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Original Research Article

Antioxidant and Antidiabetic Activities of Ethanol Extract of *Ficus botryocarpa* Miq. Fruit

Benjamart Cushnie[1]*, Autchara Promlaruk[2], Chayapim Boonchoo[2], Wanitchakorn Singban[2], Thanawut Khaodee[2], Boonyawat Boonradom[2], and Pawitra Pulburt[1]

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

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ABSTRACT

Diabetes mellitus (DM) is a major public health concern worldwide. Poorly controlled diabetes can lead to hyperglycemia and serious complications, including macrovascular and microvascular diseases of the kidneys and eyes, and a variety of clinical neuropathies. There is evidence that serious complications are associated with increased generation of free radicals. Inhibition of alpha-amylase activity can be an effective strategy for controlling carbohydrate digestion and reducing postprandial hyperglycemia. This study aimed to investigate the *in vitro* antioxidant capacity and alpha-amylase inhibitory activity of *Ficus botryocarpa* fruit ethanol extract (FBFE). Antioxidant potential was determined using a radical scavenging capacity assay (2, 2-diphenyl-1-picrylhydrazyl; DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid; ABTS), and ferric ion reducing power (FRAP) assays. The alpha-amylase inhibitory effect of the extract was investigated using 3,5 dinitrosalicylic (DNS) method. In the DPPH and ABTS assays, FBFE had IC_{50} values of 54.51±7.20 µg/mL and 5.28±0.30 µg/mL, respectively. In the FRAP assay, FBFE had FRAP value of 1.97±0.12 mM Fe^{2+}/mg dried extract. In the DNS method, the highest inhibitory effect of the extract against alpha-amylase was 97.65±5.27% at 50 µg/mL, with a half maximal inhibitory concentration (IC_{50}) value of 6.22±2.31 µg/mL. Therefore, it can be concluded that the ethanol fruit extract of *Ficus botryocarpa* demonstrated high antioxidant capacity and alpha-amylase inhibitory activity. This finding may justify its traditional use in the treatment of diabetes mellitus.

Keywords: Diabetes mellitus, *Ficus botryocarpa* Miq fruit, Antioxidant, Alpha-amylase inhibition

Introduction

The number of people with diabetes mellitus (DM) is increasing exponentially worldwide. It is a metabolic disorder that is associated with pancreatic β-cell dysfunction, insulin resistance, decreased insulin secretion, leading to hyperglycemia when the body's tissues are unable to use glucose for energy. Chronic hyperglycemia produces serious complications, including cardiovascular and microvascular diseases of the kidneys, eyes, and peripheral nervous system.[1-3] The development of these complications is generally caused by oxidative stress. High blood sugar levels over a prolonged period cause mitochondrial free radical overproduction in endothelial cells in both large and small blood vessels (macrovascular and microvascular diseases) including the myocardium.[4-6] In general, the most frequent causes of death among patients with diabetes are complications such as renal (e.g., nephritis/nephrosis, uremia), ischemic heart disease and respiratory diseases, atherosclerosis, and cerebrovascular diseases.[3] One of the keys to diabetes treatment is lowering blood sugar levels after meals, called postprandial glucose. This method is based on the principle of inhibiting carbohydrate metabolism to reduce the absorption of glucose into the body.

*Corresponding author. E mail: benjamart.w@msu.ac.th
Tel: +66956721145

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Pancreatic alpha-amylase is involved in carbohydrate digestion and is a popular therapeutic target for the treatment of postprandial glucose elevation. In clinical applications, acarbose is an inhibitor of alpha-glucosidase and -amylase, which have been shown to be an effective treatment for diabetes.[7-8] However, long-term use of this drug can cause hepatotoxicity and gastrointestinal disturbance (e.g., nausea, flatulence, indigestion, and diarrhea). Therefore, numerous researchers are interested in the search for potent alpha-amylase inhibitor plants that have fewer side effects or adverse reactions and possess antioxidant capacity to prevent serious complications and death due to diabetes.

In Thailand, traditional Thai medicine still plays a crucial role in community health-care systems, even though modern health-care systems are now easily accessible. Interestingly, only a few studies have been reported on Thai traditional medicine. *Ficus botryocarpa* (*F. botryocarpa*) Miq. is one of these plants. *F. botryocarpa* is a member of the Moraceae family and is widely used in the Philippines, Papua New Guinea, and Thailand. Fruit latex of *F. botryocarpa* is traditionally used to treat bacterial skin and microbial infections, and diarrhea.[9-12] *F. botryocarpa* is used as food (with chili dip) and for medicinal properties in Thai population. Several phytochemicals (e.g., alkaloids, ficuseptine, beta-amyrin acetate, alpha-amyrin acetate, and lupeol acetate) were found in the plant, which are associated with antibacterial effects.[9,11,13] Wau *et al*. demonstrated that the methanol extracts of the fruit and stem latex of *F. botryocarpa* exhibited a strong antibacterial effect against a wide spectrum of gram-positive and -negative bacteria.[9] In addition, some preliminary data suggested that the ethanol fruit extract of *F. botryocarpa* exerts a cytotoxic effect against a liver cancer cell line (HepG2) with an IC_{50} value of 132±25.38 µg/mL.[14] Alpha-glucosidase inhibitory activity was also found in this extract with an IC_{50} of 14.86 µg/mL.[15] However, there is

no information available regarding the antioxidant activity of *F. botryocarpa* fruit extract. Therefore, the objective of the present study was to determine the antioxidant and alpha-amylase inhibitory activities of the ethanol fruit extract of *F. botryocarpa*.

Materials and Methods

Preparation of the F. botryocarpa fruits ethanol extract (FBFE)
Raw fruits of *F. botryocarpa* Miq. were collected in March 2015 in Krabi, Thailand. The plant samples were authenticated by Assistant Professor Dr. Natsajee Nualkaew (Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand). A voucher sample (KKU-PT-5) was stored in the herbarium of the Pharmaceutical Chemistry and Natural Product Research Unit (Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, Thailand). The fruits were harvested, washed in clean water, and dried at 50 ^{o}C for 48 h in a hot air oven. The fruits (500 g) were ground to a powder and macerated in 5 L of 80% ethanol for 72 h at room temperature. The resulting extract was filtered through Whatman grade 1 filter paper. A rotary evaporator was used to concentrate the extract (at 40 ^{o}C), followed by freeze-drying. FBFE with a yield of 11.65% (w/w) was kept frozen at −20 ^{o}C before analysis.

Determination of antioxidant activity
The antioxidant activities of FBFE were investigated using two different assays: 2,2-Dipheny-1-Picrylhydrazyl (DPPH) free radical scavenging assay, and 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay.

DPPH radical scavenging assay
The DPPH scavenging activity of the sample was measured according to the methods of Taepongsorat *et al*. with minor adjustment.[16] In brief, a DPPH reaction mixture (0.15 mM) in ethanol was prepared. A 100 µL aliquot of this reaction mixture was thoroughly mixed with 100 µL of the sample (concentrations 25 − 300 µg/mL) or ascorbic acid (concentrations 1 − 20 µg/mL) in a 96-well microplate in the dark, before incubating for 30 min at room temperature. The absorbance of the reaction mixture was measured at 517 nm using a UV-visible spectrophotometer microplate reader (SPECTROstar Nano Microplate reader, BMG Labtech GmbH, Germany). Ascorbic acid was used as an antioxidant standard or positive control, DPPH solution with 0.1% DMSO was used as the negative control, and ethanol (no DPPH) with various concentrations of the sample was used as the blank. The DPPH radical scavenging activity was calculated using the following equation:

$$\%\ DPPH\ radical\text{-}scavenging\ activity = \frac{(absorbance\ of\ control - absorbance\ of\ test\ sample}{absorbance\ of\ control} \times 100$$

ABTS radical scavenging assay
The ABTS radical cation assay of the extract was conducted according to the methods of Indracanti *et al*. with minor modifications.[17] The stock of ABTS (7 mM) was mixed well with dipotassium peroxodisulfate (2.45 mM) (1:0.5). The ABTS solution was then incubated in the dark at room temperature for 16 h. Prior to analysis, the solution was diluted with ethanol to an absorbance of 0.7 (±0.02) at 734 nm. A 20 µL aliquot of the extract (concentrations 2.5 − 120 µg/mL) or ascorbic acid (concentrations 1 − 20 µg/mL) was thoroughly mixed with 180 µL of the ABTS radical reaction mixture in a 96-well microplate. The mixture was then incubated in the dark for 6 min. The absorbance was measured at 734 nm using a UV-visible spectrophotometer. Ascorbic acid was used as an antioxidant standard or positive control; ABTS solution with 0.1% DMSO was used as the negative control; and ethanol (no ABTS) with various concentrations of the extract was used as the blank. The ABTS radical scavenging activity was calculated using the following equation:

$$\%\ ABTS\ radical\ scavenging\ activity = \frac{(absorbance\ of\ control - absorbance\ of\ test\ sample}{absorbance\ of\ control} \times 100$$

Both DPPH and ABTS radical scavenging capacities are expressed as IC_{50}. A low IC_{50} value indicated that the plant was effective at low concentrations. Plotting concentration-inhibitory response curves were generated using GraphPad Prism software version 6.0. The % inhibition obtained from the DPPH and ABTS radical scavenging assays was expressed as mg of ascorbic acid or vitamin C equivalents per gram of sample based on the vitamin C standard curve, called vitamin C equivalent antioxidant capacity (VCEAC).

Ferric reducing antioxidant power assay
Ferric reducing antioxidant power (FRAP) determination was performed according to the method described by Puangpornpitag *et al*. with minor modifications.[18] In brief, 25 µL of various concentrations of extract (2.5 − 120 µg/mL) or ascorbic acid (1 − 15 µg/mL) were mixed well with 175 µL of FRAP solution (acetate buffer: ferric chloride: TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) 10: 1: 1). The mixture was then incubated at room temperature for 4 min. The absorbance of the resulting mixture was measured at 593 nm by using a UV-visible spectrophotometer microplate reader. The FRAP value was calculated using the equation obtained from the standard curve of ferrous sulfate ($FeSO_4.7H_2O$) which was expressed as mM of Fe^{2+}/mg of the extract. A high FRAP value indicated that *F. botryocarpa* is an effective antioxidant. Ascorbic acid served as an antioxidant standard, and the results were expressed as mg of ascorbic acid or vitamin C equivalents per gram of extract based on the vitamin C standard curve (VCEAC).

Alpha-amylase enzyme inhibitory assay
In vitro inhibition of alpha-amylase enzyme was tested according to the procedure of Ali *et al*. with minor modifications.[19] Briefly, a solution of 40 µL of the extract (the sample solutions were prepared in 0.1% DMSO at various concentrations (0 − 100 µg/mL)), and 200 µL of freshly prepared porcine pancreatic alpha-amylase solution in deionized water (4 U/mL) was prepared. The mixture was pre-incubated at 25 ^{o}C for 5 min. A 400 µL of 0.5% w/v potato starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and a 160 µL of distilled water were added into the mixture and then further incubated at 25 ^{o}C for 3 min. The reaction was terminated by transferring 200 µL of the reaction mixture into a new microfuge tube containing 100 µL of dinitrosalicylic acid (DNS) color reagent, followed by incubation in boiling water at approximately 85 ^{o}C for 15 min and cooled to room temperature. The absorbance of the resulting reaction was determined at 540 nm using a UV-visible spectrophotometer microplate reader (SPECTROstar Nano Microplate reader, BMG Labtech GmbH, Germany). To obtain the correct absorbance of the reaction mixture, the sample absorbance was subtracted from the absorbance of the sample blank. For the sample blank, DNS reagent was added before adding the starch solution, and the rest of the procedure was the same as that for the test. For the control, all methods were the same, except that the sample was replaced with 0.1% DMSO. *Triticum aestivum* (wheat seed), a well-known alpha-amylase inhibitor, was used at 20 U/mL as a positive control.[19] The amount of maltose present in the samples was used to determine the alpha-amylase activity. To construct a standard curve for the estimation of maltose, it was dissolved in deionized water at various mass ratios (0.01 − 0.1% w/v). Alpha-amylase inhibitory effect was expressed as IC_{50}. A low IC_{50} value indicates that the plant was effective at low concentrations. Concentration-response curves were plotted using GraphPad Prism software, version 6.0. The % alpha-amylase inhibitory activity was calculated using the following equation:

$$\%\ alpha\text{-}amylase\ inhibitory\ activity = \frac{(absorbance\ of\ control - absorbance\ of\ test\ sample}{absorbance\ of\ control} \times 100$$

Statistical analysis
Three independent experiments were performed in triplicate. The results were expressed as the mean ± standard deviation (SD), except that of the IC_{50} values which were expressed as mean ± standard error of the mean (SEM). Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.

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Statistically significant differences were indicated by a *P*-value of less than 0.05.

Results and Discussion

Antioxidant capacity of FBFE

Antioxidant activity was determined using DPPH and ABTS radical scavenging activity assays. These two methods are widely used for measuring the total antioxidant capacity of medicinal plants and are considered relatively inexpensive, effective, and sensitive to UV-visible spectrophotometry. In addition, the FRAP assay was also applied as it is easy, fast, and low-cost. This method is often used to measure the antioxidant capacity of natural products.[20] Table 1 shows the IC_{50} values and percentage inhibition of DPPH and ABTS radical scavenging of FBFE and ascorbic acid. In the DPPH and ABTS assays, FBFE had IC_{50} values of 54.51±7.20 µg/mL and 5.28±0.30 µg/mL, respectively. The concentration range of FBFE used was 2.5 − 120 µg/mL. The maximal inhibitory activity of DPPH (74.96%) was observed at 60 µg/mL and the maximal inhibitory activity of ABTS (99.11%) was also found at 60 µg/mL. In the FRAP assay, FBFE had an FRAP value of 1.97±0.12 mM Fe^{2+}/mg dried extract weight, which was lower than that of ascorbic acid (Figure 1). Figure 2 shows a comparison of the DPPH, ABTS, and FRAP assays for estimating the antioxidant activity of vitamin C or ascorbic acid. Antioxidant activity was expressed as vitamin C equivalent antioxidant capacity (VCEAC) in mg per mg of FBFE. With the DPPH radical and FRAP assays, the VCEAC of FBFE were 0.19±0.01 and 0.30±0.05 mg/mg of the extract, respectively. In contrast, with ABTS radical assay, the VCEAC was noticed to be at a higher ratio (1.61±0.15 mg/mg of FBFE). Our findings demonstrate that FBFE has a higher antioxidant capacity than vitamin C, especially when using the ABTS. These results suggest that FBFE possesses a strong ABTS scavenging activity.

Table 1: The IC_{50} values and percentage inhibition of DPPH and ABTS radical scavenging activity of FBFE and ascorbic acid

Concentration (µg/mL)	% inhibition of DPPH	% inhibition of ABTS
2.5	1.05 ± 0.09[a]	16.47 ± 2.06[a]
5	7.81 ± 0.81[a,b]	52.04 ± 1.81[b]
7.5	9.34 ± 0.34[a,b]	65.82 ± 1.05[c]
15	16.15 ± 6.15[b]	83.62 ± 3.78[d]
30	34.20 ± 7.20[c]	95.77 ± 0.15[e]
60	74.96 ± 4.96[d]	99.11 ± 0.19[e]
120	49.53 ± 9.53[c]	96.99 ± 0.30[e]
IC_{50} of FBFE	54.51 ± 7.20	5.28 ± 0.30
IC_{50} of ascorbic acid	7.62 ± 0.48	6.78 ± 0.16

Data are expressed as mean ± SD; Different superscript letters in each column indicate a significant difference (P-value < 0.05). One-way ANOVA and Tukey's post-hoc test were used.

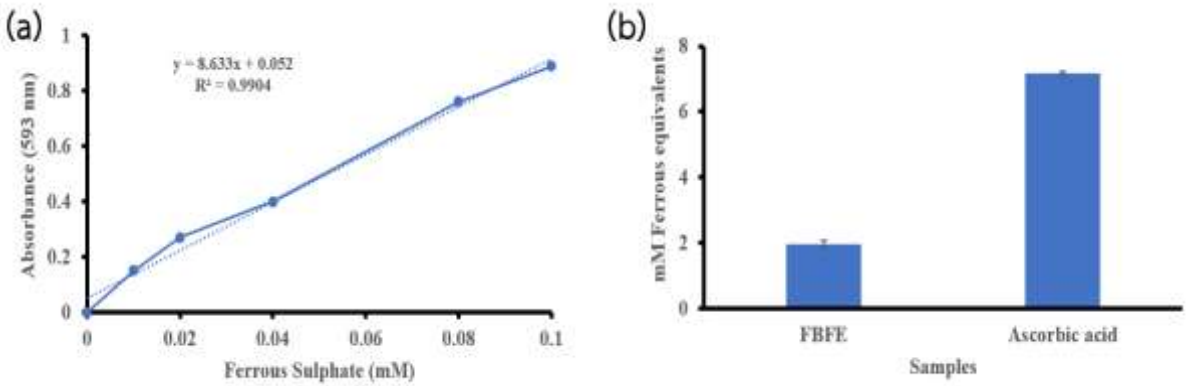

Figure 1: FRAP assay (a) Standard calibration curve for the determination of FRAP value (b) mM Ferrous equivalents or FRAP value of FBFE and ascorbic acid

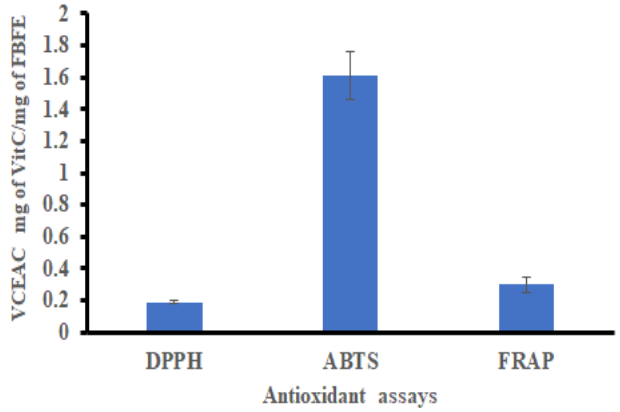

Figure 2: Comparison of DPPH, ABTS, and FRAP assays for estimating antioxidant activity from ascorbic acid or vitamin C

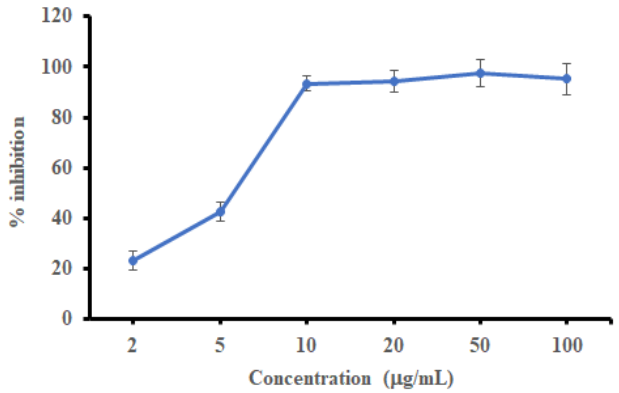

Figure 3: Evaluation of alpha-amylase inhibitory activities of FBFE

Alpha-amylase inhibitory activity of FBFE

In vitro inhibition of alpha-amylase in the extract was investigated using the DNS method. The inhibitory activities of alpha-amylase on FBFE are shown in Figure 3. FBFE demonstrated concentration-dependent inhibitory activity against the alpha-amylase effect with an IC_{50} value of 6.22±2.31 µg/mL. The maximal inhibitory activity of 97.65±5.27% was observed at a concentration of 50µg/mL. The percent inhibition value of wheat seed used as a positive control was 52.96±8.47%, which was less effective than FBFE at 10 − 100 µg/mL. This data is in accordance with the preliminary report of Puangcho *et al*. that demonstrated FBFE had an alpha-glucosidase inhibitory activity with a single IC_{50} of 14.86 µg/mL.[15] This finding suggests that FBFE is similar to acarbose, a hypoglycemic drug that inhibits both alpha-amylase and -glucosidase activities, but with undesired side effects (e.g., diarrhea, constipation, flatulence, and stomach pain). Therefore, combinations of *F. botryocarpa* fruit with acarbose may be of beneficial for diabetes treatment, with fewer side effects.

There are only few reported publications regarding the phytochemicals and pharmacological activity of *F. botryocarpa*. In contrast, other species in the genus *Ficus* have been critically reviewed.[12, 21-23] However, Wau *et al.* investigated and reported some important information regarding the identification of phytochemicals in *F. botryocarpa*. They reported the presence of ficuseptine, lupeol acetate, alpha-amyrin acetate, and beta-amyrin acetate in *F. botryocarpa*.[13] Interestingly, lupeol and beta-amyrin are the most important of the pentacyclic triterpenes found in numerous medicinal plants and these compounds have been proven to have various healthy biological activities (e.g., cardioprotective, hepatoprotective, gastroprotective, antitumor, anti-inflammatory, antimicrobial, analgesic, antiparasitic, and wound-healing), including antidiabetic activity. Recently, Tamfu *et al*. reported that the synthesis of beta-amyrin and lupeol exhibited potent inhibition against alpha-amylase and -glucosidase enzymes.[24] It is possible that the inhibitory action of the alpha-amylase enzyme found in FBFE is lupeol and beta-amyrin. However, other phytochemicals such as polyphenols[25] and flavonoids[26] have also been shown to inhibit alpha-amylase and -glucosidase enzymes.

Investigation of the phytochemical contents of total phenolics and flavonoids in FBFE needs to be confirmed in the future.

The antioxidant activities of lupeol isolated from the methanol leaf extract of *Crateva adansonii* were investigated using a variety of antioxidant methods and it was concluded that lupeol exerts antioxidant activities by scavenging free radicals. Surprisingly, Tchimene *et al*. showed that lupeol possesses strong ABTS scavenging activity[27], which is similar to the results of the present study when compared to DPPH and FRAP assays. This suggests that lupeol from FBFE may be the main bioactive compound with antioxidant properties. Analysis of the phytochemical contents associated with antioxidant activity (e.g., total phenolics, total flavonoids, tannins, and saponin)[26] in FBFE requires further investigation.

Conclusion

This study reports the *in vitro* antioxidant and antidiabetic potentials of an ethanol extract of *F. botryocarpa* fruit. It could be useful in the treatment of postprandial blood glucose, and in the reduction of complications and mortality in diabetes mellitus. However, this study suggests further investigation is required, especially of the bioactive ingredients associated with the actions of this ethanol extract. To develop a novel antidiabetic drug further *in vivo* and clinical studies are also required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Lau LH, Lew J, Borschmann K, Thijs V, Ekinci E. Prevalence of diabetes and its effects on stroke outcomes: A meta-analysis and literature review. J Diabetes Investig. 2019; 10(3):780-792.
2. Balaji R, Duraisamy R, Kumar MPS. Complications of diabetes mellitus: A review. Drug Invent Today. 2019; 12(1):98-103.
3. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of diabetes 2017. J Diabetes Res. 2018; Article ID 3086167: 4 pages.
4. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J. 2016; 24(5):547-553.
5. Giacco F and Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107(9):1058-1070.
6. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev. 2020; Article ID 8609213: 13 pages.
7. Sales PM, Souza PM, Simeoni LA, Silveira D. Alpha-amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 2012; 15(1):141-183.
8. Ye X-P, Song C-Q, Yuan P, Mao R-G. Alpha-glucosidase and alpha-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. Chinese J Nat Med. 2010; 8(5):349-352.
9. Wau J, Harakuwe A, Timi D, Sakulas H, Gubag-Sipou R, Wangiwan P. Weather Dependent Antibacterial activity of the latex from *Ficus botryocarpa*. Environ Papua New Guinea. 2009; 2(2):1-3.
10. Waruruai J, Sipana B, Koch M, Barrows LR, Matainaho TK, Rai PP. An ethnobotanical survey of medicinal plants used in the Siwai and Buin districts of the Autonomous Region of Bougainville. J Ethnopharmacol. 2011; 138(2):564-577.
11. Wau J, Timi D, Harakuwe A, Bowden B, Motti C, Sakulas H, Gubag-Sipou R. Isolation of three bioactive phenantroindolizidine alkaloids from the fruit latex of *Ficus botryocarpa* Miq. Nat Prod Chem Res. 2015; 3(6):6-10.
12. Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. Ficus spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol. 2008; 119(2):195-213.
13. Wau J. Phytochemical and antimicrobial assessment of *Ficus botryocarpa* Miq. (MORACEAE). Papua New Guinea: Papua New Guinea University of Technology; 2013.
14. Ramutton T, Phawong C, Na Ubol P, Nualkaew N, Tassaneetrithep B. Cytotoxicity study of Triphala and *Ficus botryocarpa* Miq. Isan J Pharm Sci. 2013; 9(1):220.
15. Puangcho P and Nualkaew N. Alpha-glucosidase inhibition of Thai local vegetable extracts. Isan J Pharm Sci. 2013; 9(1):218.
16. Taepongsorat L and Konsue A. Biological screening of trijannarose as a recipe from Thai traditional medicine. Phcog Res. 2019; 11(2):110-114.
17. Indracanti M, ChV S, Sisay T. A 96 well-microtiter plate ABTS based assay for estimation of antioxidant activity in green leafy vegetables. Biotechnol Int. 2019; 12(2):22-29.
18. Puangpornpitag D, Tankitijano P, Sumalee A, Konsue A. Phytochemical screening and antioxidant activities of the seedling extracts from Inca Peanut (*Plukenetia volubilis*). Pharmacogn J. 2021; 13(1):1-7.
19. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol. 2006; 107(3):449-455.
20. Munteanu IG and Apetrei C. Analytical methods used in determining antioxidant activity: A Review. Int J Mol Sci. 2021; 22(7): 30 pages.

21. Salehi B, Prakash Mishra A, Nigam M, Karazhan N, Shukla I, Kiełtyka-Dadasiewicz A, Sawicka B, Głowacka A, Abu-Darwish MS, Hussein Tarawneh A, Gadetskaya AV, Cabral C, Salgueiro L, Victoriano M, Martorell M, Docea AO, Abdolshahi A, Calina D, Sharifi-Rad J. Ficus plants: State of the art from a phytochemical, pharmacological, and toxicological perspective. Phytother Res. 2021; 35(3):1187-1217.
22. Joseph B and Raj SJ. Phytopharmacological and phytochemical properties of three ficus species - An overview. Int J Pharma Bio Sci. 2010; 1(4):246-253.
23. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry, and pharmacology of *Ficus carica*: A review. Pharm Biol. 2014; 52(11):1487-1503.
24. Tamfu AN, Munvera AM, Botezatu AV, Talla E, Ceylan O, Fotsing MT, Mbafor JT, Shaheen F, Dinica RM. Synthesis of benzoyl esters of β-amyrin and lupeol and evaluation of their antibiofilm and antidiabetic activities. Results Chem. 2022; 4: Article ID 100322:14 pages.
25. Sultana R, Alashi AM, Islam K, Saifullah M, Haque CE, Aluk RE. Inhibitory activities of polyphenolic extracts of Bangladeshi vegetables against alpha-amylase, alpha-glucosidase, pancreatic lipase, renin, and angiotensin-converting enzyme. Foods. 2020; 9(7):13 pages.
26. Zhu J, Chen C, Zhang B, Huang Q. The inhibitory effects of flavonoids on alpha-amylase and alpha-glucosidase. Crit Rev Food Sci Nutr. 2020; 60(4):695-708.
27. Tchimene MK, Nwaehujor CO, Ezenwali M, Okoli CC, Iwu MM. Free radical scavenging activity of lupeol isolated from the methanol leaf extract of *Crateva adansonii* Oliv. (Capparidaceae). Int J Pharmacogn Phytochem Res. 2016; 8(3):419-426.