Antiglycation Activity and Radical Scavenging Activity of Leaves of Artocarpus heterophyllus

J A H Erangika¹, S A Deraniyagala²*, C D Fernando³
¹(College of Chemical Sciences, Institute of Chemistry, Ceylon )
²(Department of Chemistry, University of Colombo, Sri Lanka)
³(Department of Molecular and Translational Science, Monash University, Australia)
Received 16 October 2019; Accepted 31 October 2019

Abstract: There is much interest in plant based medicine with antioxidant and anti-glycation properties. Formation of advanced glycation end products (AGEs) is a major cause of serious chronic diabetic complications. The objective of this study was to analyze the radical scavenging activity of different solvent extracts and the protein glycation inhibitory potential on fructose mediated non enzymatic glycation by the aqueous leaf extract of Artocarpus heterophyllus (ALEAH).

The ALEAH (ripe and raw) was prepared separately according to the preparation of a “Kasaya” in Ayurvedic medicine. The freeze dried powder was used for the investigations. The leaves were soaked in methanol or ethanol and shaken, filtered and evaporated in order to obtain the respective extracts.

Among the extracts the highest total phenol and flavonoid contents and the radical scavenging potential was shown in ALEAH (ripe and raw). Total phenolic content of ALEAH was 53 (ripe) and 38 (raw) mg GAE g⁻¹. Total flavonoid content of ALEAH was 32 (ripe) and 23 (raw) mg QE g⁻¹. (QE = Quercetin equivalents, GAE = Gallic acid equivalents.) DPPH radical scavenging activity at 5 - 200 µg ml⁻¹ of ALEAH (ripe) was 7-66% respectively while the ALEAH (raw) at 5-200 µg ml⁻¹ inhibited 4%, -54% respectively.

The ALEAH (ripe) at 75, 100, 125 µg ml⁻¹ inhibited AGE formation by 52%, 63%, 72% respectively and ALEAH (raw) at same concentrations inhibited 36%, 57%, and 63% respectively after two weeks of incubation in the BSA-Fructose assay.

It is noteworthy that ripe (fallen leaves) contained higher activity than the raw leaves and hence the aqueous extract may be used to combat diabetic complications as well ailments in which radicals are implicated.

Key words- Antiglycation activity, Artocarpus heterophyllus, radical scavenging activity

I. INTRODUCTION

Chronic hyperglycemia plays a vital role in the development of long-term diabetic complications by inducing protein glycation and the gradual formation of advanced glycation end products (AGEs) in various body tissues[1].

Non enzymatic reaction of sugars and free amino groups in amino acids or proteins form Schiff base structures. This process is called Maillard reaction or Advanced Glycation[2]–[6]. This Schiff base is unstable, hence it rearranges to form Amadori products via chemical transformations. These Amadori products undergo dehydration and rearrangement followed by reactions such as cyclisation, oxidation, and dehydration and cross linking to form Advanced Glycation End products (AGEs)[6],[7]. As in the case of many diseases, free radicals have also been implicated in the pathway of AGE formation[4],[8].

Accumulations of glycation end products are concomitant with various diseases. Chronic hyperglycemia resulting in AGEs facilitate diabetes related complications such as diabetic retinopathy, nephropathy, cataract, atherosclerosis etc[1], [3], [4], [9]. Aminoguanidine is the first inhibitor of AGEs discovered in 1986[10], [11]. However it may have serious toxicity when administered for diabetic nephropathy[4].

There is considerable interest in search of plant based drugs with anti-glycation activity as they may potentially inhibit AGE formation resulting in delaying and preventing the onset of diabetic complications with minimal side effects [1],[5]–[8],[11].

Artocarpus heterophyllus, also known as jack, belongs to the fig, mulberry, and breadfruit family (Moraceae). The fruit of the jack tree is the largest tree-borne fruit. Artocarpus heterophyllus grows as an evergreen tree. The height of the tree could reach 10 to 20 meters and trunk diameters are about 30 to 80 centimeters. It has a relatively short trunk with a dense treetop. The bark of the jackfruit tree is reddish-brown and smooth and in the event of injury to the bark, a milky juice is released. The ripe and unripe fleshy seeds of Jackfruit are commonly used in South and Southeast Asian cuisines. It is the national fruit of Sri Lanka[12].
Antiglycation Activity and Radical Scavenging Activity of Leaves of Artocarpusheterophyllus

The jack fruit is a rich source of potassium to lower the blood pressure while the extracts of root and bark is used in diarrhea and dysentery[13]. The heated jack leaves which possess sedative property are effective in wound healing and Jack fruit leaves burned with coconut shells is used to heal ulcers [13]. The leaves of jack tree are used in various ailments related with diabetes mellitus in Ayurvedic and traditional medicine. The infusion of mature leaves are reported to be used to treat diabetes, gall stones and relieve asthma[14].

The leaves of the Jack fruit are generally not consumed. The jack tree daily sheds a significant amount of ripe leaves and these are swept away as thrash.

The objective of this study was to analyze radical scavenging activity of different solvent extracts of the ripe and raw leaves of Artocarpusheterophyllus(AH) and to study the protein glycation inhibitory potential on fructose mediated non-enzymatic glycation of aqueous extracts of the leaves of Artocarpusheterophyllus (ALEAH).

II. MATERIALS AND METHODODLOGY

2.1 Sample preparation

Ripe and raw leaves of plant Artocarpusheterophyllus were collected from the southern province of Sri Lanka(6° 08’ 38.5” N 80° 11’ 37.4” E). The leaves were washed with running water and air dried for 24 hours at room temperature and powdered using a domestic grinder. Powdered samples were packed in air tight polythene bags and stored at 20°C[1].

2.2 Preparation of aqueous extracts

Aqueous extracts of ripe and raw leaves were prepared according to the method of “Kasaya” in Ayurvedic Medicine. 60 g (12 kalan) of the powdered sample was boiled with 960 ml (4 patha) of water to obtain final volume of 240 ml (1 patha) of extract and filtered through a fine cheese cloth. The filtrate was freeze dried to obtain a dry powder. This procedure was followed separately for two samples of ripe and raw leaves. Freeze dried samples were kept at -20°C in a tight container.[1],[5].

2.3 Preparation of solvent extracts

The stored leaves were weighed (100 g) and soaked in distilled solvent (250 ml) and kept in shaker for 48 hours using 150 rpm at room temperature. The mixture was filtered and the solvent was evaporated under reduced pressure and at 35°C using a rotary evaporator. This procedure was repeated separately for two samples of ripe and raw leaves using methanol and ethyl acetate as the solvents[15].

2.4 Total Phenolic Content (TPC)

The total phenolic content was determined using Folin-ciocalteu reagent. In brief, 0.5 ml of extract solution of particular concentration was added to 0.5 ml of Folin-ciocalteu reagent. (FC: Water volume ratio = 1:1) This mixture was incubated at room temperature for 5 minutes. To this solution 0.5 ml of 6% Na2CO3 was added and followed by addition of 2.0 ml of deionized water. The mixture was kept in dark for 60 minutes. The absorbance was measured at 765 nm using UV spectrophotometer. Similar procedure was carried out for the standard Gallic acid of varying concentrations. The total phenolic content in both samples in each of the extracts were expressed as mg GAE / g of dry weight of extract by using the standard curve prepared for Gallic acid[2],[16]. (GAE= Gallic acid equivalents)

2.5 Total Flavonoid Content (TFC)

Total flavonoid content was determined using Aluminum chloride assay. Quercetin was used as the standard of the assay. The plant extract (0.5 mL) was mixed with 2.0 mL of distilled water and 150 µL of 5% NaNO₂ solution and incubated in the dark for 5 minutes. To this solution 150 µL of 10 % AlCl₃ was added and the reaction mixture was incubated for 6 minutes. An aliquot of 1.0 mL of 1 M NaOH solution and 1.0 mL of distilled water were added to the reaction mixture and the absorbance was measured at 510 nm. The total flavonoid content was determined from the standard.

Curve constructed for Quercetin and total flavonoid content was expressed as mg QE/g of dry weight of extract[2]. (QE = Quercetin equivalents)

2.6 Percentage DPPH radical scavenging activity

The radical scavenging activity (RSA%) was examined using previously published method [5]. An aliquot of 2 mL of DPPH reagent prepared in methanol was added to 1.5 ml of the plant extract at varying concentrations. The reaction mixture was shaken well and incubated in dark for 10 min. The control sample was prepared by replacing the plant extract with methanol. The absorbance of the test solution was measured at 517 nm. The similar procedure was repeated for the standard Butylated hydroxyl toluene (BHT) using same
Antiglycation Activity and Radical Scavenging Activity of Leaves of *Artocarpusheterophyllus*

concentration series. The percentage DPPH radical scavenging activity was calculated using the equation (1) given below.

**Equation 1**

\[
\text{RSA}\% = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample}) \times 100}{\text{Absorbance of the control}}
\]

2.7 **Antiglycation activity**

Anti glycation activity of aqueous extracts were determined by the glycation induced protein cross-linking inhibitory method [1]. The solutions Bovine Serum Albumine (5ml), Fructose (4ml) and aqueous extract of each concentration (1ml) were added to a vessel and diluted up to a total volume of 20 ml with the phosphate buffered saline (0.2M, pH 7.4). The control was a mixture of BSA and Fructose which induces the formation of AGE. Corresponding control blank was prepared in the absence of fructose and the extract. A vessel containing only the sample at relevant concentration was used as the sample blank. Fluorescence emission of each mixture was measured after a week. Emission values were obtained for a period of two successive weeks. The formation of fluorescent AGE s was measured by using a fluorescence spectrophotometer at an excitation wave length 355 nm and emission wave length 450 nm. The reference standard was Aminoguanidine (AG ) [1], [2]. The percentage inhibition of protein glycation (%I) was calculated according to the equation (2) given below.

**Equation 2**

\[
\% I = \frac{(\text{Emission of control} - \text{control blank}) - (\text{Emission of sample} - \text{sample blank}) \times 100}{\text{Emission of control} - \text{control blank}}
\]

III. **RESULTS AND DISCUSSION**

3.1 **Total Phenolic Content (TPC)**

Phenols are major plant constituents which are important because of their scavenging ability due to presence of their hydroxyl groups.

The water, methanol and ethyl acetate extracts of each sample were analyzed for the phenolic content using Folin Ceocalteu reagent with Gallic acid as the standard. Figure 1 is the standard curve for Gallic acid and Table 1 gives the calculated ( as mg GAE/g) total phenol content for each extracts of raw and dry leaves of *Artocarpusheterophyllus*. According to Table 1 highest phenolic content was observed in the aqueous extract of ripe leaves of *Artocarpusheterophyllus* [ALEAH (ripe)].

![Figure 1 Standard curve of gallic acid for phenolic determination](image)

\[
y = 7.3619x + 0.1087
\]

\[
R^2 = 0.9858
\]
Antiglycation Activity and Radical Scavenging Activity of Leaves of *Artocarpus heterophyllus*

Table 1 Average TPC of each leaves extracts

<table>
<thead>
<tr>
<th>Leaves extract</th>
<th>Average TPC (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of ripe leaves</td>
<td>53.0 ± 0.2</td>
</tr>
<tr>
<td>Water extract of raw leaves</td>
<td>38.0 ± 0.4</td>
</tr>
<tr>
<td>Methanol extract of ripe leaves</td>
<td>19.2 ± 0.3</td>
</tr>
<tr>
<td>Methanol extract of raw leaves</td>
<td>15.5 ± 0.2</td>
</tr>
<tr>
<td>Ethyl Acetate extract of ripe leaves</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Ethyl Acetate extract of raw leaves</td>
<td>0.2 ± 0.0</td>
</tr>
</tbody>
</table>

3.2 Total Flavonoid Content (TFC)

Total flavonoid content of plant extracts was analyzed by Aluminium Chloride colorimetric method using Quercetin as the standard. Figure 2 gives the standard curve for Quercetin and Table 2 gives the TFC calculated (as mg QE / g) for the different extracts of the leaves of *Artocarpus heterophyllus*. The highest flavonoid content was also found in the aqueous extract of the ripe leaves ALEAH (ripe).

![Absorbance vs Concentration](image)

**Figure 2 Standard curve of Quercetin for the determination of flavonoids**

Table 2 Total Flavonoid Content for leaf extracts

<table>
<thead>
<tr>
<th>Leaves extract</th>
<th>Average TFC (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of ripe leaves</td>
<td>32.1 ± 0.6</td>
</tr>
<tr>
<td>Water extract of raw leaves</td>
<td>22.8 ± 1.3</td>
</tr>
<tr>
<td>Methanol extract of ripe leaves</td>
<td>13.4 ± 0.4</td>
</tr>
<tr>
<td>Methanol extract of raw leaves</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td>Ethyl Acetate extract of ripe leaves</td>
<td>1.3±0.5</td>
</tr>
<tr>
<td>Ethyl Acetate extract of raw leaves</td>
<td>ND *</td>
</tr>
</tbody>
</table>

*Not Detected

Phenolic compounds in plants are said to be responsible for their antioxidant activity and therefore could also contribute towards preventing AGE formation[5],[17],[18]. Flavonoids are a group of antioxidant compounds which is the largest group of polyphenols[18]. Flavonoids have antioxidant properties due to the structure of flavonoids includes an –OH group with two phenyl rings and a heterocyclic ring. Previous studies have indicated that flavonoids possess the ability to decrease oxidative stress due to presence of OH groups and number of OH groups per molecule are the significant factors of the flavonoids[19].

Previous studies have reported that flavones to be the type of flavonoids with highest antiglycation potential. Quercetin is normally used as the standard of the determination of total flavonoid content in plant extracts. Studies have shown that Quercetin which is a flavonol can significantly reduce the Amadori product formation leading to AGEs[7],[17],[20].
Antiglycation Activity and Radical Scavenging Activity of Leaves of Artocarpus heterophyllus

3.3 The DPPH free radical scavenging activity (RSA)

The DPPH radical scavenging activity of the plant extracts were determined according to standard procedure with Butylated hydroxyl toluene (BHT) as the standard.

Table 3 gives the % radical scavenging activity calculated according to the particular equation. As illustrated in Figure 3, all the extracts showed DPPH radical scavenging activity which would imply that the extracts possess antioxidant activity. The extracts of ripe leaves had higher antioxidant activity than extracts of raw leaves. ALEAH had higher radical scavenging activity than other extracts. ALEAH (ripe) had maximum %RSA of (65.7 ± 0.1) % at concentration of 0.2 mg/ml while ALEH (raw) had maximum %RSA of (54.1 ± 0.1) % at the concentration of 0.2 mg/ml.

3.4 In Vitro Protein Glycation Inhibitory Activity

The aqueous extract of Artocarpus heterophyllus (ALEAH) was used to study the antiglycation potential as it had the highest radical scavenging activity, TPC and TFC among the extracts.

Non enzymatic reaction of reducing sugars and free amino groups in amino acids or proteins form Schiff base structures[11]. The importance of this reaction in identifying diabetes was realized many years after that. It was found that the levels of hemoglobinA1c(HbA1c), minor species of hemoglobin in humans, were elevated in the patients with diabetes. It is now considered as a monitor for diabetes mellitus[21]. In the presence of elevated sugar levels, the formation of AGEs increase. These AGEs becomes responsible for the secondary complications in patients with diabetes mellitus[22].

In our experiments formation of AGEs was observed during two successive weeks incubating BSA and fructose in buffer solutions at room temperature by measuring the increase of fluorescent intensity in BSA glycated with fructose. This would occur due to the formation of early glycation product (fructosamine)[23][24].The fluorescent intensity increased throughout the period. The sample containing ALEAH demonstrated a significant reduction in the fluorescent intensity of the mixtures (Figure 4, 5). A higher yield of AGEs result from fructose compared to glucose.[22]. Furthermore, according to previously published
Antiglycation Activity and Radical Scavenging Activity of Leaves of Artocarpus heterophyllus

article[25]. AGEs resulted from fructose leads to higher fluorescence intensities compared to those resulting from glucose. Aminoguanidine, which was used as the positive AGE inhibitor in this study, has been shown to inhibit the AGE formation by trapping reactive dicarbonyl species formed prior to the Amadori products forming AGEs[26][27]. Due to the toxic effect of the synthetic amnioguanindine, which was the first anti-AGE drug, researchers became interested in plant-based remedies having lesser side effects.

The overlay fluorescent spectra showing the enhancement in fluorescent intensity on mixing BSA and fructose as well as the decrease of the fluorescent intensities in samples containing different concentrations of ALEAH(ripe) and ALEAH (raw) are shown in figure 4 and figure 5 respectively.

These figures show that ALEAH (ripe) and ALEAH (raw) bring about reduction in the formation of fluorescent AGEs when present in the BSA-fructose medium.

The inhibitory activity of ALEAH at all concentrations obtained for the first and second week after incubation were calculated according to equation 2 and tabulated in Table 4.

<table>
<thead>
<tr>
<th>Concentration µg/ml</th>
<th>%Inhibition week1 ripe</th>
<th>%Inhibition week1 raw</th>
<th>%Inhibition week 2 ripe</th>
<th>%Inhibition week 2 raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>17.2 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>26.1 ± 2.3</td>
<td>8.0 ± 0.8</td>
</tr>
<tr>
<td>75</td>
<td>45.2 ± 0.9</td>
<td>5.6 ± 1.5</td>
<td>52.2 ± 0.7</td>
<td>36.5 ±0.7</td>
</tr>
<tr>
<td>100</td>
<td>53.2 ± 0.9</td>
<td>25.1 ± 1.9</td>
<td>63.2 ± 1.5</td>
<td>57.1 ±1.4</td>
</tr>
<tr>
<td>125</td>
<td>61.3 ± 1.9</td>
<td>39.1 ± 2.0</td>
<td>72.2 ± 0.4</td>
<td>63.9 ±0.5</td>
</tr>
</tbody>
</table>
According to Table 4 the results show that ALEAH from ripe and raw leaves have exhibited protein glycation inhibitory potential during the first week of incubation itself. Inhibition increased when incubated for a further week. Inhibition was highest at the concentration of 125µg/ml each for ripe and raw leaves. ALEAH of ripe leaves showed a higher inhibition than ALEAH of raw leaves in both weeks.

The inhibitory activity of the reference standard Aminoguanidine (AG) was also obtained in particular week after incubation of same time duration. The inhibition of AG was 89.7 ± 0.5%, 94 ± 0.5%, 95 ± 0.4% in first week and 87.4 ± 0.3%, 94.9 ± 0.1%, 95.2 ± 0.4% in second week for the concentrations of 250, 750, 1250 µg/ml respectively.

This gives evidence that both of the ALEAH ripe and raw leaves have promising antiglycation activity.

IV. CONCLUSION

Ethyl acetate, methanol and aqueous extracts of the leaves of Artocarpus heterophyllus showed radicals scavenging activity which could be due to the significant flavonoid contents present in them. However, the aqueous extract gave the highest radical scavenging activity and total phenol and total flavonoid content. Therefore the leaves (ripe and raw) of Artocarpus heterophyllus may be used as remedy to suppress disease conditions arising due to oxidative stress as well as to combat aging.

Furthermore, in this study we have proven that the aqueous extract (ALEAH) prepared according to the method of preparation of “kasaya” in traditional Ayurvedha medicine has the potential to inhibit protein glycation and hence could be considered as a remedy for complications arising due to diabetes. The glycation inhibitory power of the aqueous extracts of the ripe fallen leaves was higher than that of the raw leaves. Therefore, ripe fallen leaves which are to be discarded have a potential to be developed into suitable decoctions/drugs which could combat diabetic complications.

REFERENCES


