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Research Article

Phytochemical Screening and Antioxidant, Anti-diabetic Properties Evaluation of *Lasia spinosa* L. Thwaites Stem Extracts

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Abstract

Background and Objective: *Lasia spinosa* L. Thwaites is an herbaceous perennial plant that is well-known for its multiple functions in terms of pharmacological effects. This study aimed to identify the capability to against free radicals and enzymatic glycosylation *in vitro* to examine its oxidative stress inhibition, thereby discover an innovative diabetes therapy of the stem extracts of *Lasia spinosa* L. Thwaites. **Materials and Methods:** The fractions were first used to determine the phytochemical constituents, the total content of phenolic and flavonoid. Antioxidant activities were evaluated by using various assays including DPPH, ABTS, Total Antioxidant Capacity (TAC) and Reducing Power (RP). The findings through α -amylase and α -glucosidase inhibition assays finally demonstrated the anti-diabetic capabilities of the fractions. A one-way analysis of variance experiment was carried out by applying SPSS 18.0 software. The significant differences between means which were defined by Student's t-test and p-values < 0.05 were regarded as statistically significant. **Results:** The result revealed that the extract of ethyl acetate fraction of *Lasia spinosa* L. Thwaites stems presented high levels of phenolic and flavonoid compounds. The ethyl acetate extract also had an antioxidant activity with the most efficient EC₅₀ value, compared to the remaining in DPPH, ABTS, TAC and RP assays. Further examination with enzymatic glycosylation inhibition showed a remarkable IC₅₀ value in the anti-diabetes assay of ethyl acetate fraction. **Conclusion:** The present study has given primary evidence for *Lasia spinosa* L. Thwaites stem extracts to be considered as a therapeutically valuable agent for the treatment of diabetes mellitus and oxidative stress co-morbidities.

Key words: Anti-diabetic, antioxidant, α -amylase, α -glucosidase inhibition, *Lasia spinosa* L. Thwaites

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive Oxygen Species (ROS) are also known as free radicals which play the roles of both destructive and constructive species. According to Matés *et al.*¹, ROS are naturally formed by physiological and biological metabolism in organisms. The free radicals potentially create oxidative destruction of the primary bio-molecules by the alteration of the normal oxidation-reduction state. However, besides their destructive effects on the organism, ROS plays a role in signalling elements vital for the proliferation and survival of cells². Therefore, it is necessary to keep the balance between these good and bad effects of ROS. Failure maintenance may cause negative oxidative stress to the organisms. In a recent study, the result showed that oxidative stress had a very close relationship with the development of various human diseases such as diabetes, neurodegenerative and vascular diseases, cancer, ageing, osteoporosis, inflammatory bowel diseases and obesity³.

The incidence of diagnosed diabetes has increased at an alarming rate, making it the worst health problem all over the world. Merger *et al.*⁴ found that patients with double diabetes exhibited considerably more macro vascular-related comorbidities, including coronary heart disease, stroke, diabetic foot syndrome. Also, the prevalence of microvascular diseases such as retinopathy, nephropathy was increased in those who are with double diabetes. The increase of both macrovascular and microvascular comorbidities was independent of the control of glucose. Diabetes mellitus is characterized as a metabolic disorder of carbohydrate metabolism which includes hyperglycemia without the proper regulation of insulin. Hyperglycemia may lead to pro-atherogenic events by oxidative stress in the cell. Therefore, its initial treatment is aimed at alleviating oxidative stress. Besides, regulation of ROS-sensitive signalling pathways may help mitigate cardiovascular complications of diabetes⁵.

Lasia spinosa L. Thwaites is a herbaceous plant that belongs to the family of Araceae. It is widely used as a medicinal plant in Vietnam to treat cough, inflammatory diseases, swollen lymph nodes, rheumatism and injuries. The *Lasia spinosa* L. Thwaites extracts had antioxidant properties, anti-microbial activities, cytotoxicity and anti-cancer abilities⁶⁻⁸. Rahman *et al.*⁹ indicated that the extracts of *Lasia spinosa* L. Thwaites contained an abundance of phenolic and flavonoid compounds. Its anti-oxidant constituents can combat oxidative stress through the free radical scavenging mechanism of phytochemicals such as vitamin E, vitamin C, flavonoids and polyphenols¹⁰. In this study, the crude ethanol

and fraction extracts from *Lasia spinosa* L. Thwaites stem were used to evaluate phytochemical constituents, total flavonoid and phenolic contents, anti-oxidant and anti-diabetes abilities.

MATERIALS AND METHODS

Sample collection and extraction

Plant samples: The stem of matured *Lasia spinosa* (L) Thwaites was freshly collected from An Giang province, Vietnam in September, 2019. The plant sample was authenticated by Dr. Nguyen Thi Kim Hue, Department of Biology, CanTho University. The sample was stored in the botanical laboratory with the corresponding number (Lsp-Agi67).

Extraction: The *Lasia spinosa* L. Thwaites stem was obtained, rinsed with water and shade dried at 40°C in a dryer machine. The dried stem was then pulverized by using a mechanical grinder. One hundred gram of the powdered stem was subjected to extraction with 1.0L of ethanol by maceration for 24 hrs. The extraction was filtered by using Whatman No. 1 filter paper. The concentrated extract was obtained in a rotary evaporator at 50°C by evaporating the solvent. Samples were independently prepared in triplicate. The crude extract was treated with n-hexane and ethyl acetate by liquid-liquid extraction method to obtain n-hexane, ethyl acetate and water fractions. The production of ethanolic extract and fractioned extracts were stored at -4°C for further analysis.

Phytochemical screening

Test for alkaloids: A few drops of Wagner's reagent were treated with 1 mL of acidified extracts to observe red-brown precipitate which indicates the presence of alkaloids.

Test for flavonoids: To the extracts, a few drops of concentrated sulfuric acid were added. The formation of intense yellow-orange colour was considered to confirm flavonoids.

Test for triterpenoids: In the Liebermann-Burchard reaction, the extracts were treated with 1.0 mL of acetic anhydride, 1.0 mL of chloroform and a drop of concentrated sulfuric acid. The formation of red, pink or violet determined the presence of triterpenoids.

Test for glycosides: The extracts were treated with a few drops of Fehling solution which was prepared by mixing a 1:1 ratio of Fehling's A (copper (II) sulfate solution) and Fehling's B (potassium sodium tartrate and sodium hydroxide

solution) and then boiled in a water bath for few minutes to observe the dark blue colour which indicated the presence of glycosides.

Test for tannins: To 2 mL of the extracts, five drops of 1% gelatin solution was added. The presence of tannins was determined by the formation of white precipitate.

Test for saponins: A volume of 1 mL of extracts were dissolved in 5 mL of distilled water. The solution was vigorously shaken to observe the frothing which persists. The formation of an emulsion after 15 min of incubation period at room temperature was taken as evidence for the presence of saponins.

Total content of phenolic: The total content of phenolic in ethanolic and fraction extracts was quantified by the method of Ayoola *et al.*¹¹. One mg of stock sample, 0.5 mL of Folin-Ciocalteu's reagent and 10 mL of distilled water were concocted together and incubated for 3 min. The concoction was then added by 1 mL of saturated Na₂CO₃ to get the total volume up to 25 mL. After 60 min of incubating protected from light, the absorbance of the solution was recorded at 750 nm wavelength by a spectrophotometer. The calibration curve was obtained by preparation of different concentrations of gallic acid (from 5-150 mg mL⁻¹). Samples were separately analyzed in triplicate. The mean of three tests was used to calculate the total content of phenolic content with gallic acid as standard and expressed in milligrams of gallic acid equivalent per gram of extracts (mg GAE g⁻¹).

Total content of flavonoid: The total content of flavonoids of the ethanolic and fraction extracts was quantified by the method of Thitilertdecha *et al.*¹². The stock sample (0.25 mg) was dissolved in 5 mL of distilled water, then in 0.3 mL sodium nitrite 5% solution. After 6 min of incubating protected from light, the concoction was added by 0.6 mL of 10% aluminium chloride hexahydrate solution and then incubated in the dark condition. A volume of 2 mL of sodium hydroxide 1 M was added to the concoction. The distilled water was utilized to reach the total volume of 10 mL. The absorbance of the solution was recorded at 510 nm wavelength by a spectrophotometer. Samples were independently analyzed in triplicate. The mean of three tests was used to calculate. The total flavonoid content was expressed in mg of quercetin equivalent per gram of extract (mg QE g⁻¹).

Antioxidant activity

Total Antioxidant Capacity assay (TAC): The method of Aliyu *et al.*¹³ was applied to identify the total antioxidant.

About 1 mL of reagent solution which included 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was added to 0.1 mL of stock sample. The concoction was then incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was recorded at 695 nm wavelength by a spectrophotometer. Samples were separately analyzed in triplicate. The mean of three tests was used to calculate. The results were estimated from standard curves of Trolox at 35.17 ± 0.82 µg mL⁻¹.

ABTS radical scavenging assay: A slightly modified method of Nikolaos *et al.*¹⁴ was used for the ABTS^{•+} (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of the extracts. The ABTS reagent which was a mixture of 5 mL of 7 mM ABTS and 88 µL of 140 mM potassium persulfate was incubated in dark conditions at room temperature for 16 hrs. After controlled to obtain the optical density of 0.7 ± 0.05 (1:44, v/v) at 734 nm wavelength by a spectrophotometer, the ABTS reagent (100 µL) was mixed with the sample (100 µL) and incubated for 6 min at room temperature. The absorbance of the solution was then recorded at 734 nm wavelength by a spectrophotometer after its incubation period. Samples were independently analyzed in triplicate. The mean of three tests was used to calculate. The results were estimated from standard curves of Trolox at 3.31 ± 0.05 µg mL⁻¹.

Reducing power assay: The slightly modified method suggested by Ferreira *et al.*¹⁵ using potassium ferricyanide was applied in the assay of reducing power. The concoction of 500 mL of extracts with different concentrations, including 500 µL of 0.2 M phosphate buffer (pH = 6.6) and 500 µL of 0.2 M phosphate buffer (pH = 6.6) were incubated for 20 min at 50°C. A volume of 500 µL of 10% Trichloroacetic acid was added to the concoction to discontinue the reaction, then centrifuged at 3000 rpm for 10 min. The supernatant obtained was mixed with 500 µL of distilled water and 100 µL of 0.1% ferric chloride. The absorbance of the solution was recorded at 700 nm wavelength by a spectrophotometer. Samples were separately analyzed in triplicate and the mean of three tests was used to calculate. The results were estimated from standard curves of Trolox at 1.93 ± 0.11 µg mL⁻¹.

DPPH radical scavenging assay: The DPPH radical scavenging capacity of the extracts was determined according to the method described by Ak and Gülçin¹⁶ with slight modification. Four mg of DPPH was weighed to dilute in 100 mL of ethanol to make 2 mL of DPPH reagent solution. It was then mixed with 1 mL of each concentration and incubated for 30 min at room temperature. The absorbance of each solution was

measured at 517 nm wavelength by a spectrophotometer after the incubation period. The results were calculated from standard curves of Trolox at $0.64 \pm 0.01 \mu\text{g mL}^{-1}$.

In vitro anti-diabetic activity

α -amylase inhibition assay: The α -amylase inhibition assay was performed by a slightly modified method by Xiao *et al.*¹⁷ The amount of 50 μL of extracts was diluted in 50 μL phosphate buffer (pH = 7.0) and 50 μL of 3 U mL^{-1} enzyme α -amylase. After that, the solution was incubated at 37°C for 5 min. The mixture was added by 50 μL of 2 mg mL^{-1} starch solution and conducted to incubate up to 15 min at 37°C. The volume of 200 μL of HCl 1 M solution was utilized to discontinue the reaction. After adding 300 μL of iodine reagent, the absorbance of the reaction mixture was recorded at 660 nm wavelength by a spectrophotometer. Acarbose ($45.88 \pm 0.94 \mu\text{g mL}^{-1}$) was prepared as a positive control in this assay.

α -glucosidase inhibition assay: The α -glucosidase inhibition assay was conducted by the slightly modified method of Sancheti *et al.*¹⁸. The 20 μL of 1 U mL^{-1} α -glucosidase enzyme, 100 μL phosphate buffer 100 mM (pH = 6.8) and 40 μL of the extract were mixed and then incubated for 15 min at 37°C. Then the concoction was added by 40 μL of 5 mM *p*-nitrophenyl- α -D-glucoside and incubated for up to 20 min at 37°C. After adding 100 μL sodium carbonate 0.1 M to the concoction, the absorbance of the solution was recorded at 405 nm wavelength by a spectrophotometer. Acarbose ($7.20 \pm 0.09 \mu\text{g mL}^{-1}$) was prepared as a positive control in this assay.

Statistical analysis: All data were expressed as mean \pm standard deviation from triplicate determinations. IC_{50} and EC_{50} values were inferred from interpolation. The recorded data were treated statistically using a one-way analysis of variance (ANOVA). Statistical comparisons of means followed by the Student's T-test with $p < 0.05$ considered to be statistically different. All statistical analysis was performed using SPSS software.

RESULTS

Phytochemical screening: Table 1 showed the constituents found in ethanolic and fraction extracts. Phytochemical screening showed the existence of many phytoconstituents including alkaloid, flavonoid, glycoside and tannin in all the extracts. Analysis of triterpenoid in the three extracts was

positive but a negative result was observed in the water extract. Saponins were present in the water extract but absent in the rest of the three extracts.

Total phenolic content: The total phenolic contents of ethanolic and fraction extracts were shown in Table 2. The amount of total phenolic content had various values, ranging from 14.08 ± 0.89 – $83.15 \pm 0.72 \text{ mg GAE g}^{-1}$ of the extracts. The highest quantity of phenolic compounds determined was represented to ethyl acetate extract with $83.15 \pm 0.72 \text{ mg GAE g}^{-1}$ of the extract, while n-hexane had the lowest total phenolic compounds with $14.08 \pm 0.89 \text{ mg GAE g}^{-1}$ of the extract.

Total flavonoid content: The total flavonoid content of the extracts is described in Table 2. Ethyl acetate showed the highest level of flavonoid with $590.10 \pm 51.78 \text{ mg QE g}^{-1}$ of extract. The difference between the largest ethyl acetate extract ($590.10 \pm 51.78 \text{ mg QE g}^{-1}$ of the extract) and the smallest n-hexane extract ($127.47 \pm 1.64 \text{ mg QE g}^{-1}$ of the extract) in flavonoid content was approximately 462.63 mg of flavonoid equivalents per gram of the extracts.

Anti-oxidant activity

DPPH radical scavenging activity: Anti-oxidant activities by DPPH assay of ethanolic and fraction extracts were shown in Table 3 with Trolox as a positive control ($\text{EC}_{50} = 0.64 \pm 0.01 \mu\text{g mL}^{-1}$). The effective extract against DPPH free radical was ethyl acetate with EC_{50} of $0.81 \pm 0.02 \mu\text{g mL}^{-1}$. The EC_{50} value of water extract was $10.68 \pm 0.20 \mu\text{g mL}^{-1}$, followed by the ethanol extract with EC_{50} of $81.00 \pm 0.57 \mu\text{g mL}^{-1}$ and n-hexane extract with EC_{50} of $748.08 \pm 11.68 \mu\text{g mL}^{-1}$.

Total anti-oxidant capacity: The total antioxidant capacity of the extracts was shown in Table 3. All extracts exhibited effective anti-oxidant ability, instead of the n-hexane extract ($\text{EC}_{50} = 128.38 \pm 1.86 \mu\text{g mL}^{-1}$). The extract of ethyl acetate ($\text{EC}_{50} = 10.17 \pm 0.11 \mu\text{g mL}^{-1}$) resulted in the best effect of total antioxidant capacity, even stronger than that of the standard Trolox ($\text{EC}_{50} = 35.17 \pm 0.82 \mu\text{g mL}^{-1}$), followed by the extract of water ($\text{EC}_{50} = 54.46 \pm 0.56 \mu\text{g mL}^{-1}$), ethanol ($\text{EC}_{50} = 58.13 \pm 0.99 \mu\text{g mL}^{-1}$) and n-hexane ($\text{EC}_{50} = 128.38 \pm 1.86 \mu\text{g mL}^{-1}$).

ABTS radical scavenging activity: All the extracts evaluated for antioxidant activities by ABTS assay with Trolox was a positive control ($\text{EC}_{50} = 3.31 \pm 0.05 \mu\text{g mL}^{-1}$). As presented in

Table 1: Phytochemical screening of ethanolic extract and fractioned extracts

Extracts	Alkaloid	Flavonoid	Triterpenoid	Glycoside	Tannin	Saponin
Ethanol	+	+	+	+	+	-
n-hexane	+	+	+	+	+	-
Ethyl acetate	+	+	+	+	+	-
Water	+	+	-	+	+	+

+: Present, -: Absent

Table 2: Total phenol and flavonoid contents of the stem extracts of *Lasia spinosa* (L.) Thwaites

Extracts	Total phenolic (mg GAE g ⁻¹ extract)	Total flavonoid (mg QE g ⁻¹ extract)
Ethanol	60.85±0.31 ^b	178.08±73.73 ^c
n-hexane	14.08±0.89 ^d	127.47±1.64 ^c
Ethyl acetate	83.15±0.72 ^a	590.10±51.78 ^a
Water	32.13±0.98 ^c	334.97±74.84 ^b

Different letters in superscript signify statistically significant differences from one another (p<0.05)

Table 3: Antioxidant activities of the stem extracts of *Lasia spinosa* (L.) Thwaites by using *in vitro* methods

Extracts	DPPH (µg mL ⁻¹)	ABTS (µg mL ⁻¹)	TAC (µg mL ⁻¹)	RP (µg mL ⁻¹)
Ethanol	81.00±0.57 ^b	9.49±0.07 ^b	58.13±0.99 ^b	49.03±0.11 ^b
n-hexane	748.08±11.68 ^a	100.63±0.37 ^a	128.38±1.86 ^a	391.31±17.90 ^a
Ethyl acetate	0.81±0.02 ^d	2.85±0.03 ^e	10.17±0.11 ^e	12.96±0.16 ^{cd}
Water	10.68±0.20 ^c	8.69±0.05 ^c	54.46±0.56 ^c	26.70±0.02 ^c
Trolox	0.64±0.01 ^d	3.31±0.05 ^d	35.17±0.82 ^d	1.93±0.11 ^d

Different letters in superscript signify statistically significant differences from one another (p<0.05)

Table 4: Inhibitory capacity of the stem extracts of *Lasia spinosa* (L.) Thwaites against α-amylase and α-glucosidase activities by using *in vitro* models

Extracts	α-amylase (µg mL ⁻¹)	α-glucosidase (µg mL ⁻¹)
Ethanol	80.70±3.39 ^b	0.50±0.01 ^c
n-hexane	473.83±9.77 ^a	904.80±4.84 ^a
Ethyl acetate	56.00±1.25 ^c	0.41±0.00 ^c
Water	82.81±0.94 ^b	0.85±0.01 ^c
Acarbose	45.88±0.94 ^d	7.20±0.09 ^b

Different letters in superscript signify statistically significant differences from one another (p<0.05)

Table 3, most of the extracts showed a good scavenging effect on free radicals, especially the ethyl acetate extract (EC₅₀ = 2.85±0.03 µg mL⁻¹), in comparison with the Trolox standard (EC₅₀ = 3.31±0.05 µg mL⁻¹). However, a weak inhibition of ABTS^{•+} radical was expressed in n-hexane with EC₅₀ of 100.63±0.37 µg mL⁻¹.

Reducing power activity: The investigation of the reducing power activity was shown in Table 3, with standard Trolox (EC₅₀ = 1.93±0.11 µg mL⁻¹). Ethyl acetate was the most effective extract with EC₅₀ value of 12.96±0.16 µg mL⁻¹, followed by water extract (EC₅₀ = 26.70±0.02 µg mL⁻¹) and ethanol extract (EC₅₀ = 49.03±0.11 µg mL⁻¹). The lowest reducing power activity was n-hexane with an EC₅₀ value of 391.31±17.90 µg mL⁻¹.

Anti-diabetic activity: The enzymatic inhibitory effects of the extracts on α-amylase and α-glucosidase were described in Table 4 by using Acarbose as a standard with IC₅₀ of 45.88±0.94 and 7.20±0.09 µg mL⁻¹, respectively. The ethyl acetate extract (IC₅₀ = 56.00±1.25 µg mL⁻¹) was the effective extract against α-amylase for the rest of the three extracts.

Also, moderate inhibitory action was observed in ethanol and water extracts with IC₅₀ of 80.70±3.39 and 82.81±0.94 µg mL⁻¹, respectively. The extracts of ethyl acetate, ethanol and water exhibited the most significant results against α-glucosidase with IC₅₀ of 0.41±0.00, 0.50±0.01 and 0.85±0.01 µg mL⁻¹, respectively. In contrast, the n-hexane extract showed weak suppression to both enzymes with IC₅₀ larger than 400 µg mL⁻¹.

DISCUSSION

This study aimed to evaluate the preliminary assay of crude ethanolic and stem extracts of *Lasia spinosa* L. Thwaites. Phytochemical analysis was conducted to evaluate the phytochemical composition of *Lasia spinosa* L. Thwaites. As the results are shown in Table 1, the extract of ethyl acetate fraction contained alkaloids, flavonoids, triterpenoids, glycosides and tannins but not saponins, while the water extract contained only saponins. Kumar *et al.*¹⁹ confirmed the presence of flavonoids, glycosides, tannins and phenolic compounds while water extract contained alkaloids, glucosides, saponins, tannins and phenolic compounds.

Additionally, the presence of flavonoids, terpenoids, phenolic compounds, steroids, saponins, coumarins, glycosides and anthraquinones was observed in the ethanolic extract of *Lasia spinosa* L. Thwaites²⁰.

The total flavonoid content of *Lasia spinosa* L. Thwaites stem was determined by using quercetin as a standard, while gallic acid was used for quantification of total polyphenols. The ethyl acetate fraction showed a remarkable amount of phenolic and flavonoid compounds among the rest which was about 83.15 ± 0.72 mg GAE g^{-1} and 590.10 ± 51.78 mg QE g^{-1} , respectively. Further, the methanolic extract of *Lasia spinosa* L. Thwaites stem contained lower total phenolics and flavonoids with 1.01 ± 0.12 μ g GAE g^{-1} of extract and 33.62 ± 1.10 μ g QE g^{-1} of extract, respectively²¹.

Anti-oxidant activity of the ethanolic extract and its fraction were tested by using DPPH, ABTS, TAC and RP assay assessed by EC₅₀ values. The lowest EC₅₀ concentration indicated the strongest anti-oxidant ability. All the antioxidant assays showed that the ethyl acetate fraction had a notable inhibition of free radicals, EC₅₀ = 0.81 ± 0.02 , 2.85 ± 0.03 , 10.17 ± 0.11 and 12.96 ± 0.16 μ g mL⁻¹, respectively for DPPH, ABTS, TAC and RP assay. In the DPPH assay, the fractioned extract of ethyl acetate (EC₅₀ = 0.81 ± 0.02 μ g mL⁻¹) showed a higher scavenging radical ability, compared to the study of Goshwami *et al.*⁷ (EC₅₀ = 16.42 μ g mL⁻¹). The ethanolic extract of *Lasia spinosa* L. Thwaites (EC₅₀ = 9.49 ± 0.07 μ g mL⁻¹) showed a lower EC₅₀ value than the study of Nguyen *et al.*²² with EC₅₀ of 16.47 μ g mL⁻¹ in ABTS assay. The recent study of Marisetti *et al.*²³ proved that methanolic extract from *L. spinosa* rhizome places the most important role in dose-dependent antioxidant and anti-inflammatory effects at various levels. These findings contribute to the confirmation of its antioxidant activity of *L. spinosa*.

Based on the findings from this study, the anti-diabetic ability of the extracts was expressed by IC₅₀ values determined through the inhibition of α -amylase and α -glucosidase. The α -amylase inhibitor activity of ethanolic stem extract was observed IC₅₀ value at 80.70 ± 3.39 μ g mL⁻¹ while the ethanolic leaves and roots extract inhibited 33.80 ± 3.27 and $15.37 \pm 0.53\%$ of α -amylase at 0.1 mg mL⁻¹, respectively²⁴. In the present study, the inhibitor ability of ethanolic stem extract (IC₅₀ = 0.50 ± 0.01 μ g mL⁻¹) showed a higher effect on α -glucosidase compared to ethanolic leaves, stem and root extract, IC₅₀ = 36.24 ± 0.20 , 04.21 ± 1.05 , $27.73 \pm 4.00\%$ at 0.1 mg mL⁻¹, respectively by Shafie *et al.*²⁴. Additionally, the inhibition value of methanolic root extract (IC₅₀ = 79.8 μ g mL⁻¹) by Nguyen *et al.*²⁵ was higher than the extract of ethanol, ethyl acetate and water in this study with IC₅₀ of 0.50 ± 0.01 , 0.41 ± 0.00 and 0.85 ± 0.01 μ g mL⁻¹, respectively. From another study, Hasan *et al.*²⁶ determined

the antihyperglycemic effects of methanolic and ethanolic extracts from the leaves of *L. spinosa* in oral glucose tolerance tests compared with the standard in Swiss albino mice. The experiments' results indicated that leaf extract of *L. spinosa* shows potent anti-diabetic activity in mice.

CONCLUSION

The current study illustrated a remarkable antioxidant and anti-diabetes effect of *Lasia spinosa* L. Thwaites stem compared to those of roots and leaves. Especially, its ethyl acetate fraction resulted in 18 times significantly higher compared to Acarbose against the inhibition of α -glucosidase. Noticeably, the result indicated that ethyl acetate fraction presents many phytochemical compounds including polyphenol, alkaloids, triterpenoids, glycosides and tannins as well as the highest amount of total polyphenol and flavonoids among the solvent used for extraction.

SIGNIFICANCE STATEMENT

This study discovered the biological activities in terms of antioxidant and anti-diabetes of *Lasia spinosa* L. Thwaites that can be beneficial for prospective aspects to alternative commercial medicines for the treatment of oxidative-related disease. This study will help the researchers to uncover the critical areas of antioxidant and anti-diabetes drugs from medicinal plants that many researchers were not able to explore. Thus new natural chemical compounds may be arrived at.

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