Vol 11, Special Issue 6, 2019

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

ISSN - 0975-7058

Original Article

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES BY DPPH RADICAL SCAVENGER ACTIVITY OF *RUELLIA BRITTONIANA* FLOWER

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Received: 11 Dec 2018, Revised and Accepted: 10 Mar 2019

ABSTRACT

Objective: The genus *Ruellia* has been widely used in traditional and Ayurvedic medicine as an antioxidant. This study seeks to examine the antioxidant activity of the species *Ruellia brittoniana*.

Methods: In this study, *Ruellia brittoniana* flowers were acquired from Depok, West Java, Indonesia. The flowers were cleaned and ground to form a powder, then dissolved in hexane, ethanol and ethyl acetate solvents. These three extracts were then tested for phytochemicals and thin layer chromatography (TLC) analysis. Ethanol and ethyl acetate extracts were also analyzed for antioxidants using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

Results: Phytochemical results from the three extracts proved that *Ruellia brittoniana* contains flavonoids, alkaloids, tannins, glycosides and triterpenes. These results are comparable to the results from TLC analysis, which showed the samples contained 4–5 chemical components. Furthermore, the best antioxidant activity resulted from the ethyl acetate extract of the *Ruellia brittoniana* flower with an IC50 value of 68.42 ppm.

Conclusion: An ethyl acetate extract from the *Ruellia brittoniana* flower can be used as a natural source of additional antioxidants.

Keywords: Phytochemicals, Antioxidant, *Ruellia brittoniana*, Acanthaceae, DPPH method

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s6.33531

INTRODUCTION

Living organisms produce reactive oxygen species (ROS) as the result of cell metabolism. In low to moderate concentrations, ROS work in the physiological cell process; however, in high concentrations, ROS negatively modify the cell's components, such as lipids, DNA, and proteins. Antioxidants, which are chemical substances that can interact and neutralize free radicals, can prevent the harmful effects of ROS. Normally, every aerobic organism has a system that consists of enzymatic and non-enzymatic antioxidants. However, in a pathological condition, the antioxidant system can be overwhelmed, which causes an imbalance between oxidants and antioxidants. In those cases, the human body needs an exogenous antioxidant to help ward off free radicals. Some exogenous synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) might have carcinogenic effect. Therefore, current research of exogenous natural antioxidants focuses on herbs. Spices and herbs have been identified as sources of diverse phytochemicals, and many of them have potent antioxidant activity [1-5].

Indonesia has the seventh highest plant diversity with 200 known species. Acanthaceae is one of the plant families that can be found in Indonesia, consisting of 250 genera and 2500 species [6]. Acanthaceae is often used as a decorative plant because of its beauty [6]. One of its genera is *Ruellia*, which has been used in Ayurvedic treatments and traditional medicine as an antioxidant [6]. Recently, there have been a number of studies undertaking antioxidant analysis of some species of *Ruellia* plants [6]. One study conducted antioxidant analysis of *Ruellia tuberosa* stem extracts using the DPPH method, which showed potent antioxidant activity [7]. Antioxidant analysis of aerial parts of *Ruellia prostata* showed that this plant also has potent antioxidant activity [7]. Other research has shown that aerial parts of *Ruellia brittoniana* produce an antioxidant effect of 4.2% that inhibits free radical of DPPH [8].

Previous research shows that many species of the genus *Ruellia* have strong antioxidant activity, but there has been no research done for the antioxidant activity of the *Ruellia brittoniana* flower. *Ruellia brittoniana* is a plant originally cultivated in Mexico, the Caribbean, and South America, but it can also be found in Indonesia. This plant lives in tropical and subtropical climate zones and thrives in riverine terrestrial habitats. The author is interested in examining the antioxidant activity of the *Ruellia brittoniana* flower, since there is a high probability that it also has strong antioxidant activity. The test used in this research is the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method using ethanol and ethyl acetate solvents. The aim of this research is to use phytochemical analysis to evaluate antioxidant activity of ethanol and ethyl acetate extracts of the *Ruellia brittoniana* flower [9].

MATERIALS AND METHODS

Extraction of *Ruellia brittoniana* flower

One hundred-gram samples of *Ruellia brittoniana* flowers were dried and milled into powder form. The powder was then macerated gradually three times for each solvent. The solvents used were n-hexane, ethyl acetate, and ethanol. Maceration results were then filtered and concentrated using a rotary vacuum evaporator to evaporate the solvent and form ethanol, hexane, and ethyl acetate extracts of the *Ruellia brittoniana* flower. These extracts underwent phytochemical analysis.

Phytochemicals analysis

The composition of organic compounds in ethanol, hexane, and ethyl acetate extracts were analyzed. The first step was to dissolve 5 grams of each sample with 15 ml of water, then heat and filter it until a 15 ml filtrate sample formed. Each filtrate sample underwent various tests for the chemical content of the organic compounds.

Alkaloid test

Two ml of filtrate were evaporated until a residue formed. The residue was then dissolved with HCl, 2N 5 ml. The solution formed was divided into three test tubes. The first tube was filled only with the HCl, 2N solution, three drops of Dragendorff reagent were added to the second tube, and three drops of Mayer reagent were added to the third tube. If the sample contained alkaloids, an orange precipitate would form in the second tube and a yellow precipitate in the third tube.

Flavonoid test

One ml of filtrate was put into a porcelain cup, where it was evaporated until dry. Next, the contents were moistened with acetone, and borax powder and oxalic acid were added. The resultant solution was then heated, and ether 10 ml was added. Finally, the result was observed with ultraviolet (UV) light at 366 nm. If the color was an intense yellow, it was positive for flavonoids.

Saponin test

Ten ml of filtrate were put into a test tube and shaken for ten seconds. After waiting another ten seconds, the filtrate was then observed for foam formation. If foam formed with a height of 1–10 cm and was stable for ten minutes, then one drop of HCl, 2N was added. If the foam did not disappear after the addition of HCl, 2N, the sample was positive for saponin.

Steroid and triterpene tests

For this test, a new filtrate was made by dissolving 3g of the sample with hot ethanol 10 ml, then it was filtered. Two milliliters of this filtrate were then placed in a test tube and evaporated. Next, CHCl3 0.5 ml, anhydrous acetic acid and H2SO4 2 ml were added. If a brown or violet ring formed, the sample was positive for triterpenes; whereas, if a greenish-blue ring formed, the sample was positive for steroids.

Tannin test

One ml of filtrate was placed into a test tube, and FeCl3 10% was added. A change of color to dark blue or greenish black represented the presence of tannins.

Glycoside test

One ml of filtrate was put into a test tube, then evaporated. Next, anhydrous acetic acid 5 ml and H2SO4 10 ml concentrate were added. A change of color to blue or green represented the presence of glycosides.

Thin layer chromatography (TLC) test

TLC analysis aims to evaluate how many components are contained in a mixture. The TLC procedure uses plates of glass, iron or plastic coated with a thin layer of solid adsorbent (e. g., aluminum or silica gel). The mixture to be analyzed is bottled in a small amount at the bottom of the TLC plate. This plate is then placed in a shallow solvent pool until the bottom part of the plate touches the solvent. The solvent used was a solution of chloroform and methanol with a 4:1 ratio. The solvent will begin to move past the spot that has been applied under the TLC plate. When the solution has reached the top of the plate, the plate is then taken from the pool and dried. Next, the components in the mixture are visualized. If the component is colored, direct visualization can be done. But generally, components are not colored, so UV light is needed for visualization. Finally, the retention factor (Rf) is calculated. Rf is the distance traveled by a compound compared to the distance traveled by the solvent. A compound with a greater Rf means that it has a lower polarity because of the interaction with the adsorbent on the weaker TLC plate [10].

Antioxidant activity test

Procedure of antioxidant activity evaluation of *Ruellia brittoniana* extracts by DPPH method as follows, One hundred microliters (µL) of extracted filtrate was added to 2.9 ml of DPPH 0.004% concentration in methanol. The color change of the solution from purple to yellow showed the filtrate's free radical scavenger abilities. Next, the test tube was incubated for 120 min in the dark at room temperature. The absorbance of the solution was then measured using a spectrophotometer with a wavelength of 517 nm [11].

Next, the percentage of free radical inhibition was calculated with the following formula: [11]

DPPH inhibition (%) = (Absorbance of sample-Blank absorbance)/ (Blank absorbance) x 100%

Definitions:

Blank absorbance = Absorbance of DPPH without a sample

Absorbance of samples = Absorbance of DPPH with a sample

Next, the IC50 value was calculated, which is the concentration at which the extract can counteract free radicals by 50%. This calculation is obtained using the linear equation y = a+bx. In this study the standard curve used was y = 3.93x+7.82 (R2 = 0.907) [11].

RESULTS

Extraction

In this study, the maceration process was carried out to produce extracts of ethanol, ethyl acetate, and hexane from the *Ruellia brittoniana* flower [12, 13].

Table 1: Phytochemical analysis of ethanol, ethyl acetate, and hexane extracts of *Ruellia brittoniana flower*

Metabolites	Solvent		
	Ethanol	Ethyl acetate	Hexane
Tannin	+	+	+
Glycoside	+	+	+
Triterpenoid	+	+	+
Steroid	-	-	-
Saponin	-	-	-
Alkaloid	+	+	+
Flavonoid	+	+	+

Table 2: Thin layer chromatography analysis of *Ruellia brittoniana* flower extracts with ethanol, ethyl acetate, and hexane

Extract	Rf value				
	1	2	3	4	5
Ethanol	0.250	0.389	0.750	0.833	0.9167
Ethyl acetate	0.1389	0.722	0.833	0.944	-
Hexane	0.1389	0.750	0.833	0.916	-

Table 3: Antioxidant activity of ethanol extract of *Ruellia brittoniana* flower, as determined by DPPH analysis

Concentration (ppm)	% Inhibition	SD
3.75	25.2	0.0110
6.25	25.6	0.0271
12.5	28.0	0.0013
25	27.8	0.0088
50	31.5	0.0161

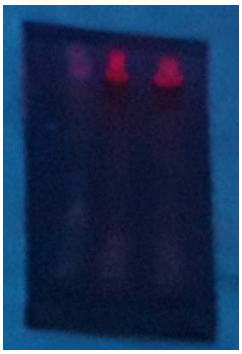

Fig. 1: TLC results of ethanol, ethyl acetate, and hexane extracts from the *Ruellia brittoniana* flower

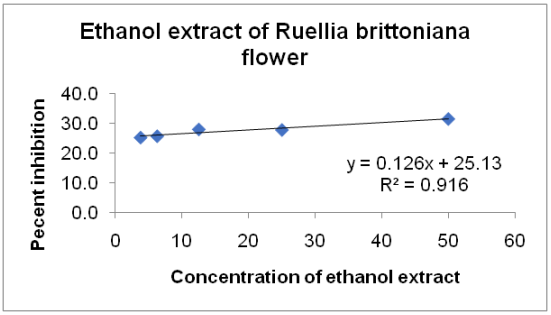

Fig. 3: The relationship between concentration of the *R. brittoniana* ethanol extract with percentage of inhibition

The IC50 value of the *Ruellia brittoniana* flower ethanol extract was calculated from the linear equation y = 0.1266x+25.135, where R2 = 0.9168, the x-axis represents the concentration of the test sample (ppm) and the y-axis represents the percent inhibition. Fig. 2 shows the relationship between the concentration of the *Ruellia brittoniana* flower ethanol extract and the percentage of DPPH activity inhibition, where a greater concentration equals a greater percentage of inhibition.

Based on the calculation from the linear equation above, where the y coefficient is the IC50 value, and the x coefficient is the concentration value of extract sought to inhibit 50% DPPH activity, the IC50 value of the ethanol extract is 196.406 ppm. The largest concentration of *Ruellia brittoniana* ethanol extract tested revealed DPPH inhibition results of only 31.5%.

Table 4 shows the results of testing the antioxidant activity of *Ruellia brittoniana* flower ethyl acetate extract.

Table 4: Antioxidant activity of *Ruellia brittoniana* flower ethyl acetate extract

Concentration (ppm)	% Inhibition	SD
3.75	18.6	0.0079
6.25	24.0	0.0827
12.5	24.3	0.0696
25	30.1	0.0402
50	16.7	0.0001

The IC_{50} value of *Ruellia brittoniana* ethyl acetate extract was obtained from the linear equation y = 0.1266x+25.135, with R2= 0.9168. The IC_{50} value of ethyl acetate extract of *Ruellia brittoniana* is 68.42 ppm, which means that the required concentration of *Ruellia brittoniana* ethyl acetate extract to inhibit 50% DPPH activity is 68.42 ppm. Ethyl acetate extract of Ruellia brittoniana is effective as an antioxidant against DPPH at the concentration below 50 ppm. As shown in table 4, percentage of inhibition increasing from 18.6% at the concentration of 3.75 ppm to 30.1% at the concentration of 25 ppm. However, the percentage of inhibition decreasing to 16.7% at the concentration of 50 ppm. Fig. 3 shows the relationship between the concentration of ethyl acetate extract of *Ruellia brittonian*a and percentage of inhibition on DPPH.

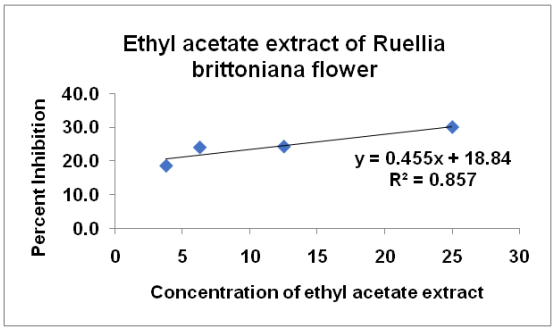

Fig. 4: The relationship between concentration of *R. brittoniana* ethyl acetate extract and percent inhibition

DISCUSSION

Extraction

The serial exhaustive extraction method was used in this research by testing various solvents ranging from non-polar (e. g., hexane) to high polarity (e. g., ethanol). Compounds with varied polarity can be extracted well because the extraction of a compound is adjusted to the polarity of the solvent. For example, extraction of lipophilic compounds, such as terpenoids and alkaloids, must use non-polar solvents like n-hexane; whereas, ethyl acetate is used for extraction of alkaloids, flavonoids and terpenoids. Polar solvents, such as methanol, ethanol and acetone are the main solvents for extraction of alkaloids, saponin, flavonol and polyphenols [12, 13].

Phytochemical analysis

Table 1 shows the phytochemical analysis results from ethanol, ethyl acetate, and hexane extracts of the *Ruellia brittoniana* flower.

Based on the phytochemical analysis above, it was found that ethanol, ethyl acetate and hexane extracts of the *Ruellia brittoniana* flower were positive for tannins, glycosides, triterpenes, alkaloids and flavonoids. Meanwhile, these three extracts did not contain steroids or saponins. Tannin or tannic acid is a water-soluble polyphenol found in many plants. Many tannin molecules show an ability to reduce the mutagenic activity of some mutagens. In addition, tannins also have anticarcinogenic effects. The anticarcinogenic and antimutagenic effect of tannins can be associated with antioxidative abilities, which play a role in protecting cells from oxidative damage. Tannins also have antimicrobial properties that can inhibit the growth of various bacteria, viruses, yeasts and fungi. In addition, they also have several physiological effects, such as blood pressure reduction, immune response modulation, and acceleration of blood coagulation and lowering of serum lipid levels. These effects are dependent on the dose and type of tannin [14].

Glycosides are compounds that consist of one or more sugars united with non-carbohydrates, such as phenol, alcohol or other complex molecules like a steroid nucleus. Glycosides exhibit antidiarrhea properties by inhibiting the release of autocoids and prostaglandins. Triterpenes, which are metabolites of isopentenyl pyrophosphate oligomers, account for the largest number of phytochemicals. It is

estimated that more than 20,000 triterpenes exist in nature. They are naturally found in various plants, fruits and herbal medicines. In many Asian countries, triterpenes are used for medicines such as analgesia, anti-inflammatories, antipyretics, cardiotonics and hepatoprotectives. Recent studies proved triterpenes can also act as antioxidants, antimicrobials, antivirals, antipruritics, antiallergics, antispasmodics, and antiangiogenics. Triterpenes are also reported to be cytotoxic to several cancer types without being toxic to normal cells. In addition, triterpenes also have antitumor properties [15, 16].

Plants produce various organic compounds called secondary metabolites (flavonoids), and most do not directly affect plant growth or development. Flavonoids have antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties. They also possess the ability to modulate the main function of the cellular enzyme; therefore, they have become an important component for pharmaceutical, cosmetic and nutraceutical purposes. Mainly, an antioxidant mechanism is carried out by preventing, delaying or removing a harmful oxidative effect. Antioxidant mechanism of flavonoid is comprehensive, including the suppression of free radical elements and chelating the metal ions that not oxidized and can bind to the human body. It also puts pressure on the enzyme associated with the generation of free radicals and stimulates an internal antioxidant enzyme. Flavonoid antioxidant activity is best described by an ability to directly counteract free radicals by donating hydrogen atoms or by single electron transfer [17, 18].

Alkaloids are natural products found in beverages, plants, tobacco smoke and well-cooked food. Many plants used as medicine for thousands of years have healing effects because of alkaloids. They are one of the important groups of phytoconstituents from natural sources and play an important role in the ecology of organisms that synthesize them. They belong to a beta-carboline group, which has anti-HIV, antimicrobial and antiparasitic properties. In some cases, alkaloids derived from plants can also become toxic and cause serious illness, injury or death. With the concentration of tannins, triterpenes and flavonoids in it, the *Ruellia brittoniana* flower has great potential to become a natural antioxidant source that can help the human body ward off free radicals [19].

Thin layer chromatography analysis

TLC tests can provide information on how many components are contained in a mixture. TLC test principles use two phases: the static phase and the mobile phase. The static phase in this research used GF 254 aluminum silica gel, while the mobile phase used a solvent that slowly rose above the TLC plate through capillary force. Table 2 shows the TLC analysis results for ethanol, ethyl acetate and hexane extracts of the *Ruellia brittoniana* flower [10, 20].

TLC analysis of *Ruellia brittoniana* flower ethanol extract shows five components with Rf values of 0.250, 0.389, 0.750, 0.833 and 0.9167. The TLC analysis of the ethyl acetate extract shows four components of chemical compounds with Rf values of 0.1389, 0.722, 0.833 and 0.944. Finally, TLC analysis of the hexane extract also showed four components with Rf values of 0.1389, 0.750, 0.833 and 0.916.

When the Rf values of these three extracts are compared, it can be seen that they have similar chemical components with the same Rf value of 0.833. In addition, the comparison between hexane extract and ethyl acetate extract shows that both of these extracts have two similar compounds since they have same spot with Rf value of 0.1389 and 0.833. Comparison between hexane extract and ethanol extract shows that both of these extracts have three similar components because of the same Rf values of 0.75, 0.833, and 0.916. Finally, the comparison between ethyl acetate and ethanol shows that these two extracts have only one similar component with an Rf value of 0.833.

Compounds with larger Rf values have a lower polarity due to weak interactions with adsorbents on the TLC plate, and smaller Rf values demonstrate higher polarity properties [10]. Based on these three extracts, the component with the lowest polarity is found in the ethyl acetate extract with an Rf of 0.944; whereas, the compound component with the highest polarity is found in the ethanol extract with an Rf of 0.25.

DPPH analysis

The DPPH method is a simple and sensitive antioxidant test method. The basic theory of this method is that antioxidants are hydrogen donors. In this method, the compound to be tested is added to DPPH•, which is a free radical in the form of a stable organic nitrogen. If the compound tested is an antioxidant, the compound will donate hydrogen to the DPPH• compound and cause the loss of the DPPH• compound in the test sample. Fig. 1 shows how the DPPH• mechanism obtains hydrogen from the antioxidant. UV spectrophotometry is used to monitor the loss or absence of DPPH• compounds in the test sample. The maximum absorbance of DPPH• compounds is at a wavelength of 517 nm (purple colored waves). If the DPPH compound is lost due to the addition of hydrogen by antioxidants, the test sample changes color from purple to yellow. This reaction is related to the number of hydrogen atoms absorbed by the DPPH compound, so this reaction is stoichiometric [21].

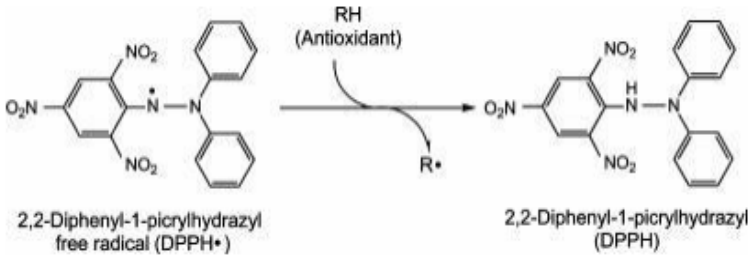

Fig. 5: Free radical conversion to DPPH by antioxidant compounds [21]

The ability of a compound to counteract free radicals is measured by calculating IC50 values, which is the amount of extract concentration that can counteract 50% of the free radicals in DPPH. This value is obtained from the linear equation y = a+bx [21]. In this study, the antioxidant activity test was carried out on *Ruellia brittoniana* flower ethanol and ethyl acetate extracts in duplicate.

One study showed that there are three classifications of antioxidant activity based on IC50 values. Compounds with IC50>150 ppm are classified as weak antioxidants, compounds with IC50 values ranging from 50–100 ppm are said to have potential antioxidants, and compounds with IC50<50 ppm have very strong antioxidant activity [22]. Based on the test results in table 5**,** the IC50 value of *Ruellia brittoniana* flower ethanol extract is 196.406 ppm, thus this ethanol extract has weak antioxidants. Meanwhile, the IC50 value of *Ruellia brittoniana* flower ethyl acetate extract is 68.42, so this extract has potential antioxidant activity. Thus, ethyl acetate extract of *Ruellia brittoniana* has stronger antioxidant activity than the ethanol extract.

Table 5: Antioxidant activity test results for the *Ruellia brittoniana* flower

Extract of *R. brittoniana*	**IC50 value (µg/ml)**
Ethanol	196.406
Ethyl acetate	68.42

CONCLUSION

The *Ruellia brittoniana* flower has antioxidant activity. With an IC50 value of 68.42 ppm, the ethyl acetate extract has stronger antioxidant activity than the ethanol extract with an IC50 value of 196.406 ppm.

ACKNOWLEDGEMENT

This article was presented at The 3rd International Conference and Exhibition on Indonesian Medical Education and Research Institute (ICE on IMERI 2018), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. We thank the 3rd ICE on IMERI Committee who had supported the peer review and manuscript preparation before submitting to the journal.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT INTERESTS

The authors declare that there is no conflict of interest

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