Journal of Applied Pharmaceutical Science Vol. 10(03), pp 101-108, March, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2020.103013
ISSN 2231-3354

Phytochemical screening, *in vitro* anti-oxidant activity, and *in silico* anti-diabetic activity of aqueous extracts of *Ruellia tuberosa* L

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ARTICLE INFO

Received on: 17/09/2019
Accepted on: 11/01/2020
Available online: 05/03/2020

Key words:
Ruellia tuberosa L., LC-HRMS, phytochemical screening, DPPH, *in silico*, α-amylase.

ABSTRACT

The study aimed to investigate the phytochemical profiles, *in vitro* antioxidant activity, and *in silico* molecular docking antidiabetic activity of the aqueous root extracts of *Ruellia tuberosa* L. The phytochemical qualitative tests revealed the positive detections of tannins, flavonoids, ascorbic acid, and phenolic compounds. Using Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) analysis, 12 compounds were tentatively identified in the extracts. The major compounds were tentatively identified as betaine, daidzein, hispidulin, α-linoleic acid, and 4-coumaric acid. The aqueous root extracts have high antioxidant activity with the IC_{50} value of 15.2 µg/ml against DPPH free radicals. The major putatively identified compounds were docked to human pancreatic α-amylase protein, to investigate their inhibitory activities to this enzyme. The interaction between betaine, daidzein, and hispidulin in docking with human pancreatic α-amylase showed different binding sites to the protein. In addition, the types of bonds involved were mostly hydrogen and hydrophobic bonds which show the interactions between three ligands and α-amylase. Energy generated from docking between betaine, daidzein, and hispidulin with α-amylase was −137.6, −245.8, and −236.7 cal/mol, respectively. This study concludes that the aqueous root extracts of *R. tuberosa* L. have prospective as an inhibitor for α-amylase protein and to be used as antidiabetic agent. Further, *in vitro* and *in vivo* studies are needed to confirm this work.

INTRODUCTION

One of the most widespread diseases due to lifestyle problems in the 21st century is diabetes mellitus (DM). This disease is generally classified as insulin-dependent or type 1 DM, which is initiated by destruction of the insulin-producing pancreatic β-cells, and nondependent insulin or type 2 DM, which is triggered by lifestyle-related obesity or other exogenous components (Thomas and Philipson, 2015). People with type 1 diabetes need to take insulin injection for survival (Bhattarai *et al*., 2019). People with type 2 diabetes are not dependent on exogenous insulin; however, they display a reduced capacity to respond to insulin or a reduced sensitivity to insulin, and the high levels of insulin produced are risk factors for cancers and cardiovascular diseases (Laakso, 2019).

The total diabetes prevalence is estimated to be 463 million people in 2019 and will be rising to 578 million by 2030 (Saeedi *et al*., 2019). The administration of oral drugs and insulin administration are currently used either alone or in combination with antidiabetic treatments. Advances in understanding the pathophysiology of the disease and the mechanism of actions of antidiabetic drugs provide an opportunity to develop safe and effective treatments of diabetes and its complications (Chaudhury *et al*., 2017).

In the prevention of diabetes, drug consumption is a complementary treatment besides diet. Oral antidiabetic drugs may be useful for people who are allergic to insulin or do not use insulin injection (Verspohl, 2012). However, the use of these drugs in the long term has disadvantages, including causing acute kidney toxicity and increasing the risk of heart attack (Hu and Jia, 2019). Therefore, many efforts to develop traditional medicines

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for diabetes are mounting, in particular, in Asian countries, where natural sources are abundant (Boy *et al*., 2018; Khan *et al*., 2011; Premanath and Nanjaiah, 2015).

One of the flora that can be exploited and contains many beneficial secondary metabolites compounds is from the family of *Acanthaceae* (Arirudran *et al*., 2011). One member of the *Acanthaceae* family is from genus *Ruellia tuberosa* L. is an indigenous tropical plant that is widely grown in Asian countries, i.e., Indonesia. Previous phytochemical determination revealed that saponins, carotenoids, flavonoids, and phenols were contained in the leaves of *R. tuberosa* L. (Manikandan and Doss, 2010), which were extracted using ethanol and water. Some nutrients, including ascorbic acid and tocopherol, also present in those extracts (Manikandan and Doss, 2010). In the previous study, n-hexane root extracts of *R. tuberosa* L. roots have shown *in vivo* antidiabetic activity (Safitri *et al*., 2019a). These decrease the blood glucose concentrations, lower the levels of malondialdehyde, and show the improvements on the kidney histopathological profiles (Safitri *et al*., 2019a). Phytochemical examination of this study shown that n-hexane *R. tuberosa* L. root extracts contained triterpenoid compounds. Furthermore, the hydroethanolic root extracts of *R. tuberosa* L. have also shown antidiabetic capacities from their positive effects on the serum and pancreatic levels of animal diabetic models (Roosdiana *et al*., 2019; Safitri *et al*., 2019b). Phytochemical screening tests conducted on this study discovered that *R. tuberosa* L. roots extracted with ethanol and water contained flavonoids, phytosterols, and phenolic compounds (Ramadhan *et al*., 2019; Safitri *et al*., 2019c). These results were also supported by Fourier transform infrared (FTIR) and LC-MS (mass spectrometry) studies (Ramadhan *et al*., 2019; Safitri *et al*., 2019c).

Most bioactive compounds, including flavonoids, and other phenolic compounds are secondary metabolite compounds that generally dissolve in the polar solvent. Hence, in this work, water is used to extract the roots of *R. tuberosa* L. In addition, the aqueous root extracts of *R. tuberosa* L. are proposed to be safer and effective which can be used as a natural remedy. This is followed by the identification and characterization of the resultant aqueous root extracts. The characterizations conducted are phytochemical test and Liquid chromatography-high-resolution mass spectrometry (LC-HRMS). Taking into account the biological functions of the *R. tuberosa* L. that demonstrated earlier, the potential of antioxidant activity of the aqueous root extracts of *R. tuberosa* L. is determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging free radical test determination. For the preliminary, the antidiabetic properties of the aqueous root extracts are determined by conducting an *in silico* analysis of the extract compounds. These are carried out by molecular docking between human pancreatic a-amylase and the major compounds confined in the aqueous root extracts of *R. tuberosa* L., as shown from LC-HRMS results. Inhibitors of the carbohydrate digesting enzymes, i.e., α-amylase, are proposed to be one of the effective treatments for DM (Nair *et al*., 2013).

MATERIALS AND METHODS

Extraction of Ruellia tuberosa L.

The plant specimens (dried root powder) of *R. tuberosa* L. were collected from *Unit Pelaksana Teknis* or technical operation unit (UPT) Materia Medica, Batu, East Java, and were enclosed with a determination letter of the species. The *R. tuberosa* L. root powder was extracted using maceration technique, with distilled water, in the volume of 4x dried weight, for 2x 24 hours. The resulted extracts were collected through filtration. To obtain the concentrated extracts, a rotary evaporator vacuum was used with slow speed at 100 rpm, at 40°C–50°C. The concentrated extracts were kept at 4°C for subsequent analysis.

Phytochemical screening test of the aqueous root extracts of Ruellia tuberosa L.

The phytochemical qualitative tests were conducted based on standard phytochemical determinations (Evans, 2009; Harborne, 2012). The test was conducted for the detection of flavonoids, phenolic compounds, terpenoids, ascorbic acids, saponins, steroids, tannins, and alkaloids.

Antioxidant activity test of the aqueous root extracts of Ruellia tuberosa L.

Antioxidant activities were calculated based on the extracts of scavenging potential of the DPPH free radicals in quantitative assay. Experiments were conducted on the basis of the methods mentioned in the previous study (Safitri *et al*., 2019c). A stock solution (1 mg/ml) of the extracts was prepared in water, from which serial diluted solutions were made to obtain a series of concentrations of 5, 10, 20, 30, 40, and 50 mg/ml. An aliquot of solution was mixed with DPPH solution (50 mg/ml), in 3:2 ratio; these mixture solutions were incubated at 37°C for 20 minutes. The absorbance was measured at 516 nm, and from these values, the percentages of response inhibitions were determined. Then, % inhibitions were drawn against concentration; the IC_{50} was calculated from the graph, using linear equation, $Y = ax + b$. The assay was carried out in triplicate. Ascorbic acid was used in the concentration range of 2–10 mg/ml, for positive control.

Liquid chromatography-high-resolution mass spectrometry analysis of the aqueous root extracts of Ruellia tuberosa L.

Liquid chromatographic separation was conducted in the *Laboratorium Sentral Ilmu Hayati* or Central laboratory for life science (LSIH) Laboratory, Brawijaya University. Column used was a Hypersil GOLD aQ 50 × 1 mm × 1.9 mm particle size column, with an injection volume of 100 ml. Solvents used were solvent $A = 0.1\%$ formic acid in water and solvent $B = 0.1\%$ formic acid in acetonitrile, with an analytical flow rate of 40 ml/min. Liquid chromatography was run for 70 minutes, and this was followed by mass spectrometric analysis in Electrospray ionization (ESI) method, positive ion mode detection, using a Thermo Scientific Q Exactive mass spectrometer. Experiments were set as follows: sheath gas (N_2) pressure = 50 psi, spray voltage = 4.5 kV, capillary temperature = 300 K, and *m/z* range = 50–750. The compounds were detected using Compound Discoverer software with mzCloud MS/MS library.

Ligand and protein preparation for in silico study

The 3D structures of the aqueous root extracts of *R. tuberosa* L. compounds, such as betaine (CID 247), daidzein (CID 5281708), and hispidulin (CID 5281628), were obtained from PubChem National Center for Biotechnology Information (NCBI) database. PyRx Virtual Screening Tool software was used to minimize their energy and convert the Structure data file (SDF) format into Protein Data Bank (PDB) format. The human pancreatic alpha-amylase protein was retrieved from the Research Collaboratory

for Structural Bioinformatics (RCSB) PDB (ID: 5kez); water and other ligands bound to protein were removed using Discovery Studio Visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/).

Molecular docking simulations

Human pancreatic α-amylase protein was docked to betaine, daidzein, and hispidulin. The HEX 8.0 software was used in this study to predict the interaction and energy binding of betaine, daidzein, and hispidulin to alpha-amylase protein. The docking results were visualized using Discovery Studio Visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/).

RESULTS AND DISCUSSION

Phytochemical screening and LC-HRMS analysis of the aqueous root extracts of Ruellia tuberosa L.

The phytochemical investigation revealed that the aqueous root extracts of *R. tuberosa* L. contained the presence of flavonoids, tannins, ascorbic acids, and phenolic compounds, whereas the tests for saponins, steroids, alkaloids, and terpenoids yielded negative results (Fig. 1). The phytochemical tests were conducted based on the color changes after extracts reacted with the standard reagents for secondary metabolite detection. Understanding the phytochemical constituents contained in the extracts is important to predict the biological and pharmacological activities of the plants. Flavonoids, phenolic compounds, and ascorbic acids are commonly known to have high antioxidant activity (Limwachiranon *et al*., 2018; Smirnoff, 2018), whereas tannins are polyphenolic compounds that can act as antimicrobial agent (Smeriglio *et al*., 2017).

In the previous study, the hydroethanolic root extracts of *R. tuberosa* L. positively contained flavonoids, steroids, phenolics, and ascorbic acids (Safitri *et al*., 2019c), whereas another study reported the positive results of tannin, lycopene, tocopherol, ascorbic acid, phenolic, and carotenoid on the hydroethanolic leaves extracts of *R. tuberosa* L. (Manikandan and Dos, 2010). These demonstrate that *R. tuberosa* L. contains many secondary metabolite compounds and also some nutrients.

Tests conducted were: (a) flavonoids, (b) phenolics, (c) ascorbic acids, (d) tannins, (e) saponins, (f) steroids, (g) terpenoids, and (h) alkaloids. Positive test results are shown in (a) to (d), and negative test results are shown in (e) to (h).

To further characterize the extracts, LC-HRMS analysis was conducted. LC-HRMS analysis led to the tentative identification of 12 compounds in the extracts (Table 1). These include phenolic acids, amino acids, flavonoids, and fatty acids. The predominant compounds contained in the extracts were tentatively identified as betaine [Retention time (RT) = 0.886 minutes], 4-coumaric acid (RT = 6.248 minutes), hispidulin (RT = 13.530 minutes), daidzein (RT = 16.675 minutes), and α-linolenic acid (RT = 56.646 minutes). Nevertheless, the compounds detected using LC-HRMS are tentatively identified, since the high-resolution MS alone is inadequate to confirm the exact identification of each compound. However, it is interesting to note that there are numerous components other than secondary metabolites such as amino acids and fatty acids found at lower concentrations in the aqueous root extracts of *R. tuberosa* L.

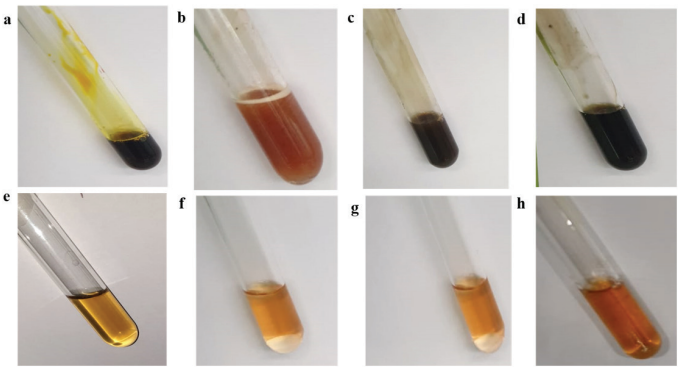

Figure 1. The phytochemical screening results of the aqueous root extracts of *R. tuberosa* L.

Betaine has a molecular formula $C_5H_{11}NO_2$, established by *m/z* 118.08607 $(M + H)^+$; 4-coumaric acid with the molecular formula $C_9H_8O_3$, appeared at *m/z* 165.05428 $(M + H)^+$; and hispidulin with molecular formula $C_{16}H_{12}O_6$, with *m/z* 301.06973 $(M + H)^+$. The molecular formula of daidzein and α-linolenic acid is $C_{15}H_{10}O_4$ and $C_{18}H_{30}O_2$, respectively, with the *m/z* emerged at 255.06450 $(M + H)^+$ and 279.23096 $(M + H)^+$, respectively (Fig. 2). These mass spectra are in correlation with the previous published literature reporting of mass spectrometry of betaine (Kimura *et al*., 2017), 4-coumaric acid (Wang *et al*., 2012), hispidulin (Yadav and Gusta, 2013), daidzein (Andres *et al*., 2015), and α-linolenic acid (Mok *et al*., 2018).

The previous studies have demonstrated that flavonoids and phenolic acids possess high biological and pharmacological activities (Wu *et al*., 2018). Hispidulin and daidzein are flavonoid compounds, whereas 4-coumaric acid is phenolic compound; these compounds have high antioxidant activity (Andres *et al*., 2015; Yadav and Gusta, 2013). A previous study showed that hispidulin inhibits apoptosis and causes autophagy in diabetic murine induced by high glucose diet (Wu *et al*., 2018). In addition, daidzein also has been reviewed to have antidiabetic activity from the evidence from clinical, preclinical, and cell culture studies (Das *et al*., 2018).

Betaine, the oxidation product of choline, can act as an organic osmolyte to protect cells under stress (Heidari *et al*., 2018). Another study pointed out that betaine has a potential application in the treatment of diabetes, by showing improvement in serum insulin and triglycerides levels of *db/db* mice (Jung *et al*., 2013).

Table 1. LC-HRMS results of the aqueous root extracts of *R. tuberosa* L. with proposed identities of compounds with mass errors of less than 5 ppm.

No	Proposed Compound	RT (minutes)	Experimental *m/z*	Calculated *m/z*	D*m* (ppm)
1	Betaine	0.886	117.07823	117.07879	4.8
2	L-phenylalanine	1.511	165.07824	165.07871	2.8
3	Isoamylamine	1.613	87.10449	87.10501	6.0
4	Phenmetrazine	2.079	177.11460	177.11501	2.3
5	DL-tryptophan	2.600	226.07304	226.07356	2.3
6	6-Methylquinoline	3.650	143.07270	143.07311	2.9
7	4-Coumaric acid	6.238	164.04644	164.04696	3.2
8	1,5-Isoquinolinediol	9.236	161.04674	161.04726	3.2
9	Hispidulin	13.530	300.06189	300.06236	1.6
10	Daidzein	16.623	254.05666	254.05707	1.6
11	Valerophenone	19.262	162.10353	162.10394	2.5
12	α-Linolenic acid	56.646	278.22312	278.22359	1.7

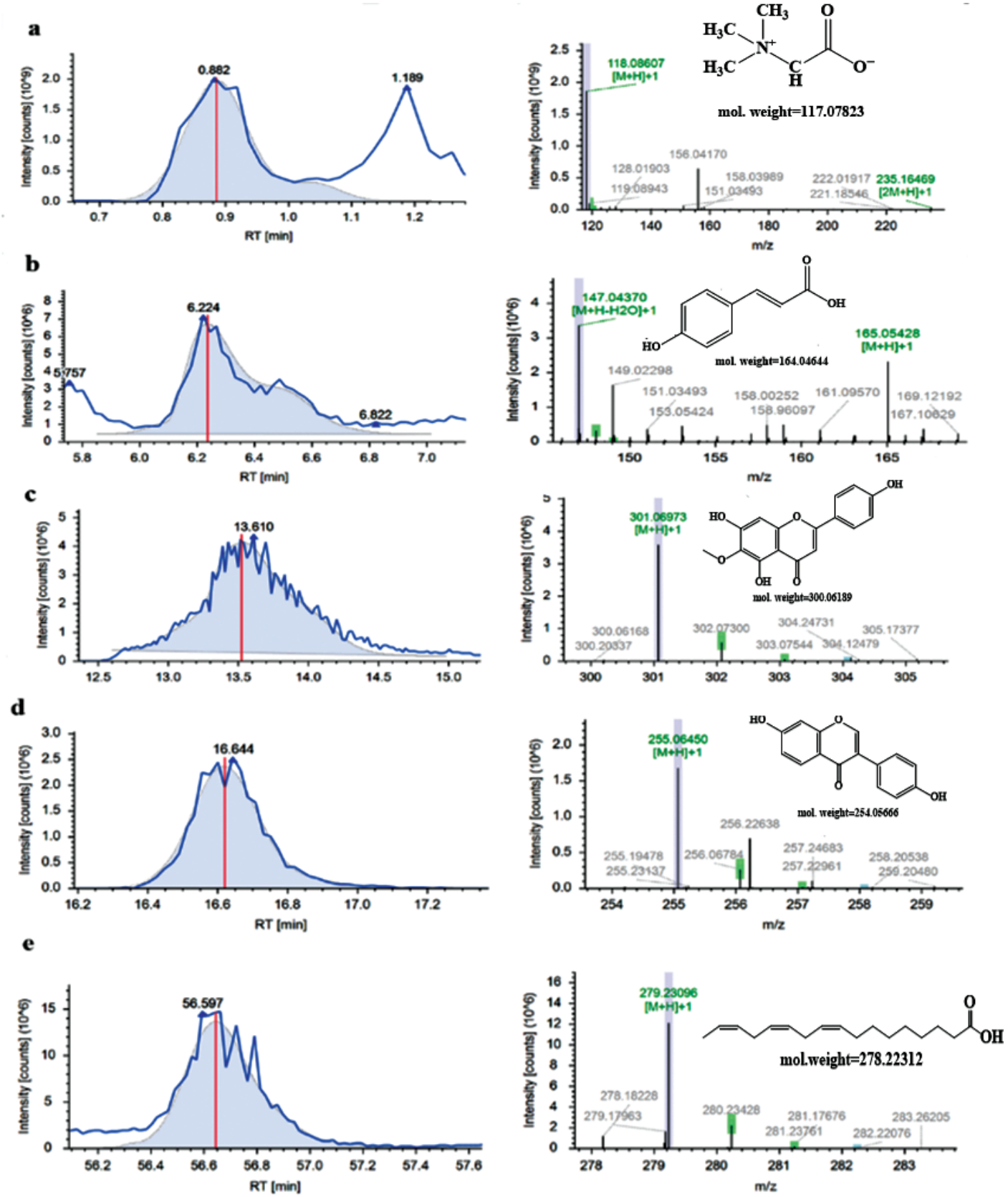

Figure 2. LC-HRMS chromatograms and mass spectra of (a) betaine, (b) 4-coumaric acid, (c) hispidulin, (d) daidzein, and (e) α-linolenic acid, of the aqueous root extracts of *R. tuberosa* L. Figures show experimental molecular weight based on the mass spectra.

Finally, α-linolenic acid is an essential fatty acid that is needed in human diet (Calder, 2015).

These have proved that water as single solvent can be applied for the extraction of secondary metabolite compounds from plants. Furthermore, since the compounds contained in the aqueous root extracts of *R. tuberosa* L have many biological capacities, the extracts have a potency to be used as natural remedy.

Antioxidant activity of the aqueous root extracts of Ruellia tuberosa L.

Many human disorders such as neurodegenerative diseases, cancer, and diabetes are frequently caused by the involvement of free radicals. In treatment of those diseases, the antioxidants through their scavenging power are needed. Many plants used for treating diabetes were reported to have antioxidant activities; therefore, it is necessary to confirm the antioxidant activities of selected medicinal plants *in vitro* using reliable methods such as radical scavenging activity and reducing power. DPPH free radical scavenging assay is a rapid and effective technique to determine the antioxidant action of a specific compound or plant extracts. The antioxidant activity of the aqueous root extracts of *R. tuberosa* L. (IC_{50} of 15.22 mg/ml) is lower compared to that the antioxidant activity of ascorbic acid (IC_{50} of 2.48 mg/ml), as shown in Table 2. However, with the IC_{50} value of 15.22 mg/ml (<50 mg/ml), the aqueous root extracts of *R. tuberosa* L. are still considered which possess high antioxidant capacity (Molyneux, 2004). These are in agreement with results from previous studies which shown that *R. tuberosa* L. extracted with organic solvents possesses biological capacities, such as antioxidant, anti-inflammation, and anticancer (Chothani *et al*., 2010).

Table 2. Antioxidant activity of the aqueous root extracts of *R. tuberosa* L.

No	Sample concentration (mg/ml)	Sample inhibition activity (%)	IC_{50} of sample (mg/ml)*	Ascorbic acid concentration (mg/ml)	Ascorbic acid inhibition activity (%)	IC_{50} of ascorbic acid (mg/ml)*
1	5	41.4		2	48.9	
2	10	47.4		4	50.4	
3	20	55.0	**15.22± 0.08**	6	53.4	**2.48 ± 0.77**
4	30	58.6	**R^2 = 0.9857**	8	56.3	**R^2 = 0.9803**
5	40	74.4		10	57.8	

*Averages and standard deviation from three replicates from two independent experiments are shown.

Table 3. Interaction of human pancreatic α-amylase with betaine, daidzein, or hispidulin by *in silico* analysis.

Compound	Interaction*	Chemistry bond	Types	Energy (cal/mol)
Betaine	**:LIG1:N** - A:ASP402:OD1	Electrostatic	Attractive charge	−137.6
	A:ARG398:HE - :LIG1:O	Hydrogen bond	Conventional hydrogen bond	
	:LIG1:H - A:GLY334:O	Hydrogen bond	Carbon hydrogen bond	
	:LIG1:H - A:GLY334:O	Hydrogen bond	Carbon hydrogen bond	
	:LIG1:H - A:THR11:O	Hydrogen bond	Carbon hydrogen bond	
Daidzein	**A:ARG389:NH1** - :LIG1	Electrostatic	Pi-Cation	−245.8
	A:ARG389:NH1 - :LIG1	Electrostatic	Pi-Cation	
	:LIG1:O - A:TRP388	Other	Pi-Lone Pair	
	A:TRP388 - :LIG1	Hydrophobic	Pi-Pi stacked	
	A:TRP388 - :LIG1	Hydrophobic	Pi-Pi stacked	
	:LIG1 - A:ARG389	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ARG389	Hydrophobic	Pi-Alkyl	
Hispidulin	**A:SER3:HG** - :LIG1:O	hydrogen bond	Conventional hydrogen bond	−236.7
	A:ASN5:HN - :LIG1:O	Hydrogen bond	Conventional hydrogen bond	
	A:GLY9:HN - :LIG1:O	Hydrogen bond	Conventional hydrogen bond	
	:LIG1:H - :LIG1:O	Hydrogen bond	Conventional Hydrogen bond	
	:LIG1:H - A:GLY334:O	Hydrogen bond	Conventional hydrogen bond	
	A:PRO4:CD - :LIG1:O	Hydrogen bond	Carbon hydrogen bond	
	A:PHE335 - :LIG1	Hydrophobic	Pi-Pi T-shaped	
	:LIG1 - A:PRO4	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:PRO4	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:PRO4	Hydrophobic	Pi-Alkyl	

*The H-donor in betaine-human a-amylase interaction; positive ion in Pi-cation, lone pair in Pi-lone pair, and Pi-orbitals in daidzein-human a-amylase interaction; and H-donor in hydrogen bonds and Pi-orbitals in hispidulin-human a-amylase interaction were written in bold letters.

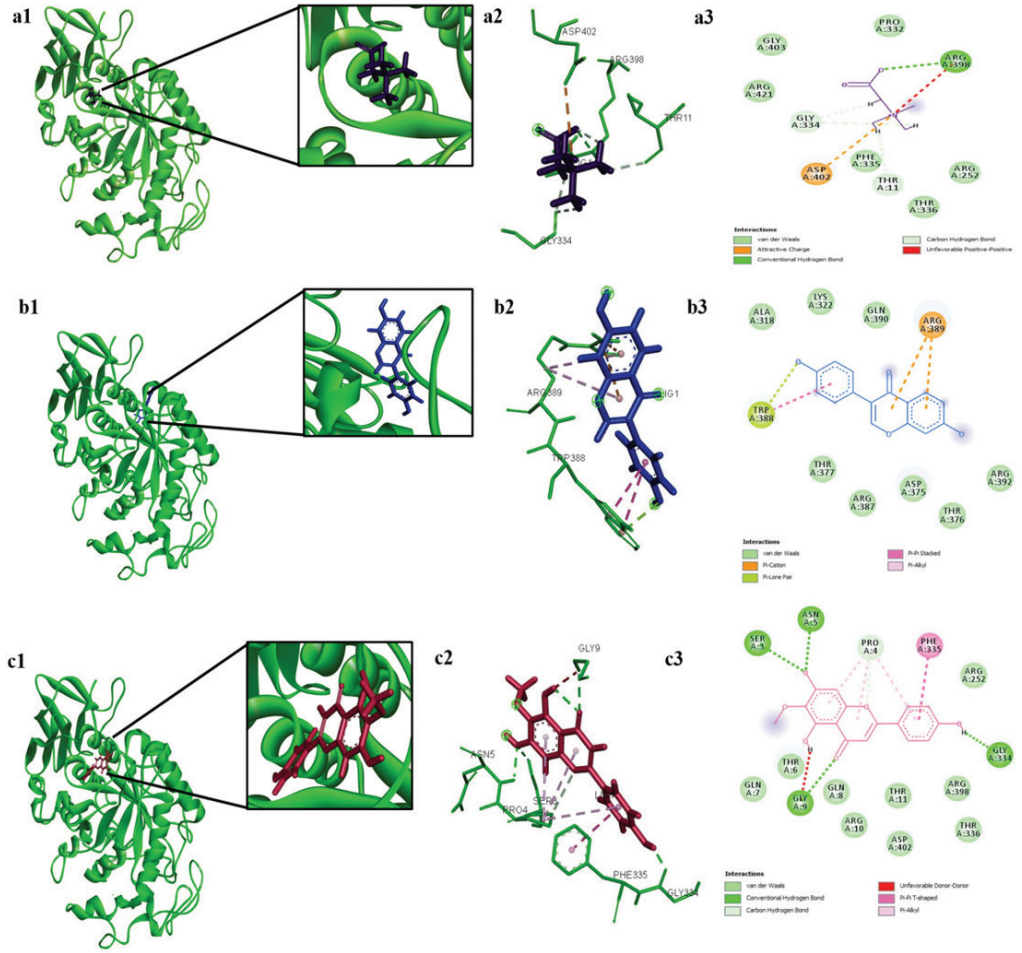

Figure 3. *In silico* molecular docking results of interactions between human pancreatic a-amylase protein with (a) betaine, (b) daidzein, and (c) hispidulin. Number 1 shows an overview of betaine/daidzein/hispidulin-human pancreatic a-amylase complex; number 2 shows the 3D structure of betaine/daidzein/hispidulin-human pancreatic a-amylase complex; and number 3 shows the 2D structure of betaine/daidzein/hispidulin-human pancreatic a-amylase complex.

In silico molecular docking analysis

The molecular docking between betaine, daidzein, and hispidulin to human pancreatic α-amylase protein has been conducted to investigate further interaction between the protein and ligands. Ligands chosen for the study are betaine, daidzein, and hispidulin. It is predicted that these compounds have the ability to bind human pancreatic α-amylase; thus, they have antidiabetic capacity.

The ligands-protein interaction was presented by the binding sites on amino acid residue and the types of chemistry bond formed as shown in Table 3. Four amino acid residues in domain A of human pancreatic α-amylase were interacted with betaine (Fig. 3a): Asp402, Arg398, Thr11, and Gly 334. The energy binding of the betaine-human pancreatic α-amylase complex was −137.6 cal/mol. Those interactions were mediated by hydrogen bond formation, either conventional hydrogen bond or carbon-hydrogen bond, and also attractive charge.

Unlike betaine, daidzein bound to different binding sites to human pancreatic α-amylase (Fig. 3b). The daidzein-human pancreatic α-amylase complex had a less number of interactions compared to those of betaine-human pancreatic α-amylase complex (Table 3). Based on the molecular docking results in Fig. 3(b), Arg389 and Trp388 were the amino acid residues that bound to daidzein by establishing Pi-alkyl and Pi-lone pair donor through hydrophobic interaction. The daidzein-human pancreatic α-amylase complex had the energy binding of −245.8 cal/mol. Intriguingly, the interaction between hispidulin and human pancreatic α-amylase occurred at different binding sites of the protein as well, different with those of betaine and daidzein (Fig. 3c). There were six amino acid residues interacted with hispidulin: Asn5, Ser3, Phe335, Gly334, Gly9, and Pro4. These interactions were maintained by hydrogen bonds and hydrophobic interaction. The hispidulin-human pancreatic amylase complex resulted in the energy binding of −236.7 cal/mol.

Alpha-amylase inhibitors are used as antidiabetic drugs since they can control carbohydrate breakdown and eventually reduce the rate of carbohydrate absorption from the gastrointestinal tract (Nair *et al*., 2013). In recent years, drugs designed to lessen blood glucose levels and to maintain glucose homeostasis from natural products are gaining popularity (Jhong *et al*., 2015). Many research has been conducted this area to search alternative drugs from natural materials that could normalize hyperglycemia through the downregulation of alpha-amylase activity. Nevertheless, the active mechanisms of action remain unclear.

The different kinds of interaction affected the binding energy (Fatchiyah *et al*., 2017). Hydrogen bonds are important contributors for the structure and interaction of protein-protein or ligand-receptor (Chen *et al*., 2016; Kostal, 2016). In the drug design, hydrogen bonds are critical to obtain the specificity of the drug to protein target (Chen, et. al., 2018). In this *in silico* analysis, betaine-human pancreatic a-amylase interaction showed the lowest number of hydrogen and hydrophobic bonds; therefore, this complex also shown the weakest binding affinity. The highest binding affinity was shown in the daidzein-human pancreatic a-amylase complex. The *in silico* study conducted has supported the identification and characterization and *in vitro* pharmacological antioxidant activity of the aqueous root extracts of *R. tuberosa* L.

The studies on the flavonoid compounds acting as alpha-amylase inhibitors through molecular docking approach have been conducted previously (Jhong *et al*., 2015; Meidinna and Fatchiyah, 2019). An *in silico* study for the compound in the n-hexane fraction of methanolic extract (HFME) of *R. tuberosa* L. has been conducted previously, also using a-amylase as protein target (Wulan *et al*., 2015). Results from this study showed that betulin, compound contained in the HFME of *R. tuberosa* L., strongly inhibited α-amylase activity, suggesting to have antidiabetic activity. Those studies have also concluded that *in silico* molecular docking is an effective and rapid method for investigating molecular interaction and mode of action of bioactive compounds targeting alpha-amylase activity.

CONCLUSION

This study implies that the aqueous root extracts of *R. tuberosa* L. have high antioxidant activity, and these extracts have potential as inhibitor for α-amylase protein. Water as single solvent can be useful for secondary metabolite extraction that possesses many biological capacities. The use of LC-HRMS tentatively identified many metabolites and compounds contained in the aqueous root extracts of *R. tuberosa* L. Further analyses, including high-resolution MS/MS and isotopic analysis, are required for positive identification of the compounds. The inhibition of alpha-amylase enzyme leads to novel discovery of plant-based therapeutic products, in particular, for diabetes. Computational molecular docking could be used as an effective supporting tool for the drug development process. To sum up, the aqueous root extracts of *R. tuberosa* L. have prospective to be utilized as natural remedy for diabetes. Further research on both the *in vitro* and *in vivo* approaches is needed to confirm this current work results.

ACKNOWLEDGMENT

This research was supported by *Hibah Penelitian Unggulan*, Universitas Brawijaya, Indonesia, grant number 697.1/UN10.C10/PN/2019.

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How to cite this article:
Safitri A, Fatchiyah F, Sari DRT, Roosdiana A. Phytochemical Phytochemical screening, *in vitro* anti-oxidant activity, and *in silico* anti-diabetic activity of aqueous extracts of *Ruellia tuberosa* L. J Appl Pharm Sci, 2020; 10(03):101–108.