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Research Article
Investigation of *in vitro* Biological Activity from Extracts of *Ruellia tuberosa*

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Abstract

Background and Objective: *Ruellia tuberosa* is a common plant in the Mekong Delta and is widely used in many Vietnamese folk remedies. This study was conducted to investigate the potential use of roots, stems, leaves of *Ruellia tuberosa* as antioxidant, antimicrobial, α-amylase and α-glucosidase inhibitors. **Materials and Methods:** The extracts were tested for their ability to inhibit the enzymes α-amylase and α-glucosidase associated with diabetes. The antioxidant activities of the extracts were evaluated using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and 2,2-Azino-Bis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC) and reducing power (RP) assays. The antibacterial activity of extracts from *Ruellia tuberosa* was evaluated by the agar well diffusion method. **Results:** The root extract of *Ruellia tuberosa* has more polyphenols (32.49±0.72 mg GAE/g extract) and flavonoids (15.48±1.32 mg QE/g extract) than the other parts. Simultaneously, the root extract of *Ruellia tuberosa* has antioxidant activity (IC_{50} values range from 117.67±2.82 to 569.20±7.68 µg/mL), inhibiting amylase (IC_{50} = 266.72±10.58 µg/mL) and glucosidase (IC_{50} = 147.13±3.58 µg/mL) enzymes more effectively than the other parts. Research results also show that extracts from *Ruellia tuberosa* are capable of inhibiting *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria with minimum inhibitory concentrations ranging from 1280 to 10240 mg/mL. **Conclusion:** These results highlighted the potential using of *Ruellia tuberosa* extracts as natural antioxidant, antimicrobial, α-amylase and α-glucosidase inhibitors agents.

Key words: Antimicrobial, antioxidant, enzyme-amylase, enzyme-glucosidase, *Ruellia tuberosa*

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Diabetes is a chronic disease caused by the ineffective functioning of insulin, often leading to increased blood glucose after meals and many other complications. Diabetes has become the main cause of death in people under 60 years of age and deaths due to diabetes account for nearly 9% of all deaths globally[1]. Therefore, finding effective treatments for diabetes and its complications is essential. A promising approach to controlling diabetes, especially type 2 diabetes, is to reduce postprandial blood glucose levels by inhibiting carbohydrate-hydrolyzing enzymes in the digestive tract. Specifically, the enzyme α-amylase participates in the process of decomposing starch and α-glucosidase decomposes oligosaccharides and disaccharides. Inhibitors of these enzymes slow carbohydrate digestion, thereby prolonging overall digestion, reducing glucose absorption and thereby reducing postprandial plasma glucose levels[2-4]. Previous experimental and clinical studies reported that oxidative stress plays a major role in the pathogenesis and development of complications of diabetes. Increased blood glucose levels promote the formation of free radicals and reduce endogenous antioxidant levels[5,6]. In addition, diabetic patients often develop amputation complications and are easily infected with pathogenic bacteria[7,8]. Plants are also considered a source of compounds with antibacterial activity[9,10]. The antibacterial activity of plants can be determined by observing the growth response of microorganisms to plant extracts. Plants possess antibacterial activity due to the presence of bioactive compounds such as glycosides, saponins, flavonoids and alkaloids[11,12].

Ruellia tuberosa is a plant species commonly distributed in the Mekong Delta provinces. Studies around the world show that *Ruellia tuberosa* possesses many important pharmacological activities, including anti-inflammatory, antioxidant, hypoglycemic and xanthine oxidase inhibitory abilities[13-16]. The *Ruellia tuberosa* contains many important secondary metabolites such as: Cirsimaritin, cirsimarin, cirsiliol 4-glucoside, sorbifolin and pepeditin, betulin, vanillic acid, indole-3-carboxaldehyde, apigenin and luteolin[17-19]. In folk medicine, *Ruellia tuberosa* is used as a diuretic, fever reducer, analgesic and hypotensive[20,21], however, scientific studies proving the biological activity of *Ruellia tuberosa* in Vietnam are still limited. This study focused on investigating the antioxidant, antibacterial and inhibitory activities of α-amylase and α-glucosidase enzymes, in order to contribute a small part to research projects so that *Ruellia tuberosa* can be applied and exploited stronger.

MATERIALS AND METHODS

Tested plants: *Ruellia tuberosa* was found in March 2024 in Phuoc Loc, Tan Loc Ward, Thot Not District, Can Tho City (coordinates 10°14'59.3"N 105°34'47.9"E). *Ruellia tuberosa* was identified using morphological traits mentioned in Pham Hoang Ho's (2003) Vietnamese herb book series, with the assistance of PhD. Thieu Van Duong (Head of Department of Biochemistry, Tay Do University). Figure 1 illustrated the morphological traits of *Ruellia tuberosa*.

Bacterial strains: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27855, *Escherichia coli* ATCC® 25922TM were provided by the Department of Biology, Faculty of Natural Sciences, Can Tho University, Vietnam.

Equipment: Drying oven (BE 200, Memmert, Germany), analytical balance (AB104-S, Mettler Toledo, Switzerland), cold centrifuge (Mikro 12-24, Hettich, Germany), incubation tank (Memmert, Germany), rotary vacuum evaporator (Heidolph, Germany), spectrophotometer (Thermo Scientific Multiskan GO, Finland), autoclave sterilizer STURDY SA-300VF (STURDY, Taiwan), biological safety cabinet JEIOTECH BC-11B (JEIOTECH, Korea) and advanced vortex mixer ZX3 (Velp, Italia).

Chemicals: Ethanol is offered by Cemaco (Vietnam). Folin-Ciocalteu's phenol reagent, sodium carbonate, dimethyl sulfoxide, potassium persulfate, potassium ferricyanide, methanol, trichloroacetic acid, gallic acid and quercetin are offered by Merck (Germany). Ascorbic acid, 2,2-azino-bis (3-ethylbanzthiazoline-6-sulphonic acid), 2, 4, 6-tripyridyl-s-triazine, 2,2-diphenyl-1-picrylhydrazyl, ferric chloride, α-amylase enzyme, α- glucosidase enzyme, acarbose and diclofenac, bovine serum albumin are provided by Sigma-Aldrich (United States). Sodium nitrite, aluminum chloride hexahydrate and ammonium heptamolybdate tetrahydrate are offered by Xilong (China).

Preparation of plant extracts: After harvest, *Ruellia tuberosa* is cleaned and split into three parts: Roots, stems and leaves. The parts will be dried at 50±2°C and pulverized into a pharmaceutical powder with a particle size of around 60 mesh. The medicinal powders of *Ruellia tuberosa* roots, stems and leaves are determined in terms of moisture by applying heat to evaporate all of the water vapor in the medicinal herbs, as defined in Vietnam Pharmacopoeia V, 2018. Medicinal powder with moisture meeting Vietnam

Fig. 1: Morphology of *Ruellia tuberosa*

Pharmacopoeia V standards will be soaked in ethanol (96%) at a material/solvent ratio of 1/10 (w/v), at room temperature for 24 hrs. The medicinal powder is soaked three times, the extracts from the soaks are collected and the solvent is evaporated using a vacuum rotary evaporator under low pressure at 50°C to yield the extracts. At Nam Can Tho University's, Department of Botany and Medicinal Materials, extracts from *Ruellia tuberosa* roots, stems and leaves were stored in glass jars that have been labeled and kept in the refrigerator.

Determination of total polyphenol content: To evaluate total polyphenol content, mix 50 µL of extract, 50 µL of deionized water and 50 µL of Folin-Ciocalteu reagent and shake well. Then, add 50 µL of 10% Na_2CO_3 and incubate for 30 min at 40°C. The spectral absorbance of the reaction mixture was determined at 765 nm. Gallic acid was employed as a positive control for creating a standard curve equation. Polyphenol content in *Ruellia tuberosa* extracts was evaluated using the gallic acid standard equation (mg GAE/g high) described by Singleton *et al.*[19].

Determination of total flavonoid content: The following method was used to determine total flavonoid content: Shake a reaction mixture containing 0.5 mL extract and 0.5 mL deionized water. The reaction mixture was combined with 100 µL of $NaNO_2$ (5%), left for 5 min, then added 100 µL of $AlCl_3$ (10%) and shook thoroughly. After 6 min of incubation, 1 mL of NaOH (1M) was added to the reaction mix. Finally, enough water was added to the reaction mixture to form 2.5 mL. The reaction mixture's absorbance was measured spectrophotometrically at 510 nm. Quercetin was utilized to create the standard curve. The total flavonoid content of *Ruellia tuberosa* extracts was measured using the quercetin standard curve equation (mg QE/g extract) reported by Bag *et al.*[6].

Investigating the neutralization effect of 2,2-diphenyl-1-picrylhydrazyl free radical: The antioxidant potential of *Ruellia tuberosa* extracts was tested using Sharma and Bhat's[22] DPPH free radical neutralization technique, with modifications. The reaction mixture included 480 µL of extract and 20 µL of DPPH solution (1000 µg/mL) in eppendorfs. Incubate in the dark for 30 min before measuring the absorbance spectra at 517 nm. The ability to neutralize DPPH free radicals is determined by the IC_{50} value (inhibitory concentration of 50 percent) and DPPH free radical neutralization efficiency. The IC_{50} value is derived using the extract's linear equation. Trolox was utilized as the positive control. The formula for calculating DPPH free radical neutralization effectiveness (%) is:

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

where, A is the spectral absorbance without trolox or extract and B is the spectral absorbance with trolox or extract.

Investigation of the ability to neutralize the free radical 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid): The antioxidant activity of *Ruellia tuberosa* extracts was measured using the $ABTS^+$ decolorization method reported by Nenadis *et al.*[20], with adjustment. The $ABTS^+$ was created by reacting 7 mM ABTS with 2.45 mM potassium persulfate. Prior to use, the mixture was incubated in the dark at room temperature for 16 hrs. After diluting the combination, the spectral absorbance at 734 nm was 0.70±0.05. To conduct the survey, add 10 µL of extract to eppendorf tube, followed by 990 µL of $ABTS^+$. Incubate at 37°C for 6 min and measure spectral absorbance at 734 nm wavelength. Antioxidant capacity is calculated using the IC_{50} value and $ABTS^{\bullet+}$ free radical neutralization efficiency. The IC_{50} value is derived using the extract's linear equation. Trolox was utilized as the positive control. The formula for calculating $ABTS^{\bullet+}$ free radical neutralization effectiveness (%) is as follows:

$$ABTS^{\bullet+} \text{ scavenging effect } (\%) = \frac{A - B}{A} \times 100$$

where, A represents the spectral absorbance in the absence of trolox or extracts and B represents the spectral absorbance in the presence of trolox or extract.

Investigation of the ability to inhibit the formation of nitric oxide free radicals: The suppression of nitric oxide generation by *Ruellia tuberosa* extracts was carried out with changes to

the method published by Sreejayan and Rao[23]. To conduct the survey, add 100 µL of extract to 200 µL of sodium nitroprusside (5 mM) and incubate at 25°C for 60 min. Then, add 300 µL of Griess reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. The spectrophotometric absorbance was measured at 546 nm. The ability to inhibit $NO^{\cdot}$ formation is determined based on the IC_{50} value and inhibition efficiency. The IC_{50} value is derived using the extract's linear equation. The inhibitory efficacy of $NO^{\cdot}$ production is estimated using the following formula:

$$\text{Efficiency inhibition of nitric oxide formation (\%)} = \frac{A - B}{A} \times 100$$

where, A is the spectral absorbance in the absence of trolox or extract and B is the spectral absorbance in the presence of trolox or extract. Trolox served as a positive control.

Ferric reducing-antioxidant power: The antioxidant capacity of *Ruellia tuberosa* extracts was measured using the ferric reducing-antioxidant power method established by Benzie and Strain[3], with modifications. To test, add 10 µL of extract to an eppendorf tube, followed by 990 µL of FRAP solution. After 30 min of incubation in the dark, the spectral absorbance at 593 nm is recorded. The $Abs_{0.5}$ value (absorbance of 0.5) is used to calculate ferric reducing-antioxidant power. The $Abs_{0.5}$ value was derived using the linear equation for the *Ruellia tuberosa* extracts. Trolox was utilized as the positive control.

Total antioxidant capacity: Total antioxidant capacity of *Ruellia tuberosa* extracts was evaluated using the phosphomolybdenum method as described by Prieto *et al.*[16], with corrections. Conduct the survey by putting 300 µL of extract into an eppendorf tube, then adding 900 µL of TAC solution, incubating for 90 min at 95°C. After incubation, measure the spectral absorbance at 695 nm. Total antioxidant capacity is determined based on the $Abs_{0.5}$ value. The $Abs_{0.5}$ value is calculated based on the linear equation of the extracts. Trolox was used as a positive control.

Reducing power: Reducing power of the extracts from *Ruellia tuberosa* was carried out according to the method of Oyaizu[15]. The reaction mixture consisted of 500 µL extract, 500 µL phosphate buffer (0.2 M, pH 6.6) and 500 µL $K_3[Fe(CN)_6]$ (1%). After the reaction mixture was incubated at 50°C for 20 min, add 500 µL CCl_3COOH (10%) and then centrifuge at 3000 rpm for 10 min. After centrifugation, 500 µL was withdrawn and added to 500 µL of water and 100 µL of $FeCl_3$ (0.1%) and shaken well. The spectral absorbance of the reaction mixture was measured at 700 nm. Reducing power is determined based on the $Abs_{0.5}$ value. The $Abs_{0.5}$ value was calculated based on the linear equation of the extracts from Ruellia tuberosa. Trolox was used as a positive control.

Investigation of the ability to inhibit α-amylase enzyme: *Ruellia tuberosa* extracts were tested for α-amylase inhibitory activity using the method published by Mohamed *et al.*[12], with some changes. The reaction mixture included 100 µL of extract at various concentrations, 100 µL of phosphate buffer (pH 7) and 100 µL of starch (2 mg/mL) incubated at 37°C for 5 min. The α-amylase enzyme (3 U/mL, 100 µL) was added to the reaction mixture and incubated at 37°C for 15 min. The reaction was halted by adding 400 µL of 1 M HCl. The absorbance of the reaction mixture was measured at 660 nm after adding 600 µL of iodine reagent. The ability to inhibit the α-amylase enzyme is assessed by the IC_{50} value and inhibition efficiency. The IC_{50} value is derived using the extract's linear equation. The ability to suppress α-amylase enzyme activity was measured based on the inhibition effectiveness:

$$\text{Inhibition effectiveness (\%)} = 100 - \frac{A - B}{A} \times 100$$

where, A represents spectral absorbance without acarbose or extract, while B represents spectral absorbance with acarbose or extract and concentration (µg/mL). Acarbose was employed as the positive control.

Investigation of the ability to inhibit α-glucosidase enzyme: *Ruellia tuberosa* extracts were tested for α-glucosidase inhibitory activity using the method published by Chipiti *et al.*[4], with some changes. Extracts (250 µL) at varying concentrations were treated with 500 µL of 1 U/mL α-glucosidase enzyme (mixed in 100 mM phosphate buffer; pH = 6.8) at 37°C for 15 min. Next, add 250 µL of 5 mM 4-nitrophenyl-D-glucopyranoside solution (mixed in 100 mM phosphate buffer; pH = 6.8) and incubate for 20 min at 37°C. The spectral absorbance of the p-nitrophenol produced during the process was measured at 405 nm. The ability to

inhibit the α-glucosidase enzyme is assessed by the IC_{50} value and inhibition efficiency. The IC_{50} value is derived using the extract's linear equation. The ability to inhibit α-glucosidase enzyme activity was evaluated based on the inhibition efficiency:

$$\text{Inhibition efficiency (\%)} = \frac{A - B}{A} \times 100$$

where, A is the spectral absorbance in the absence of acarbose or extract; B Spectral absorbance in the presence of acarbose or extract and concentration (µg/mL). Acarbose was used as a positive control.

Investigation of antibacterial activity: The antibacterial ring's diameter was determined using the agar well diffusion method. Each extract weighing 0.01 g was diluted in 1000 µL of 10% DMSO (the extract concentration at the time was 10,000 µg/mL). The extracts were diluted with 10% DMSO solvent to reach concentrations of 80, 160, 320, 640 and 1280 µg/mL. After dilution in physiological saline, bacterial fluid has an optical density of 0.5 at 600 nm. Spread 100 µL of bacterial solution on a petri dish with Luria Bertani agar medium, allow to dry, then drill 5 wells with a diameter of 7 mm. Add 50 µL of extract with concentrations of 80, 160, 320, 640 and 1280 µg/mL. To test antibacterial activity, add 50 µL of extract at different doses (80, 160, 320, 640 and 1280 µg/mL) to the wells of a petri dish containing LB agar. The test sample was incubated at 37°C for 24 hrs. The diameter of the antibacterial ring that formed around the well on the agar plate was measured 24 hrs later reported by Ngan *et al*.[8].

Statistical analysis of data: Data are reported as Mean±SEM standard deviation value, Analysis of Variance (ANOVA) and compared for significant differences between samples by test. Duncan at the 5% significance level using Minitab 16.0 software and the ANOVA method (Tukey's test).

RESULTS

Results of preparation of extracts, quantification of polyphenols, total flavonoids in extracts: *Ruellia tuberosa's* fresh roots (3200 g), stems (2300 g) and leaves (2600 g) were gathered and dried before being processed into medicinal powder. Roots (500 g), stems (500 g) and leaves (500 g), with humidity levels of 11.80, 11.20 and 10.80%. Table 1 shows the total polyphenol and flavonoid content in *Ruellia tuberosa* extracts. *Ruellia tuberosa* extracts contain polyphenols ranging from 5.56±0.17 to 32.49±0.72 mg GAE/g extract and flavonoids ranging from 8.44±0.56 to 15.48±1.32 mg QE/g extract.

Results of investigating antioxidant activity using ABTS, DPPH, NO, FRAP, RP and TAC methods: Figure 2(a-f) shows the results of testing the antioxidant potential of *Ruellia tuberosa* extracts using six methods: $ABTS^+$, DPPH, NO, FRAP, RP and TAC. Panax ginseng extracts neutralized $ABTS^{\cdot+}$, DPPH and $NO^{\cdot}$ free radicals at levels ranging from 3.83±0.86 to 84.99±1.35%. The results demonstrated that as the extract content grew, so did its ability to neutralize free radicals $ABTS^{\cdot+}$, DPPH and $NO^{\cdot}$. The free radical neutralization effectiveness of $ABTS^{\cdot+}$, DPPH and $NO^{\cdot}$ at the examined concentrations differs statistically substantially at the 5% level. *Ruellia tuberosa* extracts decrease Fe^{3+} ions to Fe^{2+} at concentrations ranging from 0.071±0.008 at 100 µg/mL to 1.33±0.016 at 1000 µg/mL, showing a substantial difference. Statistically significant ($p<0.05$) across all concentrations tested. *Ruellia tuberosa* roots extract has stronger reducing power and ferric reducing-antioxidant power than the stems and leaves of *Ruellia tuberosa*. Extracts from the roots, stems and leaves of *Ruellia tuberosa* also cause the spectral absorbance of the phosphate/Mo (V) complex to be 0.44±0.03, respectively; 0.27±0.00; 0.30±0.00 at a concentration of 100 µg/mL increased to 2.19±0.07; 1.21±0.02 and 1.57±0.02 at a concentration of 1000 µg/mL, a statistically significant difference ($p<0.05$) at all concentrations surveyed.

The antioxidant properties of *Ruellia tuberosa* extracts were compared with each other and with trolox using the IC_{50} or $Abs_{0.5}$ values presented in Fig. 3. *Ruellia tuberosa* extracts showed antibacterial activity oxidized with IC_{50} values ranging from 117.67±2.82 to 955.13±7.20 µg/mL. Among them, the root extract of *Ruellia tuberosa* has the strongest antioxidant activity with IC_{50} values in $ABTS^+$, DPPH, NO , FRAP, RP and TAC methods being 117.67±2.82, 148.33±4.30. 137.83±5.10, 569.21±7.68, 304.18±6.89 and 138.53±4.27 µg/mL, respectively.

Table 1: Total polyphenol and flavonoid content in extracts from *Ruellia tuberosa*

Content	Roots	Stems	Leaves
Polyphenol (mg GAE/g extract)	32.49±0.72[a]	15.56±0.17[c]	20.87±0.24[b]
Flavonoid (mg QE/g extract)	15.48±1.32[a]	8.44±0.56[c]	11.40±1.68[b]

[a,b,c,d]Values followed by the same letters in the same row are not statistically different ($p>0.05$)

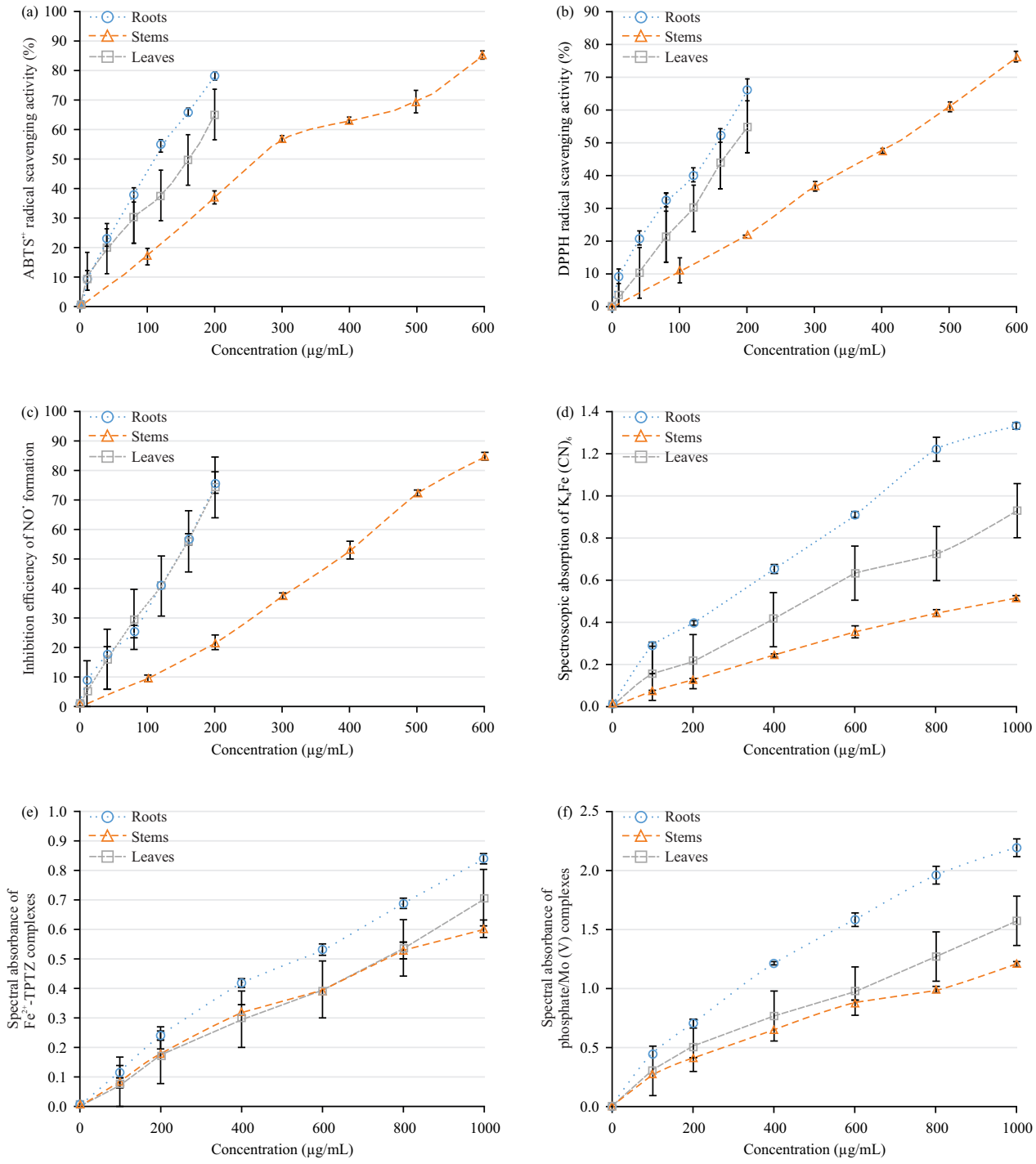

Fig. 2(a-f): *In vitro* antioxidant capacity of extracts from *Ruellia tuberosa*

Results of *in vitro* antidiabetic activity using α-amylase and α-glucosidase enzyme inhibition: The α-amylase and α-glucosidase enzyme inhibition performance of *Ruellia tuberosa* extracts was presented in Fig. 4(a-b). In this study, *Ruellia tuberosa* extracts showed good ability to inhibit α-amylase enzyme with Inhibition efficiency ranged from 5.92±1.72 to 89.35±2.40% at concentrations of 15.625 to 500 µg/mL and α-glucosidase with efficiency ranging from 3.71±0.51 to 77.19±1.66% at concentrations of 100 to 600 µg/mL.

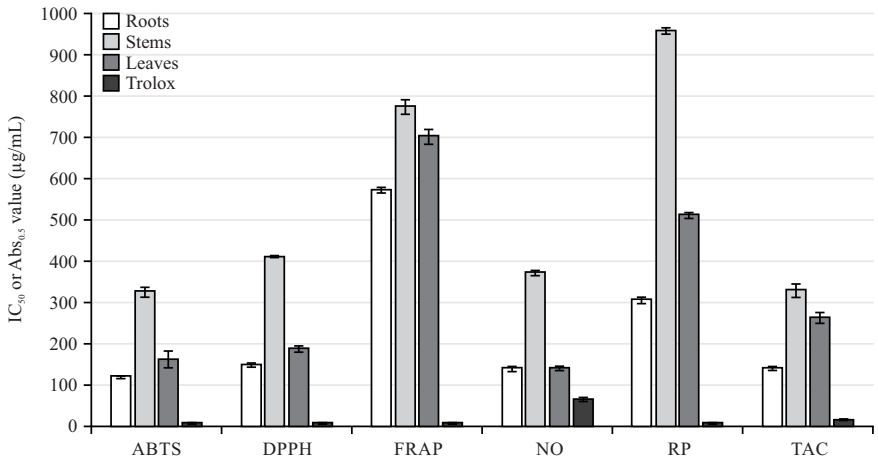

Fig. 3: IC_{50} or $Abs_{0.5}$ values of extracts from *Ruellia tuberosa* and trolox in different antioxidant methods

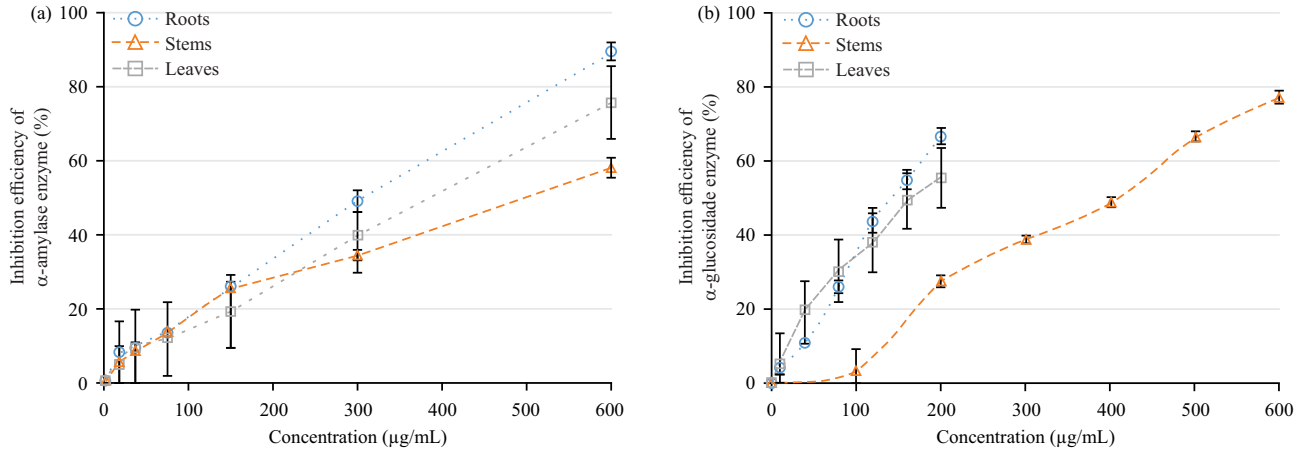

Fig. 4(a-b): Enzyme inhibitory performance of extracts from *Ruellia tuberosa*

Table 2: IC_{50} values of extracts from *Ruellia tuberosa* in α-amylase and α-glucosidase enzyme inhibition method

	IC_{50} values (μg/mL)			
Method	Roots	Stems	Leaves	Acarbose
Enzyme α-amylase	266.72±10.58^{c}	403.90±16.65^{a}	328.76±3.61^{b}	5.50±0.34^{d}
Enzyme α-glucosidase	147.13±3.58^{c}	392.80±5.10^{a}	166.28±2.60^{b}	5.04±1.03^{d}

^{a,b,c,d}Values followed by the same letters in the same row are not statistically different (p>0.05)

The IC_{50} values of extracts from *Ruellia tuberosa* in the α-amylase and α-glucosidase enzyme inhibition method were presented in Table 2. Extracts from *Ruellia tuberosa* have the ability to inhibit α-amylase and α-glucosidase enzymes with IC_{50} values ranging from 147.13±3.58 to 403.90±16.65 μg/mL. *Ruellia tuberosa* root extract (IC_{50}=266.72±10.58 μg/mL) and *Ruellia tuberosa* leaf extract (IC_{50}=328.76±3.61 μg/mL) have the ability to inhibit α-enzyme. The α-amylase is 1.51 and 1.23 times stronger than the extract from the stem of *Ruellia tuberosa* (IC_{50} = 403.90±16.65 μg/mL). *Ruellia tuberosa* root extract (IC_{50} = 147.13±3.58 μg/mL) and *Ruellia tuberosa* leaf extract (IC_{50} = 166.28±2.60 μg/mL) have the ability to inhibit α-enzyme. The α-glucosidase is 2.67 and 2.36 times stronger than the extract from the stem of *Ruellia tuberosa* (IC_{50} = 392.80±5.10 μg/mL).

Results of antibacterial activity investigation using agar well diffusion method: The antibacterial activity of extracts from *Ruellia tuberosa* was presented in Table 3. *Ruellia tuberosa* extracts have antibacterial activity, with

Table 3: Inhibition zone diameter of extracts from *Ruellia tuberosa* and amoxicillin

		Inhibition zone diameter (mm)				
Bacterial strain	Extract	640	1280	2560	5120	10240
Pseudomonas aeruginosa	Roots	-	-	-	27.60±0.53^{b*}	32.40±0.20^{a}
	Leaves	-	-	-	15.90±0.79^{b*}	27.37±0.51^{a}
	Stems	-	-	-	-	33.00±1.00^{a*}
	Amoxicillin	-	-	-	09.73±0.64^{b*}	21.00±0.92^{a}
Escherichia coli	Roots	-	-	25.10±0.96^{c*}	30.50±0.30^{b*}	32.73±0.31^{a}
	Leaves	-	-	-	30.33±0.42^{b*}	38.43±0.81^{a}
	Stems	-	-	-	22.23±0.25^{b*}	36.37±0.32^{a}
	Amoxicillin	-	-	-	12.13±0.23^{b*}	26.53±0.31^{a}
Staphylococcus aureus	Roots	-	14.37±0.71^{d*}	18.43±0.40^{c}	20.30±0.27^{b}	25.67±0.83^{a}
	Leaves	-	-	32.60±0.40^{c*}	34.73±31^{b}	38.30±0.27^{a}
	Stems	-	-	24.10±0.36^{c*}	30.40±53^{b}	34.33±0.31^{a}
	Amoxicillin	-	-	-	13.67±0.23^{b*}	28.87±1.10^{a}

^{a,b,c,d}Values followed by the same letter in the same row are not statistically different (p>0.05). -: Means there is no ability to inhibit, *Minimum inhibitory concentration (MIC), the antibiotic amoxicillin used in the study was investigated for its antibacterial activity at concentration ranges of 80, 160, 320, 640 and 1280 µg/mL, the minimum inhibitory concentration of the antibiotic amoxicillin against the bacterial strains *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* is 640 µg/mL

antibacterial rings measuring 0 to 38.43±0.81 mm. Table 3 shows the minimal inhibitory doses of extracts from *Ruellia tuberosa*. *Ruellia tuberosa* extracts inhibit Gram-negative bacteria at minimum concentrations of 2560 to 10240 µg/mL. *Ruellia tuberosa* extracts inhibit Gram-positive bacterial strains at minimum doses of 1280 to 2560 µg/mL.

DISCUSSION

Oxidative stress is caused by an imbalance of oxidant and antioxidant activity. Humans have an antioxidant defense system in their bodies. When reactive oxygen species and reactive nitrogen species accumulate beyond the body's innate antioxidant capability, excessive oxidation occurs, causing damage to cells or tissue[22-24]. Extracts of *Ruellia tuberosa* include a wide range of secondary metabolites with antioxidant properties. Indeed, extracts from *Ruellia tuberosa* have been found to effectively neutralize ABTS and DPPH free radicals while inhibiting the generation of NO free radicals. Humans create abiotic free radicals such as ABTS and DPPH to help screen medicinal plants for novel drugs. Nitric oxide is a free radical that plays a significant role in inflammatory processes and interacts strongly with specific proteins and other free radicals. Free nitric oxide is created when sodium nitroprusside interacts with oxygen to make nitrite. *Ruellia tuberosa* extracts prevent nitrite production by directly competing with oxygen in the interaction with nitric oxide. The reducing power and ferric reducing-antioxidant power of extracts from *Ruellia tuberosa* can be evaluated based on their potential to form complexes with metal ions, especially iron and copper. Among them, iron-containing complexes are often widely used in evaluating antioxidant activity in the direction of reducing Fe^{3+} to Fe^{2+} ions. Total antioxidant activity was determined based on the reduction of Mo (VI) to Mo (V) with antioxidant compounds and the formation of a blue phosphate/Mo (V) complex. Research results show that the spectral absorbance of the phosphate/Mo(V) complex increases proportionally to the concentration of the extract. Cheonga *et al*.[25] used methanol to extract *Ruellia tuberosa* leaves and stems. The antioxidant activity survey found that methanol *Ruellia tuberosa* leaves extract and methanol *Ruellia tuberosa* extract can neutralize DPPH free radicals at IC_{50} values of 800 and 1050 µg/mL, respectively. Our investigation found that the leaf and stem extract of *Ruellia tuberosa* extracted with 96% ethanol solvent was more effective in neutralizing DPPH free radicals than Cheonga *et al*.[25] study. Current findings were similar to those of Cheonga *et al*.[25], who found that the antioxidant impact of *Ruellia tuberosa* leaves was larger than that of *Ruellia tuberosa* roots.

Controlling glucose levels is an important goal for diabetes individuals since it helps to reduce health issues and maintain stable blood glucose. Inhibiting α-amylase and α-glucosidase enzymes reduces carbohydrate digestion, slows glucose absorption and lowers blood glucose. Plants can reduce blood glucose levels by suppressing α-amylase and α-glucosidase enzyme activities. *Ruellia tuberosa* extracts inhibited α-amylase and α-glucosidase enzymes more effectively as the concentration increased.

The antioxidant activity and inhibition of α-amylase and α-glucosidase enzymes in *Ruellia tuberosa* extracts increased gradually in the order of stem extract <leaf extract<roots, according to the survey results. This finding is consistent with the polyphenol and flavonoid concentration of each of the extracts examined before. Extracts from *Ruellia tuberosa*

exhibit antioxidant activity and inhibit α-amylase and α-glucosidase enzymes based on their polyphenol and flavonoid content. Polyphenols and flavonoids are powerful antioxidants that inhibit α-amylase and α-glucosidase enzymes, according to numerous studies. Compounds from the polyphenol and flavonoid groups can donate electrons to free radicals or decomplex harmful chemicals to make them stable, non-toxic, or less toxic for biological macromolecules in the body.

Furthermore, research results reveal that *Ruellia tuberosa* extracts are more effective against Gram-positive bacterial strains than Gram-negative bacterial strains. The reason could be that Gram-positive bacteria have a cell wall made primarily of peptidoglycan, whereas Gram-negative bacteria have a cell wall made of a thin coating of peptidoglycan and an outside membrane coated in lipopolysaccharide. Extracts from *Ruellia tuberosa* must be able to reach the cell membrane in order to fight bacterial strains; however, the cell membrane of Gram-negative bacteria is protected by a lipopolysaccharide layer, making inhibition more difficult. Furthermore, research findings indicate that *Ruellia tuberosa* root extracts have higher antibacterial activity than stems and leaves.

CONCLUSION

The study evaluated the polyphenol, flavonoid, antioxidant, antibacterial, α-amylase and α-glucosidase enzyme activity of *Ruellia tuberosa* extracts. *Ruellia tuberosa* extracts contain antioxidant action and inhibit the activity of α-amylase and α-glucosidase enzymes. *Ruellia tuberosa* extracts are more effective at inhibiting Gram-positive bacterial strains than Gram-negative strains. *Ruellia tuberosa* root extract has superior antioxidant, antibacterial and α-amylase and α-glucosidase enzyme inhibitory properties compared to other extracts. This is presumably due to the high polyphenol and flavonoid content of each kind. Extracts from *Ruellia tuberosa* have antioxidant, antibacterial and enzyme inhibitory properties, making them potentially useful in health care.

SIGNIFICANCE STATEMENT

This study is very important in evaluating the antioxidant, antibacterial and inhibitory activities of α-amylase and α-glucosidase enzymes, in order to contribute to further research so that *Ruellia tuberosa* plants can be more powerful applications and exploits. This is a very necessary source of raw materials for research into the preparation of natural products that support diseases related to diabetes, antibacterial and anti-oxidation. This is the first time research has been conducted on *Ruellia tuberosa* species in Vietnam about these valuable activities. At the same time, these results help Vietnamese scientists as well as scientists around the world orient further research on this easy-to-grow and abundant medicinal herb.

REFERENCES

1. Zhang, L., Z.C. Tu, T. Yuan, H. Wang, X. Xie and Z.F. Fu, 2016. Antioxidants and α-glucosidase inhibitors from *Ipomoea batatas* leaves identified by bioassay-guided approach and structure-activity relationships. Food Chem., 208: 61-67.
2. Kashtoh, H. and K.H. Baek, 2022. Recent updates on phytoconstituent alpha-glucosidase inhibitors: An approach towards the treatment of type two diabetes. Plants, Vol. 11. 10.3390/plants11202722.
3. Benzie, I.F.F. and J.J. Strain, 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., 239: 70-76.
4. Chipiti, T., M.A. Ibrahim, M. Singh and M.S. Islam, 2015. *In vitro* α-amylase and α-glucosidase inhibitory effects and cytotoxic activity of *Albizia antunesiana* extracts. Pharmacogn. Mag., 11: S231-S236.
5. Matough, F.A., S.B. Budin, Z.A. Hamid, N. Alwahaibi and J. Mohamed, 2012. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ. Med. J., 12: 5-18.
6. Bag, G.C., P.G. Devi, and T. Bhaigyabati, 2015. Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three hedychium species of Manipur valley. Int. J. Pharm. Sci. Rev. Res., 30: 154-159.
7. Matijević, T., J. Talapko, T. Meštrović, M. Matijević, S. Erić, I. Erić and I. Škrlec, 2023. Understanding the multifaceted etiopathogenesis of foot complications in individuals with diabetes. World J. Clin. Cases, 11: 1669-1683.
8. Ngan, L.T.M., J.K. Moon, J.H. Kim, T. Shibamoto and Y.J. Ahn, 2012. Growth-inhibiting effects of *Paeonia lactiflora* root steam distillate constituents and structurally related compounds on human intestinal bacteria. World J. Microbiol. Biotechnol., 28: 1575-1583.
9. Sun, W. and M.H. Shahrajabian, 2023. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules, Vol. 28. 10.3390/molecules28041845.
10. Fialová, S.B., K. Rendeková, P. Mučaji, M. Nagy and L. Slobodníková, 2021. Antibacterial activity of medicinal plants and their constituents in the context of skin and wound infections, considering European legislation and folk medicine-A review. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms221910746.

11. Mujeeb, F., P. Bajpai and N. Pathak, 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. BioMed Res. Int., Vol. 2014. 10.1155/2014/497606.
12. Mohamed, E.A.H., M.J.A. Siddiqui, L.F. Ang, A. Sadikun and S.H. Chan *et al*., 2012. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. BMC Complementary Altern. Med., Vol. 12. 10.1186/1472-6882-12-176.
13. Chothani, D.L. and S.H. Mishra, 2012. *In vitro* anti-oxidant activity of *Ruellia tuberosa* root extracts. Free Radicals Antioxid., 2: 38-44.
14. Ahmad, A.R., B. Elya and A. Mun'im, 2017. Antioxidant activity and isolation of xanthine oxidase inhibitor from *Ruellia tuberosa* L. leaves. Pharmacogn. J., 9: 607-610.
15. Oyaizu, M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet., 44: 307-315.
16. Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.
17. Rao, A.G.R. and S.S. Subramania, 1974. Apigenin glycosides from *Thunbergia fragrans* and *Ruellia tuberosa*. Curr. Sci., 43: 480-480.
18. Nair, S.S., V. Kavrekar and A. Mishra, 2013. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur. J. Exp. Biol., 3: 128-132.
19. Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In: Methods in Enzymology, Burslem, G.L. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780121822002, pp: 152-178.
20. Nenadis, N., L.F. Wang, M. Tsimidou and H.Y. Zhang, 2004. Estimation of scavenging activity of phenolic compounds using the $ABTS^{\cdot+}$ assay. J. Agric. Food Chem., 52: 4669-4674.
21. Grubešić, R.J., D. Kremer, M.Z. Končić, J.V. Rodríguez and M. Randić, 2012. Quantitative analysis of polyphenols and antioxidant activity in four *Daphne* L. species. Open Life Sci., 7: 1092-1100.
22. Sharma, O.P. and T.K. Bhat, 2009. DPPH antioxidant assay revisited. Food Chem., 113: 1202-1205.
23. Sreejayan and M.N.A. Rao, 1997. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol., 49: 105-107.
24. Vona, R., L. Pallotta, M. Cappelletti, C. Severi and P. Matarrese, 2021. The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. Antioxidants, Vol. 10. 10.3390/antiox10020201.
25. Cheong, B.E., M.Z. Waslim, F.F. Lem and P.L. Teoh, 2013. Antioxidant and anti-proliferative activities of Sabah *Ruellia tuberosa*. J. Appl. Pharm. Sci., 3: 20-24.