J Herbmed Pharmacol. 2024; 13(3): 472-481.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2024.51498

Journal of Herbmed Pharmacology

In vitro antiglycation and antioxidant properties of ethanolic extracts of *Ficus botryocarpa* and *Ficus racemosa* fruits

Benjamart Cushnie[1*], Achida Jaruchotikamol[1], Jantarawimol Laihakhot[2], Apinya Chotson[2]

[1]Pharmaceutical Chemistry and Natural Products Research Unit, Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, Thailand.
[2]PharmD student, Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, Thailand

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 14 March 2024
Accepted: 2 May 2024

Keywords:
Ficus botryocarpa
Ficus racemosa
Phenolics
Flavonoids
Antiglycation
Antioxidation

ABSTRACT

Introduction: The formation of advanced glycation end products (AGEs) due to prolonged high blood sugar levels and oxidative stress is crucial for accelerating several chronic diseases, including diabetic complications. Edible Thai plants, such as *Ficus botryocarpa* (FB) and *F. racemosa* (FR), are abundant in phytochemicals and exhibit antioxidant, antidiabetic, and antimicrobial properties. This study aimed to evaluate the potential effects of ethanolic extracts derived from the fruits of FB and FR (referred to as FBE and FRE, respectively) on glycation and oxidative stress *in vitro*. In addition, the total phenolic and flavonoid compounds in the extracts were measured.

Methods: Phenolic and flavonoid contents were determined using the Folin-Ciocalteu and aluminium chloride methods, respectively. To evaluate their antiglycation capabilities, the extracts were tested along with aminoguanidine as a positive control using a fructose-induced bovine serum albumin (BSA) glycation model. Fluorescent AGE and fructosamine levels were quantified. The inhibition of lipid peroxidation and superoxide anion radicals was evaluated using thiobarbituric acid-reactive substances (TBARS) and a riboflavin–light–nitroblue tetrazolium assay, respectively.

Results: The phenolic and flavonoid compounds in FBE extract were greater than those in the FRE. FBE inhibited AGEs and produced a significant reduction in fructosamine levels more effectively than FRE ($P<0.05$). In lipid peroxidation and superoxide radical scavenging assay, FBE exhibited a significantly higher ($P<0.05$) inhibition percentage than FRE.

Conclusion: FBE has the potential to act as an antiglycation agent and to protect glycation modifications of albumin from oxidative damage.

Implication for health policy/practice/research/medical education:
The ethanolic extract of *Ficus botryocarpa* fruit exhibited the potential to inhibit glycation and reduce oxidative stress *in vitro*. These results indicate its potential benefits in reducing glycation and protein oxidation, especially in the context of diabetes and its associated complications. However, further investigation of its properties is required.

Please cite this paper as: Cushnie B, Jaruchotikamol A, Laihakhot J, Chotson A. *In vitro* antiglycation and antioxidant properties of ethanolic extracts of *Ficus botryocarpa* and *Ficus racemosa* fruits. J Herbmed Pharmacol. 2024;13(3):472-481. doi: 10.34172/jhp.2024.51498.

Introduction

Glycation, also known as Maillard reaction, involves interactions between reducing sugars (such as glucose and fructose) and proteins via a spontaneous non-enzymatic pathway. The process of forming advanced glycation end products (AGEs) begins with an initial step involving the nucleophilic attachment reaction of free amino groups of proteins with the carbonyl group of reducing sugars. This reaction produces a Schiff base product, which can reversibly convert into a stable fructosamine residue (ketoamine) through an Amadori rearrangement. These early glycation products, Schiff's base, and fructosamines,

***Corresponding author**: Benjamart Cushnie,
Email: benjamart.w@msu.ac.th

can undergo further transformations, including oxidation, rearrangement, dehydration, and cyclization, leading to the formation of stable agents known as AGEs. Glycation causes tissues, including blood vessels, tendons, and the skin, to become less elastic and lose their ability to function as proteins. Glycation occurs at an accelerated rate in the presence of hyperglycemia and oxidative stress in the tissues. Previous studies have demonstrated that AGEs and oxidative stress are closely associated. AGEs accumulation can trigger oxidative stress by directly producing reactive oxygen species (ROS) or indirectly by stimulating oxidative stress pathways in cells. In contrast, oxidative stress can lead to increased AGEs production (1). The interaction between increased glycation and oxidative stress plays a significant role in the development of several diseases, such as diabetes complications, aging, Alzheimer's disease, cerebrovascular diseases, and atherosclerosis (2). A synthetic compound, aminoguanidine, which is a hydrazine derivative with antiglycation properties, has been shown to block AGE formation. Previous studies have indicated that aminoguanidine can prevent the progression of vascular complications in animal models of diabetes. Nonetheless, clinical findings have indicated that aminoguanidine may trigger serious toxicities, such as arrhythmias, heart failure, myocardial infarction, anemia, and gastrointestinal discomfort, thus making it inappropriate for human use (3). Therefore, the identification of novel antiglycation agents is essential for the development of protein glycation inhibitors.

Certain compounds can inhibit AGE formation through various mechanisms including scavenging free radicals, chelating metal ions, masking protein glycation sites, and lowering blood sugar levels (4). There is growing interest in exploring medicinal plants that possess the ability to inhibit protein glycation and delay the modification of protein activity, which contributes to degradation and conversion to AGEs, while maintaining minimal toxicity. Inhibition of AGEs is closely linked to their antioxidant activity (5). Current scientific evidence suggests that certain active phytochemicals, such as polyphenols, terpenoids, polysaccharides, alkaloids, and vitamins, are promising novel agents for inhibiting AGE formation (4). Polyphenols, including phenolic acids and flavonoids, have been studied extensively. Plant-derived polyphenols are believed to protect against AGE-induced health complications through various mechanisms such as regulating blood glucose metabolism, exhibiting antioxidant properties, chelating metal ions, and trapping dicarbonyl compounds. Polyphenols have shown promise in decreasing AGE formation in both laboratory experiments and animal models (5).

Fig trees, which are part of the *Ficus* genus in the Moraceae family, predominantly flourish in tropical areas and have their origins in Southeast Asia and the Eastern Mediterranean. Their fruits are rich in phytochemicals and are well-known for their several health benefits. Several species have been widely used in traditional medicine and Ayurveda (6). Both *Ficus botryocarpa* Miq. and *F. racemosa* Linn. are cultivated in Thailand, with their fruits commonly consumed as traditional food, either served with chili dip or incorporated into various dishes. Furthermore, *F. botryocarpa* has been discovered to exhibit antioxidant, antidiabetic effects (7), as well as antibacterial effects (8), attributed to active compounds such as naringenin, quercetin, rutin, triterpenoids, and alkaloids (9). Similarly, *F. racemosa* has been found to possess significant antioxidant, antidiabetic (10), anti-inflammatory, and anticarcinogenic properties due to its abundance of phytochemical components, including polyphenols, triterpenes, phytosterols, tannins, and steroids (11,12).

Our research team recently documented the antioxidative activities of ethanolic extracts obtained from the fruits of *F. botryocarpa* (FBE) and *F. racemosa* (FRE) using DPPH, ABTS, and FRAP techniques. Additionally, we reported the antidiabetic properties of these extracts through the inhibition of α-amylase, a key enzyme in carbohydrate metabolism, which is analogous to that of the antidiabetic medication acarbose (7,10). It would be more attractive if candidate plants exhibit antioxidant, antidiabetic, and antiglycation characteristics, providing the dual benefits of reducing blood sugar levels and decreasing complications in patients with diabetes. Scientific reports thus far have not documented their effective use in preventing the development of diseases associated with protein glycation and oxidative stress, nor have they explored value addition to these plants. Therefore, an effort was made to investigate both *F. botryocarpa* and *F. racemosa* concerning their phytochemical compositions, antiglycation, and antioxidative stress effects, and prospects, aiming to shed light on their potentials.

Materials and Methods

Plant materials and preparation of ethanolic extracts

In July 2022, raw *Ficus botryocarpa* (FB) and *F. racemosa* (FR) fruits were collected from Surat Thani and Roi Et provinces, respectively, in Thailand. Dr. Nuttapong Wichai, a botanist, authenticated the plant materials, and the voucher samples (PH-FB-01-2022 and PH-FR-01-2022) were securely stored at the Faculty of Pharmacy, Mahasarakham University. The collected plant materials were cleaned followed by a 72-hour drying process in a hot-air oven at 50 °C after which they were finely ground. To produce the ethanolic extracts, 500 g of each plant was macerated twice at room temperature over a 7-day period using 2.5 L 80% (v/v) ethanol. The resulting solution was passed through a filter paper. The solvent was removed using a rotary evaporator, and the residual material was then dried with a freeze dryer to yield a concentrated extract with 11.33% (w/w) yield for the ethanolic extracts derived from the fruits of *F. botryocarpa* (FBE) and 12.09% (w/w) yield for *F. racemosa*

(FRE). These extracts were stored at −20 °C until further analyses were performed.

Qualitative assessment of phytochemical components

The purpose of qualitatively analyzing the extract samples was to detect different chemical groups, including anthraquinones, carbohydrates, flavonoids, phenolics, saponins, tannins, and terpenoids. This identification process utilizes color reactions. Phytochemical analysis was based on established methods and involved studying a solution of the plant extract, which consisted of 50 mg of extract dissolved in 10 mL of ethanol.

Test for anthraquinones (Borntrager's test)

Initially, 2 mL of chloroform was mixed with 2 mL of the extract solution, and the mixture was filtered. Subsequently, 1 mL of a 10% (w/v) ammonia solution was introduced to the filtered material and vigorously stirred for 30 s. The development of a pink/violet/red color in the ammonia layer signified the presence of anthraquinones (13).

Test for carbohydrates (Molisch's test)

Carbohydrates were identified by the appearance of a purple ring at the interface between the examined substance and the acid. This process was performed by adding two drops of Molisch's reagent (20% [w/v] α-naphthol dissolved in ethanol) to 2 mL of the extract solution, followed by the addition of a small quantity of concentrated sulfuric acid (14).

Test for flavonoids (Shinoda's test)

After a short interval, a mixture of five drops of concentrated hydrochloric acid and 2 mL of the extract solution was combined with 5–8 fragments of magnesium. The appearance of a pink/crimson red color indicated the presence of flavonoids (14).

Test for phenolics (Ferric chloride test)

To identify phenolic compounds, 2 mL of the extract solution was combined with a small quantity of a neutral 2% (w/v) ferric chloride solution. A dark green color was considered positive for the presence of phenolic compounds (14).

Test for saponins (Foam test)

Fifty milligrams of the extract was dissolved in 10 mL distilled water and vigorously shaken. This mixture was thoroughly mixed with a small amount of concentrated hydrochloric acid. The presence of saponins was verified by the formation of foam (14).

Test for tannins (Braymer's test)

Deionized water (2 mL) was combined with a small quantity of 5% (w/v) ferric chloride solution containing 2 mL of extract solution. Tannins were detected by the appearance of a brownish-green or blue-black color (14).

Test for terpenoids (Salkowski's test)

To detect terpenoids, 5 mL of the extract solution was mixed with 1 mL chloroform. Subsequently, concentrated sulfuric acid (0.5 mL) was added to 2 mL of this mixture. Terpenoids were confirmed by the appearance of a reddish/brown color at the boundary (14).

Evaluation of total phenolic content

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu assay (15,16) in a 96-well plate. A reaction mixture was prepared by mixing 20 µL of the extract solution, 0.1 mL of 10% Folin–Ciocalteu's reagent dissolved in distilled water, and 80 µL of a 7.5% (w/v) sodium carbonate solution. Gallic acid was used as the reference phenolic compound. After a 15-minute incubation in the absence of light at room temperature, the optical density (OD) of the mixture was measured at 765 nm using a microplate reader. TPC determination was based on a standard curve established using gallic acid, and the findings were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Evaluation of total flavonoid content

The determination of total flavonoid content (TFC) was determined using a modified aluminum chloride colorimetric assay (17). The extract (0.5 mL) was combined with 0.075 mL of a 5% (w/v) sodium nitrite solution and 0.15 mL of 10% (w/v) aluminum chloride solution. After a 5-minute incubation in the absence of light, 0.5 mL of 1 M NaOH and 0.275 mL distilled water were introduced to the reaction mixture. Quercetin was used as the reference flavonoid. Following a 15-minute incubation period, the OD of the reaction mixture was assessed at a wavelength of 510 nm using a microplate reader. TFC was determined based on a standard curve constructed with quercetin, and the findings were presented in milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

Glycation of bovine serum albumin

This study used a glycation model with fructose and bovine serum albumin (BSA) to assess the antiglycation effects of the extract, as outlined in a previously documented procedure (18) with minor modifications. Briefly, BSA and fructose solutions were prepared in 0.1 M phosphate-buffered saline (PBS) at pH 7.4, with the inclusion of 0.02% (w/v) sodium azide to inhibit bacterial contamination. A mixture of 0.35 mL of 1.1 M fructose solution, 0.6 mL of BSA solution (50 mg/mL), and 50 µL of the extract at various concentrations, were incubated for 14 days at 37 °C. Following that step, 0.2 mL of the resulting reaction mixture, which contained glycated BSA, was placed in a black 96-well plate for the measurement of fluorescent AGEs. A microplate fluorescence reader was used to measure fluorescence at excitation and

emission wavelengths of 355 and 460 nm, respectively. Aminoguanidine, a synthetic compound known for its antiglycation properties, served as the positive control at a concentration of 0.5 mg/mL. The percent inhibition of glycation was determined using the following formula:

$$\text{Percent inhibition of glycation} = \frac{[(FC - FCB) - (FS - FSB)]}{FC - FCB} \times 100$$

where FC represents the fluorescence detected in the glycated control without any sample, and FCB is the fluorescence of the blank control, which is the reaction mixture of BSA without fructose. FS represents the fluorescence of the glycated sample, whereas FSB represents the fluorescence of the blank sample, which is the reaction mixture of BSA and a sample without fructose.

Determination of fructosamine

The nitroblue tetrazolium (NBT) assay was used to assess fructosamine concentration in glycated samples, following a previously described method (19) with minor modifications. After a 14–day incubation period, 180 µL of 500 µM NBT solution (in 100 mM carbonate buffer at pH 10.4) was thoroughly mixed with 20 µL of the glycated samples. Following a 15–min incubation, the OD of the mixture was measured at 530 nm using a microplate reader. Fructosamine levels were calculated and compared with various concentrations of a reference compound, namely 1-deoxy-1-morpholino-fructose (1-DMF), based on the standard curve, y = −0.0258x + 0.436, R^2 = 0.9473, and presented as DMF equivalent in micromolar fructosamine concentration.

Lipid peroxidation assay

To investigate the production of lipid peroxides, we used a thiobarbituric acid-reactive substances (TBARS) assay (20), in which malondialdehyde (MDA), a byproduct of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA). In a test tube, 50 µL of the extract solution (1 mg/mL) and standard ascorbic acid (0.2 mg/mL) were mixed with 400 mL of the egg yolk homogenate (diluted 1:10 in PBS). Following this step, 150 μL of 75 mM $FeSO_4$ was introduced into the reaction mixture, and incubation was conducted at 37 °C for 30 minutes to induce lipid peroxidation. To stop the reaction, 200 μL of 15% trichloroacetic acid and 0.5% TBA were introduced. The resulting mixture was vortexed and subsequently subject to heating at 95 °C in a boiling water bath for 15 minutes. After cooling, centrifugation was performed at 3500 rpm for 15 minutes, and the absorbance of the supernatant was recorded at 532 nm. The percent inhibition of MDA was calculated using the absorbance values with the equation shown below:

$$\textit{Percent inhibition of MDA} = \frac{[A_{control} - (A_{sample} - A_{blank})]}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control reaction, which comprises all reagents excluding the extract); A_{sample} represents the absorbance of the sample when the extract is present; A_{blank} represents the absorbance of the sample blank, consisting the reaction solution added to the extract without TBA.

Superoxide radical scavenging assay

A modified method was used to evaluate the scavenging capability of superoxide ($O_2^{\bullet}$) anion radical. This approach relies on the ability of the extract to inhibit the photochemical reduction of NBT (21). Briefly, the reaction mixture consisted of 20 µL of 750 µM NBT, 20 µL of 1 mM ethylenediaminetetraacetic acid (EDTA), 100 µL of 266 µM riboflavin, 20 µL of 50 mM potassium PBS (pH 7.4), and 40 µL of test sample solution (1 mg/mL), in addition to a reference agent, ascorbic acid (0.2 mg/mL), in a 96-well plate. After 10-minute of exposure to fluorescent light, absorbance was measured at 590 nm. The percentage inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with those of the reaction mixture containing the test sample.

$$\text{Percent inhibition of superoxide radical} = \frac{[A_{control} - (A_{sample} - A_{blank})]}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control reaction, which contains all reagents excluding the extract; A_{sample} represents the absorbance of the sample when the extract is present; A_{blank} represents the absorbance of the sample blank, which consists of the reaction solution added to the extract without riboflavin.

Statistical analysis

The results were presented as mean ± standard error of the mean (SEM) from three independent experiments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test. Statistical significance was set at *P* value < 0.05 and the analysis was performed using GraphPad Prism (version 9.0).

Results

The results of the phytochemical screening of the FBE and FRE extracts are presented in Table 1. Both extracts contained anthraquinones, flavonoids, phenolics, tannins, and terpenoids; however, no saponins were detected. TPC, measured using Folin-Ciocalteu's method and expressed as gallic acid equivalent (GAE), was determined based on a standard curve (y = 0.036x – 0.119, R^2 = 0.99). TFC was analyzed using an aluminum chloride colorimetric assay and expressed as quercetin equivalents (QE). TFC was referenced against a standard curve (y = 0.0031x – 0.055, R^2 = 0.99). TPC was 2.5 times greater in the FBE extract than in the FRE extract (Figure 1A). Likewise, the TFC in the FBE was approximately 1.73 times higher than that in

Table 1. Phytochemical screening of the ethanolic extracts obtained from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively)

Phytochemical constituents	Plant extracts*	
	FBE	FRE
Anthraquinones	+	+
Carbohydrates	+	–
Flavonoids	+	+
Phenolics	+	+
Saponins	–	–
Tannins	+	+
Terpenoids	+	+

Note: *Chemical compositions are: (–) absence; (+) presence.

the FRE extract, with a statistically significant difference observed in both analyses ($P < 0.05$) as shown in Figure 1B.

In vitro antiglycation activities

Figure 2 (A and B) display the percentage of antiglycation effects of a standard reagent called aminoguanidine, which inhibits the formation of AGEs. The concentration of aminoguanidine ranged from 2.5 to 500 µg/mL. Additionally, the ethanolic extracts were tested at concentrations ranging from 100 to 1000 µg/mL). These experiments were conducted using BSA-fructose derived AGEs after a 14–day incubation period. Aminoguanidine, a standard inhibitor, exhibited the most potent AGE inhibitory effect with the lowest IC_{50} value of 55.45 ± 10.37 µg/mL. Conversely, FRE showed the least AGE inhibitory action, with the highest IC_{50} value of 539.83 ± 21.95 µg/mL. The intermediate IC_{50} value of FBE was determined to be 228.91 ± 14.32 µg/mL. Both FBE and FRE demonstrated increasing antiglycation activity with increasing concentration (in a concentration-dependent manner). At 1000 µg/mL, FBE and FRE demonstrated the highest inhibitory activities at 87.42 ± 1.23 and 58.02 ± 0.49, respectively, whereas aminoguanidine at 500 µg/mL showed an inhibitory activity of 95.35 ± 0.90.

Figure 3 illustrates the effects of FBE, FRE, and aminoguanidine at a concentration of 500 µg/mL following a 14-day incubation in a fructose-mediated BSA glycation model and compared to the control (BSA/fructose) with respect to fructosamine levels. These findings indicate that FBE notably reduced fructosamine levels by approximately 83.97%. Interestingly, both FRE and aminoguanidine inhibited the increase in fructosamine levels by approximately 64.67% and 63.33%, respectively, with no significant difference between them.

In vitro antioxidative stress activities

In this study, we explored the antioxidant potentials of FBE and FRE using assays to evaluate lipid peroxidation and superoxide anion radical scavenging effects. These assessments are relevant for evaluating oxidative stress associated with AGE-mediated chronic diseases in humans. An extract concentration of 1 mg/mL exhibited the most significant anti-AGE effects (Figure 2B), prompting us to select this concentration for evaluating the antioxidative stress properties of the extracts and confirming their effects against glycation. This approach yielded valuable insights into potential therapeutic applications.

The *in vitro* lipid peroxidation assay is a commonly used technique for evaluating the oxidative breakdown of lipids in biological samples. The TBARS assay quantifies the levels of MDA, which is a byproduct of lipid oxidation and acts as an indicator of potential harm to cell membranes. Figure 4 shows the percentage of MDA inhibition by FBE and FRE extracts at a concentration of 1 mg/mL in comparison to ascorbic acid at a concentration of 0.2 mg/mL. At a concentration of 1 mg/mL, both FBE and FRE produced a notable reduction in MDA inhibition in comparison to ascorbic acid at 0.2 mg/mL, which represents the standard reference. Nevertheless, FBE exhibited approximately a 2.28 times greater suppression

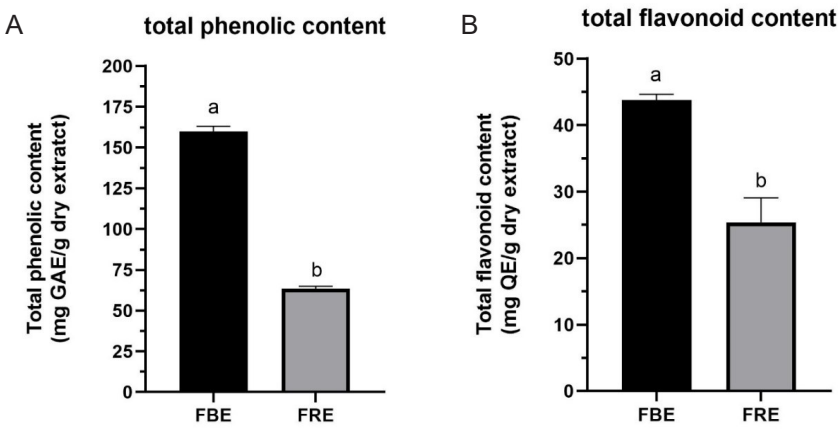

Figure 1. Quantitation of (A) total phenolic and (B) total flavonoid compounds (TPC and TFC, respectively) in the ethanolic extracts obtained from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively). Data values are expressed as mean ± standard error of the mean (SEM). Different letters on each bar signify a notable distinction at $P < 0.05$. Abbreviations: GAE/g: Milligrams gallic acid equivalent per gram; QE/g: Milligrams quercetin equivalent per gram.

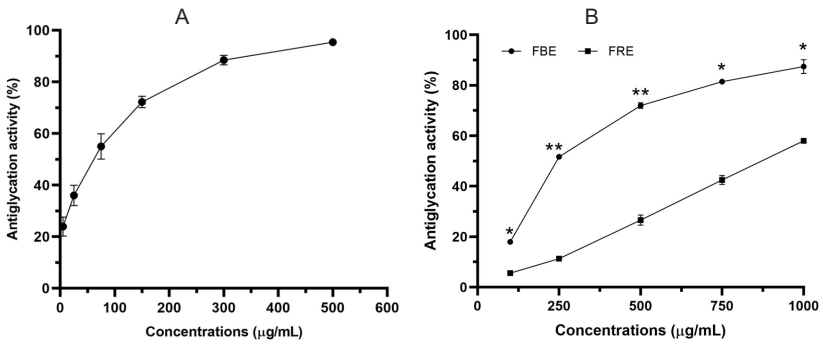

Figure 2. The percentage inhibition of advanced glycation end products (AGEs) by (A) a standard inhibitor aminoguanidine and (B) the ethanolic extracts obtained from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively). This measurement was conducted after a 14–day incubation in a fructose-mediated bovine serum albumin (BSA) glycation model. The percent inhibition is presented as mean ± standard error of the mean (SEM) from triplicate experiments. Note: **P* < 0.05 and ***P* < 0.001 indicate significant differences between the extracts at various concentrations.

of MDA than FRE. These results agree with previous findings of similar impacts on the inhibition of AGE (Figure 2) and fructosamine (Figure 3).

The superoxide radical scavenging assay assesses the ability of substances to scavenge highly ROS known as superoxide radicals. This study evaluated the percentage of superoxide radical inhibition achieved by FBE and FRE extracts at a concentration of 1 mg/mL, with ascorbic acid at 0.2 mg/mL. Remarkably, FBE demonstrated superoxide radical inhibition almost identical to that of ascorbic acid (approximately 88%). In contrast, FRE exhibited the least significant inhibitory effect on superoxide radicals, as shown in Figure 5.

Discussion

Prolonged hyperglycemia can modify protein structures in many organs such as the heart, brain, kidneys, eyes, and coronary arteries. Protein structural changes arise from non-enzymatic glycation, which results in impaired protein and mitochondrial functions (1,2). Traditional medicine has identified certain medicinal herbs that appear to efficiently lower high blood sugar levels and inhibit the formation of AGEs, which may provide potential benefits for patients with diabetes and prevent chronic diseases (5,22). Phytochemical screening is a crucial tool for identifying the secondary metabolites present in medicinal plants, as many of these compounds play vital roles in therapeutic actions. Polyphenols, such as phenolic acids and flavonoids, are noted for their significant contribution to various biological activities, including antimicrobial, anti-inflammatory, antidiabetic, antiglycation, and antioxidant properties (4,5,22). Phytochemical screening of crude ethanolic extracts of *F. botryocarpa* (FBE) and *F. racemosa* (FRE) revealed

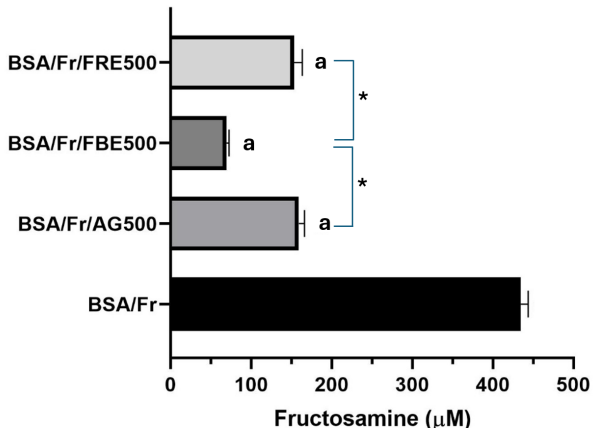

Figure 3. The effects of a standard inhibitor aminoguanidine (AG) and ethanolic extracts derived from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively) at a concentration of 500 μg/mL on the levels of fructosamine. This assessment was conducted following a 14–day incubation in a fructose-mediated bovine serum albumin (BSA) glycation model. Fructosamine levels are presented as mean ± standard error of the mean (SEM) obtained from triplicate experiments. Note: [a] *P* < 0.05 when compared to BSA/Fr (Fructose), * *P* < 0.05 when compared between each group

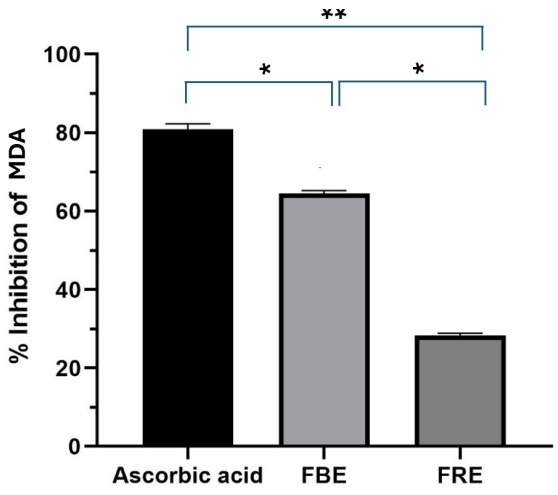

Figure 4. The percentage inhibition of malondialdehyde (MDA) by ethanolic extracts obtained from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively) at a concentration of 1 mg/mL compared to ascorbic acid at a concentration of 0.2 mg/mL. The reported percentage inhibition values represent the mean ± SEM, which were obtained from three independent experiments. Note: **P* < 0.05 and ***P* < 0.01 indicate significant differences between the groups.

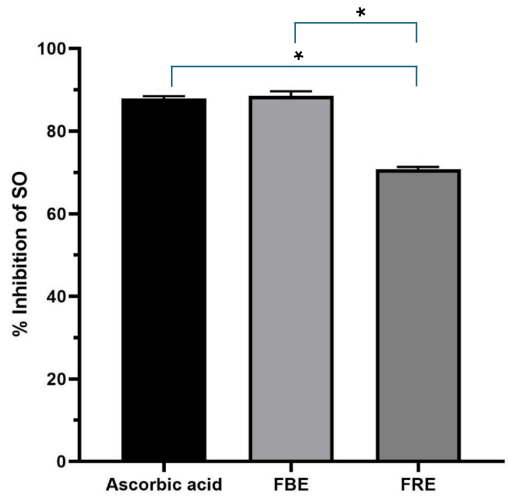

Figure 5. The percentage inhibition of superoxide (SO) radicals by ethanolic extracts obtained from the fruits of *Ficus botryocarpa* and *F. racemosa* (FBE and FRE, respectively) at a concentration of 1 mg/mL compared to ascorbic acid at a concentration of 0.2 mg/mL. The reported percentage inhibition values represent the mean ± SEM, which were derived from three separate experiments. Note: *$P < 0.05$ indicates significant differences between the groups.

the presence of anthraquinones, flavonoids, phenolics, tannins, and terpenoids, whereas saponins were absent. Our phytochemical screening results are consistent with those reported in previous studies on these plants (9,12,24). In the quantification assay, FBE significantly exhibited a higher content of phenolics at 159.85 ± 3.14 mg GAE/g dry extract compared to FRE. However, Buamard et al reported a TPC value of 615.58 ± 3.53 mg GAE/g dry extract for 80% ethanolic extract (9), which is higher than the TPC value found in the current study. Possible reasons for these differences could be variations in the extraction methods, timing of plant harvesting, and/or changes in climatic and soil conditions (23). Regarding the TFC, FBE also exhibited a higher level at 43.77 ± 0.85 mg QE/g dry extract compared to FRE (25.36 ± 3.70 mg QE/g dry extract). Unfortunately, there is no available data on FBE for direct comparison. In a previous study, Munira et al showed that the methanolic extract of FR fruits had a TPC of 24.63 mg GAE/g dry extract and a TFC of 32.49 mg QE/g dry extract (24). The observed levels of TPC (63.21 ± 1.68 mg GAE/g dry extract) and TFC (25.36 ± 3.70 mg QE/g dry extract) in our investigation, which used an ethanolic extract, were only slightly different from these. Variations in phenolic profiles were noted based on the solvents used for extraction. Ethanol, which is considered safe for human consumption, is an effective solvent for the extraction of polyphenols. Methanol is generally more effective in extracting polyphenols with lower molecular weight but it is not considered safe for consumption due to its toxicity.

The main objective of our study was to assess the potential inhibitory effects of FBE and FRE on the development of fructosamine and AGEs, which serve as indicators of the early and late stages of glycation, respectively. Elevated levels of these substances in the body have been associated with a range of chronic conditions, including cardiovascular diseases, kidney disorders, and diabetes mellitus (1,2). Our study aimed to determine the levels of fluorescent AGEs and fructosamine under the most favorable experimental conditions. Within this specific context, we examined the formation of fluorescent AGEs and fructosamine after 14–day incubation of a solution containing fructose and BSA at 37 °C. The experimental conditions closely resembled the physiological environment of the body.

In this study, we employed a conventional antiglycation agent, aminoguanidine, which is commonly used because of its potential pharmacological effects, particularly its ability to inhibit glycation. Aminoguanidine showed an IC_{50} value of 55.45 ± 10.37 µg/mL, which closely aligns with the findings of Grzegorczyk-Karolak et al. (63 µg/mL) (25) and Ávila et al. (82.6 ± 0.4 µg/mL) (26). FBE exhibited a significantly lower potency as an inhibitor, as evidenced by its IC_{50} value of 228.91 ± 14.32 µg/mL, which is 4.13 times higher than that of aminoguanidine. When comparing FRE and aminoguanidine, the IC_{50} value for FRE was found to be 9.74 times higher, indicating a significantly lower potency. Regarding the effect on fructosamine levels, it was noted that at a concentration of 500 µg/mL, FBE considerably inhibited fructosamine formation to a greater degree than both aminoguanidine and FRE, exhibiting a 20% difference. It appears that FBE primarily influences the early stage of glycation by targeting fructosamine rather than AGEs. However, plant extracts have not yet been characterized. In the literature, a previous study revealed a similar occurrence. It was found that 100 µg/mL lupeol, the main active compound extracted from the ethanolic extract of banana flowers, inhibited fructosamine by 86% and decreased AGEs production by 71% (27). Lupeol, recognized as a triterpenoid compound found in a variety of fruits, including *F. botryocarpa* (8), could potentially contribute to the observed effects of FBE. Flavonoids such as quercetin and rutin exhibit superior and stronger antiglycation properties than aminoguanidine at all stages of glycation. Interestingly, both quercetin and rutin are present in *F. botryocarpa* fruits (5).

Oxidative stress can initiate AGE formation by stimulating glycation reactions. To confirm the relevant mechanisms, it is critical to examine the roles of ROS scavengers, including lipid peroxidation and superoxide anions, which occur at various stages of glycation and contribute to the reduction of structural changes in proteins (28). For the evaluation of antioxidant activity, 1 mg/mL of the plant extracts was chosen for experimentation because of its potent antiglycation effectiveness. These findings have significant implications for the prospective health benefits of these extracts.

FBE showed a strong inhibitory effect on MDA and

superoxide anion radicals, which was comparable to the reference standard ascorbic acid but more potent than FRE did. Consistent with the results of our previous investigations, FBE effectively reduced ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and scavenged radicals 2,2-Diphenyl-1-picrahydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (DPPH and ABTS, respectively) (7,10).

FBE contained higher levels of phenolic and flavonoid compounds than FRE. Furthermore, it has been hypothesized that these bioactive compounds are responsible for their potent antiglycation, antioxidant, and antidiabetic properties (22,29,30). Buamard et al identified and characterized compounds in a 60% ethanolic extract of *F. botryocarpa* fruits, including phenolic compounds, such as naringenin, quercetin, rutin, and chlorogenic acid (9). These compounds are known for their antioxidant, antiglycation, antidiabetic, and anti-inflammatory properties (31-33).

In terms of antiglycation and antioxidant effects, as well as phytochemical constituents, FBE appears particularly promising for further investigation of its mechanism of action. Our results indicate that FBE can reduce lipid peroxidation and superoxide radical formation and prevent protein glycation, and all activities are strongly correlated with each other. Based on the AGE inhibitory assay, our findings demonstrated that increasing the concentration of the extracts led to increased antiglycation activity. Therefore, it appears that the antioxidant and antiglycation capabilities of FBE are most likely due to its phenolic and flavonoid contents. No information on the antiglycation activity of either extract has been documented previously. FBE appears to have a strong capacity for reducing oxidative stress and preventing glycation. These results, in conjunction with those of previous studies on both plants, confirm the positive effects of the extracts on antioxidative stress and antiglycation. However, further experiments using *in vitro* systems (such as metal chelation and aldose reductase), animal models, and clinical trials are needed to explore these effects more comprehensively.

Conclusion and recommendations

Our research presents initial evidence of the *in vitro* antiglycation and antioxidant properties of ethanolic extracts derived from the fruits of *F. botryocarpa* and *F. racemasa*. When comparing FBE to FRE, FBE had a higher ability to suppress lipid peroxidation, as well as the generation of superoxide radicals, fructosamime, and AGE formation. The increased concentrations of phenolic and flavonoid compounds in FBE are likely involved in mechanisms that prevent protein glycation and protein oxidation. In summary, FBE shows potential as an inhibitor of AGE-mediated pathogenesis, especially in patients with diabetes who are at a high risk of developing chronic complications. Furthermore, FBE protects the proteins from oxidative damage. Therefore, additional exploration of FBE is highly recommended.

Authors' contribution

Conceptualization: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Data curation: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Formal analysis: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Funding acquisition: Benjamart Cushnie.
Investigation: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Methodology: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Project administration: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Resources: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Software: Benjamart Cushnie, Jantarawimolwan Laihakhot and Apinya Chotson.
Supervision: Benjamart Cushnie and Achida Jaruchotikamol.
Validation: All authors.
Visualization: All authors.
Writing–original draft: Benjamart Cushnie, Jantarawimolwan Laihakhot, and Apinya Chotson.
Writing–review & editing: All authors.

Conflicts of interests

The authors declare no conflict of interest concerning this work.

Ethical considerations

The authors have considered all ethical issues, including duplicates, and the manuscript has been checked for plagiarism.

Funding/Support

This research project was financially supported by the Faculty of Pharmacy at Mahasarakham University, Thailand. (S#2/2566).

References

1. Khalid M, Petroianu G, Adem A. Advanced glycation end products and diabetes mellitus: mechanisms and perspectives. Biomolecules. 2022;12(4):542. doi: 10.3390/biom12040542.
2. Koska J, Saremi A, Howell S, Bahn G, De Courten B, Ginsberg H, et al. Advanced glycation end products, oxidation products, and incident cardiovascular events in patients with type 2 diabetes. Diabetes Care. 2018;41(3):570-6. doi: 10.2337/dc17-1740.
3. Pasten C, Lozano M, Rocco J, Carrión F, Alvarado C, Liberona J, et al. Aminoguanidine prevents the oxidative stress, inhibiting elements of inflammation, endothelial activation, mesenchymal markers, and confers a renoprotective effect in renal ischemia and reperfusion injury. Antioxidants (Basel). 2021;10(11):1724. doi: 10.3390/antiox10111724.
4. Song Q, Liu J, Dong L, Wang X, Zhang X. Novel advances in inhibiting advanced glycation end product formation using natural compounds. Biomed Pharmacother. 2021;140:111750. doi: 10.1016/j.biopha.2021.111750.
5. Anwar S, Khan S, Almatroudi A, Khan AA, Alsahli MA, Almatroodi SA, et al. A review on mechanism of inhibition of advanced glycation end products formation

by plant derived polyphenolic compounds. Mol Biol Rep. 2021;48(1):787-805. doi: 10.1007/s11033-020-06084-0.
6. Singh B, Sharma RA. Updated review on Indian *Ficus* species. Arab J Chem. 2023;16(8):104976. doi: 10.1016/j.arabjc.2023.104976.
7. Cushnie B, Promlaruk A, Boonchoo C, Singban W, Khaodee T, Boonradom B, et al. Antioxidant and antidiabetic activities of ethanol extract of *Ficus botryocarpa* Miq. fruit. Trop J Nat Prod Res. 2022;6(7):1119-23. doi: 10.26538/tjnpr/v6i7.13.
8. Wau J, Timi D, Harakuwe A, Bowden B, Motti C, Sakulas H, et al. Isolation of three bioactive phenantroindolizidine alkaloids from the fruit latex of *Ficus botryocarpa* Miq. Nat Prod Chem Res. 2015;3(6):197. doi: 10.4172/2329-6836.1000197.
9. Buamard N, Singh A, Zhang B, Hong H, Singh P, Benjakul S. Ethanolic extract of Duea ching fruit: extraction, characterization and its effect on the properties and storage stability of sardine surimi gel. Foods. 2023;12(8):1635. doi: 10.3390/foods12081635.
10. Cushnie B, Promlaruk A, Boonchoo C, Singban W, Khaodee T, Boonradom B, et al. Alpha-amylase inhibition and antioxidant activities of ethanolic crude extract of *Ficus racemosa* Linn. fruit. Thai Sci Techno J. 2019;27(5):951-63. doi: 10.14456/tstj.2019.75.
11. Pahari N, Majumdar S, Karati D, Mazumder R. Exploring the pharmacognostic properties and pharmacological activities of phytocompounds present in *Ficus racemosa* Linn.: a concise review. Pharmacol Res Mod Chin Med. 2022;4:100137. doi: 10.1016/j.prmcm.2022.100137.
12. Sharma H, Pathak R, Jain S, Bhandari M, Mishra R, Reena K, et al. *Ficus racemosa* L: a review on its important medicinal uses, phytochemicals and biological activities. J Popul Ther Clin Pharmacol. 2023;30(17):213-27. doi: 10.47750/jptcp.2023.30.17.018.
13. Godlewska K, Pacyga P, Najda A, Michalak I. Investigation of chemical constituents and antioxidant activity of biologically active plant-derived natural products. Molecules. 2023;28(14):5572. doi: 10.3390/molecules28145572.
14. Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. J Intercult Ethnopharmacol. 2017;6(2):170-6. doi: 10.5455/jice.20170403094055.
15. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol. 2023;15:51-62. doi: 10.2147/jep.s379805.
16. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel). 2019;8(4):96. doi: 10.3390/plants8040096.
17. Jirasripongpun K, Jirakanjanakit N, Pola S, Obsuwan K. Antioxidation and anti-melanogenesis of three colored flowers of *Dendrobium hybrids*. Sci Eng Health Stud. 2022;16:22030006. doi: 10.14456/sehs.2022.22.
18. Zuo GL, Kim HY, Guillen Quispe YN, Wang ZQ, Hwang SH, Shin KO, et al. Efficient separation of phytochemicals from *Muehlenbeckia volcanica* (Benth.) Endl. by polarity-stepwise elution counter-current chromatography and their antioxidant, antiglycation, and aldose reductase inhibition potentials. Molecules. 2021;26(1):224. doi: 10.3390/molecules26010224.
19. Adisakwattana S, Sompong W, Meeprom A, Ngamukote S, Yibchok-Anun S. Cinnamic acid and its derivatives inhibit fructose-mediated protein glycation. Int J Mol Sci. 2012;13(2):1778-89. doi: 10.3390/ijms13021778.
20. Upadhyay R, Chaurasia JK, Tiwari KN, Singh K. Antioxidant property of aerial parts and root of *Phyllanthus fraternus* Webster, an important medicinal plant. ScientificWorldJournal. 2014;2014:692392. doi: 10.1155/2014/692392.
21. Al Mahmud Z, Bachar SC, Hasan CM, Emran TB, Qais N, Uddin MM. Phytochemical investigations and antioxidant potential of roots of *Leea macrophylla* (Roxb.). BMC Res Notes. 2017;10(1):245. doi: 10.1186/s13104-017-2503-2.
22. Hassanpour SH, Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 2023;13(4):354-76. doi: 10.22038/ajp.2023.21774.
23. Bibi N, Shah MH, Khan N, Al-Hashimi A, Elshikh MS, Iqbal A, et al. Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. Plants (Basel):950. 2022;11(7):950. doi: 10.3390/plants11070950.
24. Munira M, Uddin M, Islam M, Aktar K, Muhit A. Evaluation of antioxidant, cytotoxic, antibacterial and thrombolytic activity of the methanolic extracts of *Ficus racemosa* fruits. J Complement Altern Med Res. 2018;5(2):1-13. doi: 10.9734/jocamr/2018/40429.
25. Grzegorczyk-Karolak I, Gołąb K, Gburek J, Wysokińska H, Matkowski A. Inhibition of advanced glycation end-product formation and antioxidant activity by extracts and polyphenols from *Scutellaria alpina* L. and *S. altissima* L. Molecules. 2016;21(6):739. doi: 10.3390/molecules21060739.
26. Ávila F, Cruz N, Alarcon-Espósito J, Nina N, Paillan H, Márquez K, et al. Inhibition of advanced glycation end products and protein oxidation by leaf extracts and phenolics from Chilean bean landraces. J Funct Foods. 2022:98;105270. doi: 10.1016/j.jff.2022.105270.
27. Ávila F, Cruz N, Alarcon-Espósito J, Nina N, Paillan H, Márquez K, et al. Inhibition of advanced glycation end products and protein oxidation by leaf extracts and phenolics from Chilean bean landraces. J Funct Foods. 2022;98:105270. doi: 10.1016/j.jff.2022.105270.
28. Ramu R, Shirahatti PS, Zameer F, Ranganatha LV, Nagendra Prasad MN. Inhibitory effect of banana (*Musa* sp. var. Nanjangud rasa bale) flower extract and its constituents umbelliferone and Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. S Afr J Bot. 2014;95:54-63. doi: 10.1016/j.sajb.2014.08.001.
29. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194-222. doi: 10.3390/biom5010194.
30. Sun C, Liu Y, Zhan L, Rayat GR, Xiao J, Jiang H, et al. Anti-diabetic effects of natural antioxidants from fruits.

Trends Food Sci Technol. 2021;117:3-14. doi: 10.1016/j.tifs.2020.07.024.
31. Cepas V, Collino M, Mayo JC, Sainz RM. Redox signaling and advanced glycation endproducts (AGEs) in diet-related diseases. Antioxidants (Basel). 2020;9(2):142. doi: 10.3390/antiox9020142.
32. Swallah MS, Sun H, Affoh R, Fu H, Yu H. Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. Int J Food Sci. 2020;2020:9081686. doi: 10.1155/2020/9081686.
33. Yeh WJ, Hsia SM, Lee WH, Wu CH. Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings. J Food Drug Anal. 2017;25(1):84-92. doi: 10.1016/j.jfda.2016.10.017.
34. Khan M, Liu H, Wang J, Sun B. Inhibitory effect of phenolic compounds and plant extracts on the formation of advance glycation end products: a comprehensive review. Food Res Int. 2020;130:108933. doi: 10.1016/j.foodres.2019.108933.