

## RESEARCH ON PHYTOCHEMICAL AND ALFA-GLUCOSIDASE INHIBITORY ACTIVITY OF ETHYL ACETATE FRACTION OF *RUELLIA TUBEROSA*

Pham Thi Nhat Trinh<sup>1</sup>, Pham Ngoc An<sup>2</sup>, Nguyen Trong Tuan<sup>3</sup>,  
Nguyen Thi Le Thuy<sup>4</sup>, Tong Thanh Danh<sup>5</sup>, Le Tien Dung<sup>2, 6, \*</sup>

<sup>1</sup>Faculty of Natural Sciences, Tien Giang University, 119 Ap Bac – My Tho – Tien Giang

<sup>2</sup>Institute of Applied Materials Science, VAST, 01A TL29, Dist. 12, Ho Chi Minh city

<sup>3</sup>Department of Chemistry, College of Natural Sciences, Can Tho University,  
Dist. Ninh Kieu, Can Tho

<sup>4</sup>Department of Food Technology, Faculty of Biotechnology, Open University Ho Chi Minh City,  
97 Vo Van Tan, Dist. 3, Ho Chi Minh City

<sup>5</sup>Department of Organic Chemistry, Faculty of Chemical Engineering,  
Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet, Dist. 10, Ho Chi Minh City

<sup>6</sup>Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi

\*Email: [inpcdung@yahoo.com](mailto:inpcdung@yahoo.com)

Received: 20 September 2018; Accepted for publication: 10 October 2018

### ABSTRACT

*Ruellia tuberosa* L. is a folk remedy, which is used in the treatment of diabetes mellitus, antiinflammation and diuretic agent. However, its  $\alpha$ -glucosidase inhibitory activity has not been investigated so far. In the present study, the antidiabetic activities of the hexane, ethyl acetate and methanol fraction of crude ethanolic extract of this plant were investigated. Our preliminary results showed that the crude ethanol extract as well as ethyl acetate and methanol fractions of *Ruellia tuberosa* showed  $\alpha$ -glucosidase inhibitory activity with  $IC_{50}$  of 15.84, 4.73 and 8.27  $\mu$ g/ml, respectively. From the ethyl acetate extract of whole plant of *Ruellia tuberosa*, four compounds, including rutin (**1**), syringaresinol (**2**), catechin (**3**) and pulmatin (**4**) were isolated and identified by column chromatography. Their structures were elucidated by spectroscopic method including MS, 1D and 2D- NMR and comparison with the literature values.

**Keywords:** *Ruellia tuberosa* L., flavonoid, anthraquinone glycoside,  $\alpha$ -glucosidase inhibitory activity.

### 1. INTRODUCTION

Diabetes mellitus is a chronic disease which relates to hyperglycemia as a result of defects in insulin secretion, insulin action, or both. The goal of treatment of diabetes is to control and keep blood glucose levels at normal or near-normal levels, to reduce postprandial blood glucose

[1, 2]. To control blood glucose, people are used to drugs, including inhibitors  $\alpha$ -glucosidase enzyme. The  $\alpha$ -glucosidase inhibitors prevent the digestion of carbohydrates, lead to reduce postprandial absorption of glucose, thereby delaying the increase in blood glucose. Public interest in alternative therapies, including the use of plants and natural dietary supplements, has risen throughout the world. *Ruellia tuberosa* L., is widely distributed in Vietnam, and is a folk remedy in the treatment of diabetes mellitus, antihypertensive, analgesic, anti-inflammation and diuretic agent.

*Ruellia tuberosa* L. possessed significant blood glucose lowering effect in alloxan-induced diabetic rat and rabbit [3, 4], antioxidant, xanthine oxidase inhibitory [5], anticholinesterase [6], hypolipidemic [7], antinociceptive and anti-inflammatory [8] activities. It was reported that five flavonoids including: cirsimaritin, cirsimarin, cirsilol 4-glucoside, sorbifolin, and pedalin along with betulin, vanillic acid, and indole-3-carboxaldehyde were isolated from the ethyl acetate fraction of methanolic extracts of *R. tuberosa* L. [9]. Phenylethanoid and flavone glycosides included apigenin, 3,5-diglucoside, apigenin-7-O-glucuronide, apigenin glucoside, apigenin rutinoside, luteolin glucoside, verbascoside, isoverbascoside, nuomioside were also reported in *R. tuberosa* L. [10-12]. In addition, our previously published result showed that the main chemical components were flavonoid and phenyl ethanoid [13]. The aim of this present work is to evaluate the inhibition of  $\alpha$ -glucosidase and isolate phytochemicals from active fraction of *Ruellia tuberosa*.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and reagents

Solvents: *n*-hexane, chloroform, ethyl acetate, methanol and ethanol 96 % were purchased from Chemsol company (Viet Nam). Silica gel 60 (0.063–0.200 mm, Merck) and silica gel C-18 were used for column chromatography. TLC F<sub>254</sub> plate (Merck) and TLC RP-18 F<sub>254</sub> plate (Merck) were used for thin layer chromatography. NMR spectra were recorded on a Bruker AM500 FT-NMR Spectrometer of Institute of Chemistry, VAST.

### 2.2. Sample treatment and preparation

*Ruellia tuberosa* L. were collected at July, 2017 in My Tho, Tien Giang, Vietnam. The plants were authenticated and voucher specimens (S072017) has been preserved in the Institute of Applied Materials Science. The whole plants were washed away from muds and dust; after that samples were dried in an oven at about 50 °C until well-dried.

### 2.3. Extraction and isolation

The dried powder of whole plant (5 kg) was soaked in 96% ethanol at room temperature for four times (4 × 20 L) and filtered. The filtrate was concentrated under reduced pressure to give crude ethanol extract (350 g). The extract was then fractionated over flash column chromatography eluting successively with hexane, ethyl acetate, and methanol, respectively to yield hexane (80 g), ethyl acetate (95 g), and methanol fractions (148 g).

The ethyl acetate fraction was subjected to a silica gel column chromatography and eluted with Hexane -EtOAc to obtain six subfractions (E1-6). Subfraction E1 was further separated on a silica gel CC and eluted under gradient hexane-EtOAc (from 30:1 to 10:1, v/v) to yield five

subfractions (E1.1-7). Fraction E1.2 was rechromatographed on silica gel CC, eluting with  $\text{CHCl}_3$ : MeOH (30:1 to 5:1, v/v) to obtain six subfractions (E1.2.1-6). Subfraction E1.2.4 was chromatographed on silica gel using  $\text{CHCl}_3$ : MeOH (8:1, v/v) as eluent, then purified by sephadex LH-20 (MeOH 100 %) to give compound **3** (13.8 mg). Subfraction E1.2.3 was further chromatographed on Rp18 CC, eluted with MeOH:  $\text{H}_2\text{O}$  (1:1, v/v) and was then subjected to a sephadex LH20 CC ( $\text{CHCl}_3$ :MeOH 1:1) to give compound **2** (5.6 mg).

Fraction E5 was further separated on a silica gel column, eluted with  $\text{CHCl}_3$ :MeOH (from 50:1 to 5:1, v/v) to yield nine subfractions (E5.1-9). Subfraction E5.4 was repeatedly chromatographed on silica gel CC Rp18 eluted with  $\text{CHCl}_3$ : MeOH (15:1 to 7:1, v/v) to obtain four subfractions (E5.4.1-4). The fraction E5.4.4 was further chromatographed on silica gel Rp18, eluted with MeOH: $\text{H}_2\text{O}$  (1:3, v/v) to obtain three subfractions (E5.4.4.1-3). Fraction E5.4.4.1 was repeatedly separated on a silica gel Rp18 with MeOH:  $\text{H}_2\text{O}$  (1:4, v/v) to obtain compound **1** (17.2 mg). Fraction E5.4.4.3 was purified through a sephadex LH 20 column using MeOH as eluent to yield compound **4** (8.5 mg).

Compound **1**:  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ ),  $\delta$  (ppm),  $J$  (Hz): 6.22 (1H, s, H-6); 6.41 (1H, s, H-8); 7.68 (1H, s, H-2'); 6.90 (1H, d, 7.5Hz, H-5'); 7.65 (1H, d, 7.5Hz, H-6'); 5.13 (1H, d, 7.0 Hz, H-1''); 4.54 (1H, s, H-1''').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ ),  $\delta$  (ppm): 159.3 (C-2); 135.6 (C-3); 179.3 (C-4); 162.9 (C-5); 99.9 (C-6); 165.9 (C-7); 94.8 (C-8); 158.4 (C-9); 105.6 (C-10); 123.0 (C-1'); 117.6 (C-2'); 145.8 (C-3'); 149.7 (C-4'); 116.0 (C-5'); 123.5 (C-6'); 104.7 (C-1''); 75.7 (C-2''); 78.1 (C-3''); 71.3 (C-4''); 77.1 (C-5''); 68.5 (C-6''); 102.3 (C-1'''); 72.0 (C-2'''); 72.2 (C-3'''); 73.9 (C-4'''); 69.6 (C-5'''); 17.8 (C-6''').

Compound **2**: **ESI-MS**:  $m/z$  417.0[M-H] $^-$ .  $^1\text{H-NMR}$  (500 MHz, acetone- $d_6$ ),  $\delta$  (ppm),  $J$  (Hz): 6.68 (4H, s, H-2, H-2', H-6, H-6'); 4.61 (2H, d, 4.0 Hz, H-7, H-7'); 3.10 (2H, m, H-8, H-8'); 4.24 (2H, dd, 8.5 and 6.5 Hz, H-9a, H-9a'); 3.87 (2H, dd, 3.0 and 9.0 Hz; H-9b, H-9b'); 3.82 (12H, s,  $\text{OCH}_3$ -10, 10', 11, 11').  $^{13}\text{C-NMR}$  (125 MHz, acetone- $d_6$ ),  $\delta$  (ppm): 133.2 (C-1, C-1'); 104.4 (C-2, C-2'); 148.6 (C-3, C-3'); 136.2 (C-4, C-4'); 148.6 (C-5, C-5'); 104.4 (C-6, C-6'); 86.7 (C-7, C-7'); 55.3 (C-8, C-8'); 72.3 (C-9, C-9'); 56.6 (C-10, C-10', C-11, C-11').

Compound **3**:  $^1\text{H-NMR}$  (500 MHz,  $\text{DMSO-}d_6$ ),  $\delta$  (ppm),  $J$  (Hz): 4.59 (1H, d, 8.0 Hz, H-2); 4.01 (1H, m, H-3); 2.89 (1H, dd, 5.5; 16.0 Hz, H-4a); 2.55 (1H, dd, 8.5; 16.0 Hz, H-4e); 5.88 (1H, d, 2.5 Hz, H-6); 5.95 (1H, d, 2.5 Hz, H-8); 6.86 (1H, d, 2.0 Hz, H-2'); 6.79 (1H, d, 8.0, H-5'); 6.74 (1H, dd, 2.0; 8.0 Hz, H-6').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{DMSO-}d_6$ ),  $\delta$  (ppm): 82.8 (C-2); 68.8 (C-3); 28.5 (C-4); 157.5 (C-5); 95.5 (C-6); 156.9 (C-7); 96.3 (C-8); 157.8 (C-9); 100.8 (C-10); 132.2 (C-1'); 115.2 (C-2'); 146.2 (C-3'); 146.2 (C-4'); 116.1 (C-5'); 120.0 (C-6').

Compound **4**: **ESI-MS**:  $m/z$  416.91 [M+H] $^+$ .  $^1\text{H-NMR}$  (500 MHz,  $\text{DMSO-}d_6$ ),  $\delta$  (ppm),  $J$  (Hz): 7.71 (1H; s, H-2); 7.54 (1H; s, H-4); 7.66 (1H; dd; 8.0; 1.0 Hz, H-5); 7.74 (1H; t; 8.0 Hz, H-6); 7.35 (1H; dd; 8.0; 1.0 Hz, H-7); 2.47 (3H; s, H<sub>3</sub>-11); 5.16 (1H; d; 7.5 Hz, H-1').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{DMSO-}d_6$ ),  $\delta$  (ppm): 158.4 (C-1); 122.8 (C-2); 147.4 (C-3); 121.3 (C-4); 118.3 (C-5); 136.2 (C-6); 124.3 (C-7); 161.4 (C-8); 187.7 (C-9); 182.1 (C-10); 118.3 (C-1a); 134.5 (C-4a); 132.5 (C-5a); 116.8 (C-8a); 21.8 (C-11); 100.5 (C-1'); 73.4 (C-2'); 76.6 (C-3'); 69.6 (C-4'); 77.3 (C-5'); 60.7 (C-6').

#### 2.4. $\alpha$ -glucosidase inhibitory activity assay

The  $\alpha$ -glucosidase inhibitory activity was determined according to the described method [5]. A reaction mixture containing 500  $\mu\text{l}$  of 67 mM phosphate buffer (pH 6.8), 20  $\mu\text{l}$  of 0.3 U/ml fungal  $\alpha$ -glucosidase in cold deionized water and 20  $\mu\text{l}$  of sample was pre-incubated in

athermoregulator for 5 minute at 37 °C, and then 50  $\mu$ l of 5 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) solution was added to the mixture. After further incubation at 37 °C for 30 min, the reaction was stopped by adding 2440  $\mu$ l of 100 mM Na<sub>2</sub>CO<sub>3</sub> (pH 9.6). The released pNP was monitored spectrophotometrically by measuring UV absorbance at 400 nm. Tannic acid was used as positive control.

The percentage of  $\alpha$ -glucosidase enzyme inhibition by the sample was calculated by the following formula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

where A<sub>C</sub> is the absorbance of the control and A<sub>S</sub> is the absorbance of the tested sample.

### 3. RESULTS AND DISCUSSION

#### 3.1. Assay for $\alpha$ -glucosidase inhibitory activity

The yeast  $\alpha$ -glucosidase is used to investigate the inhibitory activity of the crude extract and fractions. The  $\alpha$ -glucosidase inhibitory activities of all extracts against  $\alpha$ -glucosidase were determined using *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) as a substrate and these were compared with tannic acid (Table 1).

Table 1. The  $\alpha$ -glucosidase inhibitory activity of *Ruellia tuberosa*.

No.	Sample	IC <sub>50</sub> ( $\mu$ g/ml)
1	ethyl acetate fraction	4.73
2	Methanol fraction	8.27
3	crude ethanol	15.84
4	hexane fraction	46.76
5	tannic acid	7.66 (ng/mL)

All samples showed inhibitory activity against  $\alpha$ -glucosidase enzyme significantly. IC<sub>50</sub> values of samples of plant extracts are higher than tannic acid because in the extract, pharmacological active compounds are not pure. Of which, the ethyl acetate fraction was the most potent, with IC<sub>50</sub> of 4.73  $\mu$ g/ml.

#### 3.2. Structural elucidation

The EtOAc portion was repeatedly chromatographed on silica gel, Sephadex LH-20, and silica gel reversed-phase to afford rutin (**1**), syringaresinol (**2**), catechin (**3**) and pulmatin (**4**). These compounds were identified by comparison of their spectroscopic data with literature values (Fig. 1).

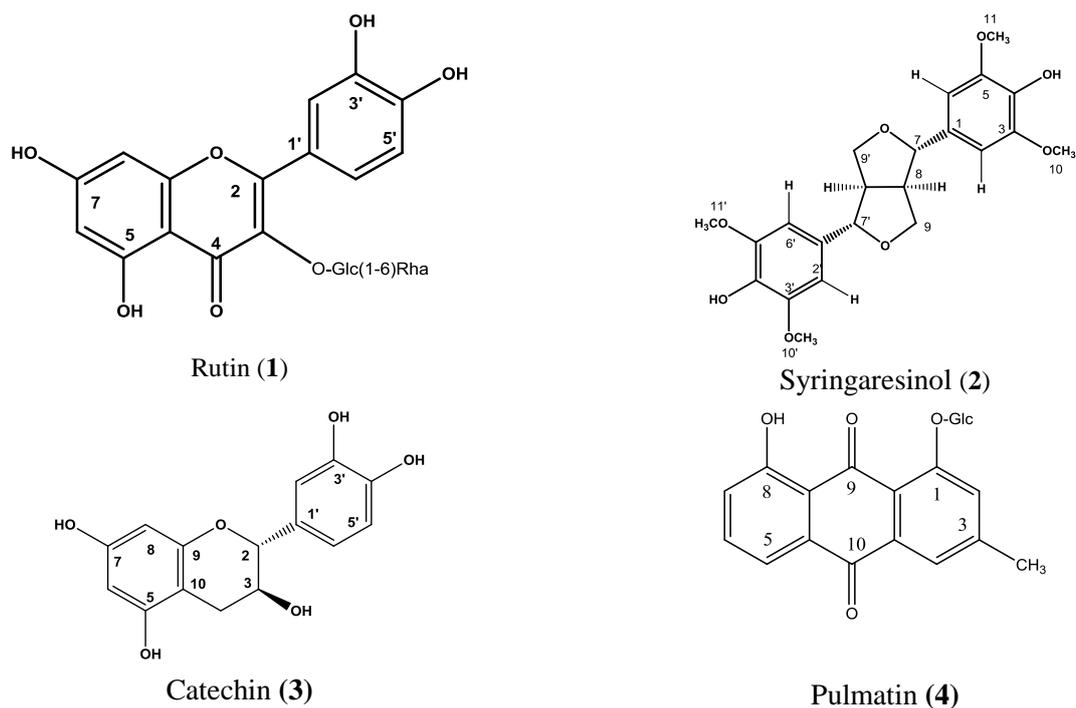


Figure 1. Structures of isolated compounds.

Compound **1** was obtained as a yellow powder. The  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 500MHz) indicated the presence of a tetrasubstituted benzene and a trisubstituted benzene at  $\delta_{\text{H}}$  [6.22 (1H, s); 6.41 (1H, s); 6.90 (1H, d, 7.5 Hz); 7.65 (1H, d, 7.5 Hz) and 7.68 (1H, s)]. Two anomeric protons were observed at  $\delta_{\text{H}}$  5.13 (1H, d, 7.0 Hz, H-1'') and  $\delta_{\text{H}}$  4.54 (1H, s, H-1'''), along with oxymethine protons at 3.28- 3.83 ppm and a methyl group at  $\delta_{\text{H}}$  1.14 (3H, d, 6 Hz). Two singlet signals at [ $\delta_{\text{H}}$  6.41 (1H, s); 6.22 (1H, s)] were assigned to H-8 and H-6, respectively. Three proton signals of a ABX pattern at [ $\delta_{\text{H}}$  7.68 (1H, s); 7.65 (1H, d, 7.5 Hz); 6.90 (1H, d, 7.5 Hz)] were assigned to H-2', H-6' and H-5'.

In the  $^{13}\text{C-NMR}$  spectrum, 27 carbon signals were observed, of which 15 carbons belong to a flavonol skeleton. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data proved that two monosaccharide were  $\beta$ -D-glucose and  $\alpha$ -L-rhamnose. In HMBC spectrum, there are correlations between rhamnose anomeric proton  $\delta_{\text{H}}$  4.54 (1H, s) with glucose C-6'' and glucose anomeric proton  $\delta_{\text{H}}$  5.13 (1H, d, 7.0 Hz) with C-3 of flavonol framework. From these evidences above, compound **1** was determined as rutin [14].

Compound **2** was isolated as a white prisms, m.p 175-176  $^{\circ}\text{C}$ . The ESI-MS spectrum showed a pseudomolecular ion  $[\text{M-H}]^-$  at  $m/z$  417.0, consistent with molecular formula  $\text{C}_{22}\text{H}_{26}\text{O}_8$ . The  $^1\text{H-NMR}$  spectrum (500 MHz, acetone- $d_6$ ) of **1** exhibited the resonance signals of two hydroxyl protons at  $\delta_{\text{H}}$  7.16 (2H, s), four aromatic protons at  $\delta_{\text{H}}$  6.68 (4H, s), two oxymethine protons at  $\delta_{\text{H}}$  4.67 (2H, d, 4 Hz), two methine protons at  $\delta_{\text{H}}$  3.10 (2H, m), two oxymethylene protons at [ $\delta_{\text{H}}$  4.24 (2H, dd, 8.5 and 6.5 Hz) and 3.87 (2H, d, 3 Hz)] and four methoxy groups at  $\delta_{\text{H}}$  3.82 (12H, s). The  $^{13}\text{C-NMR}$  together with DEPT spectra revealed 22 carbon signals including four methoxy groups at  $\delta_{\text{C}}$  56.6, 12 aromatic carbons, two oxymethine at  $\delta_{\text{C}}$  86.7, two methine at  $\delta_{\text{C}}$  55.3 and two oxymethylene at  $\delta_{\text{C}}$  72.3. NMR data suggested that **2** possessed lignan skeleton. Two aromatic protons and two methoxy protons appeared only at  $\delta_{\text{H}}$

6.68 and 3.82, respectively, proving that the structure of 2 was symmetry. Based on the NMR data and in comparison with reported literature, compound 2 is syringaresinol [15].

Compound 3 was isolated as a white needle. The  $^1\text{H-NMR}$  spectrum showed signals of a trisubstituted benzene at [ $\delta_{\text{H}}$  6.86 (1H, *d*, 2.0 Hz, H-2'); 6.79 (1H, *d*, 8.0 Hz, H-5') and 6.74 (1H, *dd*, 2.0 & 8.0 Hz, H-6')], a tetrasubstituted benzene at [ $\delta_{\text{H}}$  5.95 (1H, *d*, 2.5 Hz, H-8) and 5.88 (1H, *d*, 2.5 Hz, H-6)], two oxymethine groups at [ $\delta_{\text{H}}$  4.59 (1H, *d*, 8.0 Hz, H-2) and 4.01 (1H, *m*, H-3)], and 1 methylene  $\delta_{\text{H}}$  2.89 (1H, *dd*, 5.5 & 16.0 Hz, H-4a) and 2.55 (1H, *dd*, 8.5 and 16.0 Hz, H-4b)]. The  $^{13}\text{C-NMR}$  spectrum presented 15 signals including 12 aromatic, 2 oxymethine and 1 methylene carbons. From the above NMR data along with previous literature reports led to a conclusion that 3 was catechin. Moreover, specific rotation of 3 is  $[\alpha]^{25}_{\text{D}} + 25.7$  (*c* 0.01, methanol), chemical structure of 3 is (+)-catechin [16].

The  $^1\text{H-NMR}$  of 4 appeared two meta protons at  $\delta_{\text{H}}$  7.71 (1H, *s*) and 7.54 (1H, *s*); 3 protons of trisubstituted benzene at  $\delta_{\text{H}}$  7.76 (1H, *t*, 8.0 Hz); 7.67 (1H, *d*, 8.0 Hz) and 7.36 (1H, *d*, 8.0 Hz), together with a methyl group at  $\delta_{\text{H}}$  2.50 (3H, *s*) and an anomeric proton at  $\delta_{\text{H}}$  5.17 (1H, *d*, 8.0 Hz) as well as oxymethine signals from 3 to 4 ppm. The  $^{13}\text{C-NMR}$  indicated 21 carbon signals of 12 aromatic carbons, 2 carbon carbonyl signals, 1 methyl carbon and 6 carbon signals of glucose. The beta configuration of glucose is determined through J-coupling of anomeric proton. Moreover, the appearance of two carbonyl groups proves that 4 is an anthraquinone glycoside. The chelated proton signal at  $\delta_{\text{H}}$  12.94 (1H, *s*) indicated the presence of a hydroxyl group at C-8. The carbon chemical shift of two carbonyls at 187.6 and 182.1 ppm implied that C-1 and C-8 attached two hydroxyls. HMBC correlation between anomeric protons with C-1 confirmed glucose moiety linked to C-1. MS spectrum showed a pseudomolecular ion  $[\text{M}+\text{H}]^+$  at *m/z* 416.91, consistent with molecular formula  $\text{C}_{21}\text{H}_{20}\text{O}_9$ . The glucose moiety was confirmed through fragment *m/z* 254.3  $[\text{M-glucose}]^+$ . Based on the above evidence, compound 4 was determined as pulmatin [17].

#### 4. CONCLUSIONS

Our findings indicate that *Ruellia tuberosa* L. extracts show inhibitory activity against  $\alpha$ -glucosidase enzyme significantly. Among that, the ethyl acetate fraction is the most potent, with  $\text{IC}_{50}$  of 4.73  $\mu\text{g/ml}$ . From this fraction, by column chromatography four compounds as rutin (1), syringaresinol (2), catechin (3) and pulmatin (4) have been isolated and identified. Their structures were elucidated by spectroscopic method including MS, 1D and 2D- NMR and comparison with the literature values. This is the first time these compounds were found in *Ruellia tuberosa*.

**Acknowledgements.** This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2017.54.

#### REFERENCES

1. Unwin N., Alberti K. G. - Chronic non-communicable diseases, *Ann Trop Med Parasitol* **100** (5-6) (2006) 455-464.
2. Ezenwaka C. E., Kalloo R., Uhlig M., and Eckel J. - Relationship between adiponectin and metabolic variables in Caribbean offspring of patients with type 2 diabetes mellitus, *Hormone and Metabolic Research* **36** (4) (2014) 238-242.

3. Rajan M., Kishor K. V. K., Satheesh K. P. S., Swathi K. R., and Haritha S. - Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of *Ruellia tuberosa* Linn leaves in normal and alloxan induced diabetic rats, *Journal of Chemical and Pharmaceutical Research* **4** (6) (2012) 2860–2868.
4. Shahwara D., Ullah S., Ahmad M., Ullah S., Ahmad N., and Khan M. A. - Hypoglycemic activity of *Ruellia tuberosa* Linn (Acanthaceae) in normal and alloxan-induced diabetic rabbits, *Iranian Journal of Pharmaceutical Sciences* **7** (2) (2011) 107–115.
5. Ahmad A. R., Elya B., Mun'im A. - Antioxidant activity and isolation of xanthine oxidase inhibitor from *Ruellia tuberosa* L. leaves, *Pharmacognosy Journal* **9**(5)(2017) 607-610.
6. Khachitpongpanit S., Singhatong S., Sastraruji T., Jaikang C. - Phytochemical study of *Ruellia tuberosa* chloroform extract: antioxidant and anticholinesterase activities, *Pharmacia Lettre* **8** (6)(2016), 238-244.
7. Sravanthi K., Reddy R. B., Kumar V. V. - Evaluation of hypo lipidemic and antioxidant properties of methanolic extract of leaves of *Ruellia tuberosa* Linn on mithionine and triton induced models in wister albino rats, *Indo American Journal of Pharmaceutical Sciences* **3** (5) (2016) 462-472.
8. Alam M. A., Subhan N., Awal M. A., Alam M. S., Sarder M., Nahar L., Sarker S. D. - Antinociceptive and anti-inflammatory properties of *Ruellia tuberosa*, *Pharmaceutical Biology* **47** (3) (2009) 209-214.
9. Lin C. F., Huang Y. L., Cheng L. Y., Sheu S. J., and Chen C.C. - Bioactive flavonoids from *Ruellia tuberosa*, *The Journal of Chinese Medicine* **17** (3) (2006) 103–109.
10. Nair A. G. R. and Subramanian S. S. - Apigenin glycosides from *Thunbergia fragrans* and *Ruellia tuberosa*, *Current Science* **43** (1974) 480.
11. Wagner H., Danninger H., Iyengar M. A., Seligmann O., Farkas L., Subramanian S. S., Nair A. G. - Synthesis of glucuronides in the flavonoid-series. 3. Isolation of apigenin-7-D-glucuronide from *Ruellia tuberosa* L. and its synthesis, *Chemische Berichte* **104** (9) (1971) 2681–2687.
12. Phakeovilay C., Disadee W., Sahakitpichan P., Sitthimonchai S., Kittakoop P., Ruchirawat S., Kanchanapoom T. - Phenylethanoid and flavone glycosides from *Ruellia tuberosa* L. *Journal of Natural Medicines* **67** (1) (2013) 228-233.
13. Dung L. T., An N. H., Tri M. D., Trinh P. T. N., Ngoc T. T. – Flavonoid and phenyl ethanoid compounds from *Ruellia tuberosa*, *Vietnam Journal of Chemistry* **51** (6ABC) (2013) 119-121.
14. Le T. M. C., Vu D. H., Nguyen T. M. T., Tran D. T. - Chemical constituents of the rhizomes of *Zingiber collinsii* mood & theilade (Zingiberaceae) growing in Viet Nam, *Vietnam Journal of Science and Technology* **54** (4A) (2016) 283-289.
15. Hyun B. P., Kyu H. L., Ki H. K., Il K. L., Hyung J. N., Sang U. C., Kang R. L. - Lignans from the Roots of *Berberis amurensis*, *Natural Product Sciences* **15** (1) (2009) 17-21.
16. Cai Y., Evans F. J., Roberts M. F., Phrltpson J. D., Zenk M. H., Gleba Y. Y. - Polyphenolic compounds from *Croton lechleri*, *Phytochemistry* **30** (6) (1991) 2033-2040.
17. Isao K., Yoshihiro M., Iwang S., Soediro S., Soelaksono S. - Cytotoxic anthraquinones from *Rheum pulmatum*, *Phytochemistry* **31** (3) (1992) 1063-1065.