cation reduces it

to ABTS, to an extent and on a time scale depending on the antioxidant activity, the concentration of the antioxidant and the duration of the reaction. Thus the extent of decolorization as percentage inhibition of the ABTS radical cation is determined as a function of concentration and time and calculated relative to the reactivity of Trolox as a standard under the same conditions<sup>[15]</sup>.

**Table 3:** Antiglycation activity of *Osbeckia octandra* leaf decoction

Location of sample	Antiglycation activity( $\mu\text{g/ml}$ )
Galle (Southern Province)	23.0 $\pm$ 1.2 <sup>b</sup>
Rukmale (Western Province)	25.2 $\pm$ 2.2 <sup>b</sup>
Bandarawela (Uva Province)	28.5 $\pm$ 1.3 <sup>a</sup>
Arbutin	65.2 $\pm$ 2.3

Different letters in a column indicate significant differences at 95% confidence interval

Antiglycation activity is also high in the sample collected from the Southern province (23.0 $\pm$ 1.2  $\mu\text{g/ml}$ ) followed by the samples from Western province (25.2 $\pm$ 2.2  $\mu\text{g/ml}$ ) and Uva province (28.5 $\pm$ 1.3  $\mu\text{g/ml}$ ). All samples showed higher activity than the positive control Arbutin. Recent studies have highlighted the benefits of using medicinal plants with combined antiglycation and antioxidant properties in diabetic patients<sup>[16]</sup>. Both synthetic and natural products have been evaluated as inhibitors against the formation of Advanced Glycation End Products. Many plant derived products have been shown to possess hypoglycemic, hypolipidemic as well as antioxidant properties<sup>[17]</sup>. Some important compounds such as phenolics, oligosaccharides and polysaccharides, carotenoids, unsaturated fatty acids and many others have been reported to possess antiglycation activity<sup>[18, 19]</sup>.

**Table 4:** Total phenolic content of *Osbeckia octandra* leaf decoction

Location of sample	Total phenolic content mg/GAE/g
Galle (Southern Province)	608 $\pm$ 4.8 <sup>a</sup>
Rukmale (Western Province)	666 $\pm$ 5.1 <sup>a</sup>
Bandarawela (Uva Province)	483 $\pm$ 3.2 <sup>b</sup>

Different letters in a column indicate significant differences at 95% confidence interval

The highest total phenolic content was found in sample collected from Western province followed by samples from Southern Province and Uva province. Phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions<sup>[20]</sup>. Several studies have demonstrated that the anti-glycation activity correlates significantly with the phenolic content of the tested plant extracts<sup>[21]</sup>. Polyphenols are the most abundant dietary antioxidants. The differences in phenolics might be due to the location or the maturity level of the plants.

### 5. Conclusion

The results of the present study have confirmed the long history of the use of aqueous leaf extract of *Osbeckia octandra* in traditional medicine for diabetes mellitus and further studies on the compounds with high antiglycation and antioxidant activities will be highly important in drug development industry.

### 6. Acknowledgement

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