DOI: https://doi.org/10.59294/HIUJS2025025

Antiglycemic and antihypertension potential of extract from *Syzygium malaccense* (L.) Merr. & L.M.Perry leaf

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ABSTRACT

Background: Diabetes and hypertension are diseases of an increased rate in humans. The target enzymes of two diseases are α-glucosidase, α-amylase, dipeptidyl peptidase-IV (DPP-IV), and angiotensin-converting enzyme (ACE). Objective: The aim of this study is to determine the inhibition ability of inhibition ability α-glucosidase, α-amylase, dipeptidyl peptidase-IV (DPP-IV), and angiotensin-converting enzyme (ACE) of extract from S. malaccense leaves. Materials and method: S. malaccense leaves were extracted with 96% alcohol at a ratio of 1:25 (w/v) to obtain total extract (TP). A quantity of total extract was fractionated with ethyl ether, ethyl acetate, and n-butanol solvents to obtain ethyl ether extract (EE extract), ethyl acetate extract (EA extract), n-butanol (BU extract), and the remaining aqueous solution (WA extract). TP extract and fractionated extracts were determined for enzyme inhibitory activity. Results: BU extract has the most potent inhibitory activity on α-amylase enzymes with an IC_{50} value of 265.23 µg/mL. The ethyl acetate (EA) extract was found with the highest inhibitory activity on α-glucosidase, DPP-IV, and ACE activity at IC_{50} values of 107.34 µg/mL, 160.07 µg/mL, and 186.32 µg/mL, respectively. Conclusion: The research indicates that leaf extracts can be applied to support the treatment of hypertension and diabetes.

Keywords**:** *Syzygium malaccense, α-glucosidase, α-amylase, angiotensin-converting enzyme (ACE), Dipeptidyl peptidase IV (DPP-IV)*

1. INTRODUCTION

Diabetes is always a global concern, especially type II diabetes (T2DM). Some complications, such as eye damage, kidney failure, and heart failure, reduce the patient's quality of life when infected. To treat T2DM, carbohydrate hydrolytic enzymes like *α*-amylase and *α*-glucosidase must be stopped from breaking down carbohydrates after a meal. Diabetes medications often cause many dangerous side effects, such as hypoglycemia, digestive disorders, weight gain, and hepatotoxicity. Currently, the incretin pathway for DM treatment is widespread. Incretins include Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Peptide-1 (GLP-1). However, GLP-1 is strongly cleaved by Dipeptidyl peptidase IV [1].

Recent studies have also shown that natural compounds can treat diabetes by inhibiting *α*-amylase, *α*-glucosidase, and Dipeptidyl peptidase IV to minimise these substances' toxicity and side effects. Inhibitors are being used to control the disease because therapeutic concentrations are maintained, and there are fewer side effects. Baraik and colleagues studied *A. aspera* for treating several diseases such as hypertension, diabetes, inflammation, pneumonia, … [2]. Nowadays, the use of medicinal herbs in the treatment of diabetes and hypertension is prevalent because it has few side effects and can be used long-term.

Hypertension is one of the main risks that can have a substantial impact on cardiovascular diseases in patients with type II diabetes; arteriosclerosis is a typical example [3]. ACE inhibitors are the first line of treatment for uncomplicated hypertension, as well as when it coexists with other diseases like diabetes, coronary sclerosis, heart failure, and kidney failure. ACE inhibitors act directly on the renin-angiotensin

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system (RAS), causing ACE inhibition, reducing the conversion of angiotensin I (active form) to angiotensin II (inactive form), while inhibiting the degrading bradykinin causes vasodilation to lower blood pressure, such as Captopril, Quinapril, Benazepril, Lisinopril, Enalapril, ... [4]. However, the drug still has side effects such as kidney failure, hyperkalemia, fetal malformations, persistent dry cough (0 - 44%), angioedema, and patients treated with Captopril will often experience complications. taste disorders, leukopenia, ... [5]. On the contrary, herbs cause fewer side effects, so the trend of using herbs to inhibit ACE to support the treatment of hypertension is becoming popular [6].

(a) flower (b) leaves and fruit

Figure 1. *Syzygium malaccense*

Myrtaceae is a plant family with many species used in the healthcare field. However, *S. malaccense,* belonging to this family, is rarely studied. Batista and colleagues determined that the shell, seeds, and leaves of *S. malaccense* contain phenolics, flavonoids, and carotenoids and have antioxidant activity. In many countries, *S. malaccense* fruit is used as a nutritious food, and the leaves are used as medicine to support the treatment of hypertension and diabetes [7]. Ethanol extract from *S. malaccense* leaves in Malaysia can inhibit blood sugar enzymes such as *α*-glucosidase and *α*-amylase [8]. Herbs of the *Syzygium* genus can also help treat hypertension [9].

The content of this study is to confirm the ability of *S. malaccense* to inhibit *in vitro* diabetes and *in vitro* hypertension by determining the ability to inhibit the enzymes *α*-amylase, *α*-glucosidase, Dipeptidyl Peptidase IV (DPP-IV), and angiotensin-converting enzyme (ACE) of total ethanol extract (TP) and fractions such as ethyl ether (EE), ethyl acetate (EA), *n*-butanol (BU), and water (WA).

2. MATERIALS AND METHODS

2.1. Materials

Leaves of *S. malaccense* were harvested in Tien Giang province (Vietnam), and approached by MSc. Tran Huu Thanh. The dried leaves had a moisture content of 9%, were ground and filtered through a 710 µm sieve to obtain powder for extraction.

Chemicals for this study such as 96% ethanol, ethyl ether, ethyl acetate, *n*-butanol from Fisher, ACE (2.0 units/mg protein) from rabbit lungs, hippuryl histidyl-leucine (HHL), captopril, *α*-glucosidase (from Saccharomyces cerevisiae, ≥ 10 units/mg protein), *p*-Nitrophenyl *α*-D-glucopyranoside (pNPG), *α*-amylase (from porcine pancreas, ≥ 5 units/mg), acarbose, dipeptidyl Peptidase (DPP-IV), Gly-Pro *p*-nitroanilide hydrochloride, and diprotin A, were purchased from Sigma Chemical Co. (USA).

UV-VIS spectrophotometer and standard lab equipment.

2.2. Method

2.2.1. Extraction of S. malaccense leaves

600 grams of medicinal herbs are extracted with 96% alcohol at a ratio of 1:25 (w/v) in 72 hours at room temperature. Gather the alcohol solution and condense it into total extract (TP), a solid form. A portion of the TP extract is mixed with water to prepare the extraction of the fractional extracts. The fractionation process was carried out in turn with solvents as ethyl ether, ethyl acetate, and n-butanol. Consequently, *n*-butanol (BU), ethyl ether (EE), ethyl acetate (EA), and residual water extract (WA) were obtained as four different types of extracts. The extracts were concentrated to prepare for studies on biological activity.

2.2.2. Method for α-amylase inhibitory activity

200 µL of sample was mixed with 40 µL of *α*-amylase enzyme (5 U/mL), 0.36 mL of 0.02 M sodium phosphate buffer at pH 6.9, and 6 mM sodium chloride. The mixture was incubated at 37°C for 20 minutes. Then, 300 µL of 1% starch was added and incubated at 37°C for 20 minutes. 0.2 mL of 1% DNSA reagent was added to the mixture. Stirred and boiled for five minutes, then cooled to room temperature. Distilled water was added to make 10 ml and measured at 540 nm. Acarbose was used as a control. All tests were performed in triplicate with Equation (1), and each test was performed in triplicate [10].

$$\alpha - \text{amylase inhibition}\ (\%) = \frac{A - B}{B}.100\%$$

Where A is the absorbance of the control, and B is the absorbance of the sample.

2.2.3. Method for α-glucosidase inhibitory activity

100 µL of extract was added to 2,200 µL of sodium phosphate buffer (0.01 M) and 100 µL of 1.0 U/mL α-glucosidase enzyme to start the reaction and left at 37°C for 5 min. Add 100 µL of 3 mM pNPG, stir well, and incubate for 30 min at 37°C. Finally, add 15.50 µL of 0.1 M Na_2CO_3 solution to terminate the reaction. Finally, use UV-VIS measurement at 405 nm to determine the *α*-glucosidase inhibitory activity by calculating the p-nitrophenol generated from pNPG during the response. The positive control used was acarbose. With Equation (2), each assay was conducted in triplicate [11].

$$\alpha - \text{glucosidase inhibition}\ (\%) = \frac{A - B}{B} \times 100\%$$

Where A is the absorbance of the control, and B is the absorbance of the sample.

2.2.4. Method for DPP-IV inhibitory activity

25 µL of the sample solution was mixed well with 25 µL of 12 mM Gly-Pro p-nitroaniline and incubated for 10 min at 37°C. Add 50 µL of DPP-IV (0.02 U/mL) and continue incubation for 30 min at 37°C after 10 min. Add 100 µL of 1M sodium acetate buffer to terminate the reaction at pH 4.0. The wavelength of 405 nm was used to measure the samples. Diprotin A served as the reference point. With Equation (3), each assay was conducted in triplicate [12].

$$\text{DPP} - \text{IV inhibition}\ (\%) = \frac{A - B}{B} \times 100\%$$

Where A is the absorbance of the control, and B is the absorbance of the sample.

2.2.5. Method for ACE inhibitory activity

50 µL of the extract was mixed with 50 µL of ACE solution (25 mU/mL), incubated at 37°C for 10 min. Next, 150 µL of HHL substrate was mixed into the above solution and left at 37°C for half an hour. To extract hippuric acid, 250 µL of 1M HCl and 500 µL of ethyl acetate were added, mixed well, and centrifuged at 3000 rpm for 10 min. For 30 min, 200 µL of the ethyl acetate layer was dried with the upper hippuric acid solution layer at 60°C and measured at 228 nm after dilution with 2 mL of distilled water. As a control, captopril was used. With Equation (4), each assay was performed in triplicate [13].

$$\text{ACE inhibition}\ (\%) = \frac{A - B}{B} \times 100\%$$

Where A is the absorbance of the control, and B is the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1. Results of medical extraction

600 g of *S. malaccense* leaves (deducted for the moisture content of 10.77 ± 0.33%) were used to make 138.24 g of TP extract (with the moisture content of 19.93 ± 0.95%).

By using 60 g of TP extract and shaking the fraction to obtain 21.20 g of EE extract (with the moisture content of 8.47 ± 0.61%), 20.51 g EA extract (with the moisture content of 16.60 ± 0.35%), 11.1 g of BU extract (with the moisture content of 18.13 ± 0.31%), and 7.15 g of WA extract (with the moisture content of 19.20 ± 0.72%).

3.2. *α*-amylase inhibitory activity of *S. malaccense* leaf extract

The *α*-amylase inhibitory activity revealed that the extract with the highest inhibitory activity was BU, followed by extracts from EA, EE, TP, and WA. The IC_{50} value of 740.36 (µg/mL) for total extract (TP) is 1.13 times higher than that of Acarbose (IC_{50} = 657.73 µg/mL) (Figure 2).

The *α*-amylase inhibitory activity of TP extract of *S. malaccense* leaves was higher than that of the study conducted by Arumugam et al. [8]. This could be attributed to the impact of growing conditions

on the content of secondary compounds in leaves, as the TP content of leaves extracted using the same solvent from *S. malaccense* leaves grown in Malaysia was lower [8].

The fractionated extracts with α-amylase inhibitory activity that are 2.48 and 1.50 folds higher than acarbose, respectively, are BU and EA extracts. The potent inhibitory activity of BU and EA may be related to the presence of compounds such as flavonoids or phenolics. In contrast, the water fraction (WA) showed the weakest activity, indicating that the main inhibitory compounds were not well soluble in water. This suggests that BU and EA are the most potential fractions for exploiting inhibitory α-amylase activity from *S. malaccense* leaves.

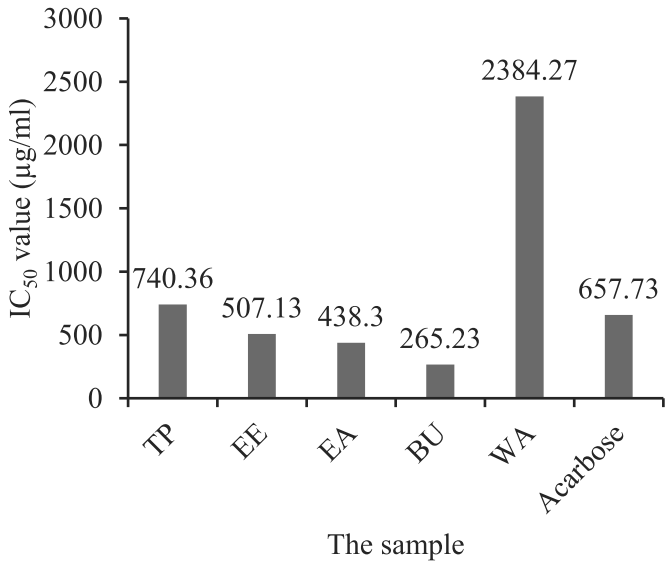

Figure 2. IC_{50} value (µg/mL) of α-amylase inhibitory activity of total extract and fractions of *S. malaccense* leaves

3.3. α-glucosidase inhibitory activity of *S. malaccense* leaf extract

After studying the α-glucosidase inhibitory activity of *S. malaccense* leaf extracts, EA extract was found to have the best ability to inhibit α-glucosidase, with BU, EE, TP, and WA extracts following (Figure 3). The α-glucosidase inhibitory activity of TP extract is 1.91 folds greater than that of acarbose. The highest amount of α-glucosidase inhibitory activity was found the EA fraction, which is 1.43 times more than that of the acarbose positive control.

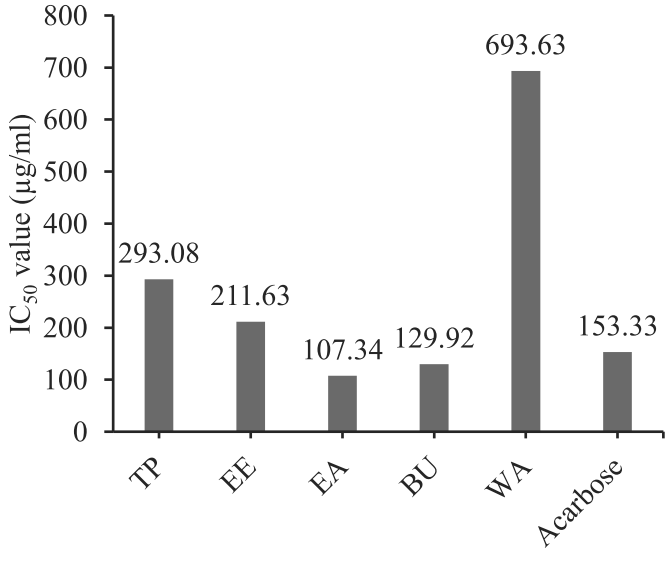

Figure 3. IC_{50} value (µg/mL) of α-glucosidase inhibitory activity of total extract and fractions of *S. malaccense* leaves

The α-glucosidase inhibitory activity of *S. malaccense* leaves ethanolic extract is less than that of Arumugam et al. (2014) [8]. This could be because growing conditions impact how active secondary compounds form in leaves.

This result may reflect the concentration of flavonoid compounds in the EA fraction, which is known to have potent activity on this enzyme. The fact that TP had lower activity than EA suggests that fractionation helps to concentrate the more active compounds [14]. Therefore, EA is a promising fraction for application in postprandial glycemic control.

3.4. DPP-IV inhibitory activity of *S. malaccense* leaf extract

EA extract has the highest DPP-IV inhibitory activity, with an IC_{50} value of 160.07 (µg/mL), and is low, according to the findings of surveying the DPP-IV inhibitory activity of extracts from *S. malaccense* leaves (Figure 4). The IC_{50} value of the WA extract is 1302.30 (µg/mL). With an IC_{50} for DPP-IV inhibitory activity of 1.60 (µg/mL), the TP extract and fraction extracts obtained from *S. malaccense* leaves exhibit greater levels than the Diprotin A positive control.

Medicinal plants such as *Syzygium cumini*, which inhibit DPP-IV, have anti-diabetic properties and belong to the Myrtaceae family. *Syzygium cumini* seed extract extracted with 70% alcohol shows an intense DPP-IV inhibitory action [15]. Based on research on α-amylase, α-glucosidase, and DPP-IV inhibition, *S. malaccense* is a Myrtaceae plant that has the potential to be used medicinally to treat diabetes.

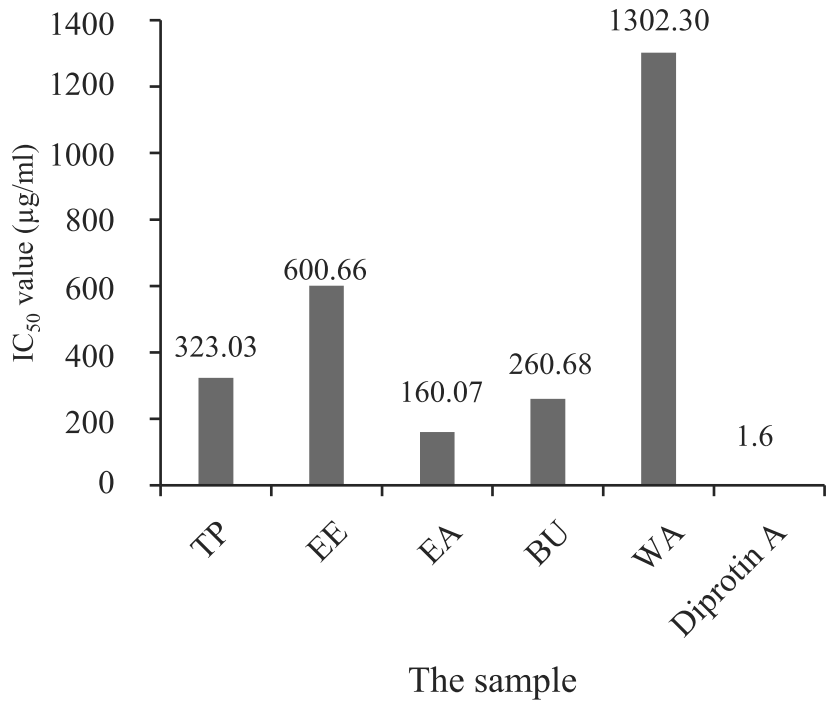

Figure 4. IC_{50} value (µg/mL) of DPP-IV inhibitory activity of total extract and fractions of *S. malaccense* leaves

3.5. ACE inhibitory activity of *S. malaccense* leaf extract

According to the research findings displayed in Figure 4, extracts from *S. malaccense* leaves exhibit ACE inhibitory activity ranging from 186.32 to 951.65 µg/mL. With an IC_{50} (µg/mL) = 186.32, the EA extract exhibits the most ACE inhibition capacity, followed by the EE, TP, and BU extracts, while the WA extract exhibits the lowest (Figure 5).

The IC_{50} values of the extracts in this experiment were all higher than captopril, indicating that *S. malaccense* leaves still had a low ACE enzyme inhibitory activity level.

The results showed that EA remained the most potent ACE inhibitory fraction (IC_{50} = 186.32 µg/mL), significantly lower than other fractions and TP. However, this IC_{50} value was still higher than that of captopril - the standard ACE inhibitor, indicating

limited activity compared to the synthetic drug. Fractions EE and BU had moderate efficacy, while WA showed the weakest activity. Although not as effective as captopril, the potential of EA in inhibiting ACE is still noteworthy, especially considering the advantage of having few side effects of the drug.

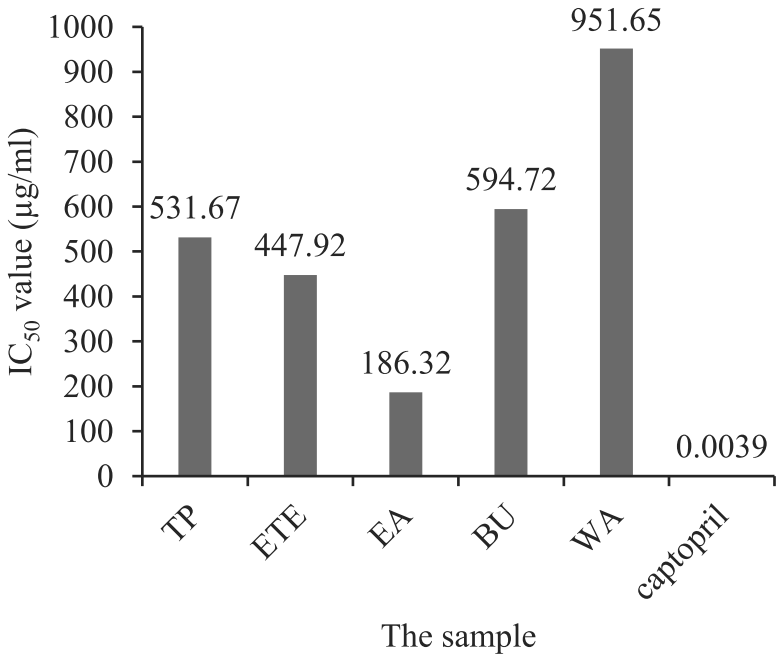

Figure 5. IC_{50} value (µg/mL) of ACE inhibitory activity of total extract and fractions of *S. malaccense* leaves

4. CONCLUSION

Research results show that extracts from *Syzygium malaccense* leaves have potential applications to support the treatment of diabetes and hypertension. *Syzygium malaccense* leaf extracts can inhibit *in vitro* enzymes such as *α*-amylase, *α*-glucosidase, Dipeptidyl Peptidase IV, and Angiotensin-converting enzyme.

However, it is still necessary to understand the enzyme inhibition mechanisms of compounds isolated from extracts of *S. malaccense* leaves and test *in vivo* activity on mice in subsequent studies to aim for producing these drug products to support the treatment of hypertension and diabetes in humans.

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Tiềm năng hạ đường huyết và hạ huyết áp của chiết xuất từ lá Điều đỏ (*Syzygium malaccense* Merr. & L.M.Perry)

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TÓM TẮT

Đặt vấn đề: Đái tháo đường và tăng huyết áp là những bệnh có tỷ lệ gia tăng ở người. Các enzyme mục tiêu của hai bệnh này là α-glucosidase, α-amylase, dipeptidyl peptidase-IV (DPP-IV) và enzyme chuyển đổi angiotensin (ACE). Mục tiêu nghiên cứu: Mục tiêu của nghiên cứu này là xác định khả năng ức chế α-glucosidase, α-amylase, dipeptidyl peptidase-IV (DPP-IV), enzyme chuyển đổi angiotensin (ACE) của chiết xuất từ lá S. malaccense. Đối tượng và phương pháp nghiên cứu: Lá S. malaccense được chiết với cồn 96 theo tỉ lệ 1:25 (w/v) thu được cao toàn phần (TP). Một lượng cao toàn phần được chiết phân đoạn lần lượt với các dung môi là ethyl ether, ethyl acetate và n-butanol để thu nhận được các cao là cao ethyl ether (cao EE), ethyl acetate (cao EA), n-butanol (cao BU) và dịch nước còn lại (cao WA). Cao TP và các cao phân đoạn sẽ được xác định hoạt tính ức chế các enzyme. Kết quả: Cao phân đoạn BU có hoạt tính ức chế mạnh nhất đối với enzyme α-amylase với giá trị IC_{50} là 265.23 µg/mL. Cao phân đoạn etyl acetat (EA) có hoạt tính ức chế cao nhất đối với α-glucosidase, DPP-IV và ACE với giá trị IC_{50} lần lượt là 107.34 µg/mL,

160.07 µg/mL và 186.32 µg/mL. Kết luận: Cao phân đoạn EA và BU có tiềm năng trong việc tạo ra các chế phẩm hỗ trợ điều trị đái tháo đường và tăng huyết áp.

Từ khóa: *Syzygium malaccense, α-glucosidase, α-amylase, angiotensin-converting enzyme (ACE), Dipeptidyl peptidase IV (DPP-IV)*

Received: 27/3/2025
Revised: 16/6/2025
Accepted for publication: 17/6/2025