Pharmaciana
Vol.10, No.1, March 2020, Page. 77-88
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v10i1.13636

The ethanol extract of the bastard cedar (*Guazuma ulmifolia* L.) as antioxidants

Sijani Prahastuti [1], Meilinah Hidayat[1], Stella Tinia Hasiana[1], Wahyu Widowati*[1], Wahyu Setia Widodo[2], Rr. Anisa Siwianti Handayani[2], Rizal Rizal[2], Hanna Sari Widya Kusuma[2]

[1]Medical Research Center, Faculty of Medicine, Maranatha Christian University
Jl. Prof. Drg. Surya Sumantri 65, Bandung, West Java, Indonesia
[2]Aretha Medika Utama, Biomolecular and Biomedical Research Center,
Jl. Babakan Jeruk II No. 9, Bandung, West Java, Indonesia

Submitted: 19-07-2019 ***Reviewed: 21-11-2019*** ***Accepted: 31-03-2020***

ABSTRACT

Guazuma ulmifolia, or commonly known as the bastard cedar, has many pharmaceutical activities. Therefore, it is claimed as a source of various plant-based medicines. This research was purposed to identify the antioxidant activities of the ethanolic extract of *G. ulmifolia* (EEGU) by phytochemical screening assay, total flavonoid and total phenolic testing, and comparative analysis between the antioxidant activities of EEGU and epicatechin. The qualitative phytochemical screening assay of EEGU detected the availability of phenols, flavonoids, alkaloids, tannins, and terpenoids, but not saponins and triterpenoids. Meanwhile, the total phenolic content was 32.24 µg GAE/mg extract, and the total flavonoid content was 6.48 µg QE/mg extract. The role of antioxidants examined by FRAP, DPPH, H_2O_2, and ABTS assays. These assays are proved that the IC_{50} values of EEGU are higher than epicatechin. For DPPH scavenging, H_2O_2 scavenging, and ABTS reduction activities, EEGU resulted IC_{50} 45.70 µg/mL, 162.93 µg/mL, and 35.96 µg/mL, while epicatechin only yielded IC_{50} 0.56 µg/mL, 57.91 µg/mL, and 16.74 µg/mL respectively. Otherwise, the highest reduction in FRAP activities were shown at 50 µg/mL concentration of epicatechin and EEGU were 236.33 and 202.71 µM Fe (II)/µg respectively. Based on these results, EEGU is concluded as an active natural product because it exhibited antioxidant activities.

Keywords: antioxidant, *Guazuma ulmifolia*, phytochemical, total flavonoid, total phenolic

Corresponding author:
Wahyu Widowati
Medical Research Center, Faculty of Medicine, Maranatha Christian University
Jl Prof. Drg. Surya Sumantri 65, Bandung , West Java, Indonesia
E-mail: wahyu_w60@yahoo.com

INTRODUCTION

Excess of free radicals and oxidants increases oxidative stress, which have a negative impact on a variety of cellular structures, including, proteins, lipids, membranes, and DNA (Pizzino *et al*., 2017). Antioxidants are known to be potential protective agents to reduce oxidative damages to the human body. Most importantly, they are naturally abundant in fruits and can neutralize free radicals by giving an electron and transforming it into harmless molecules. Also, with the reactive free radical quenching properties, these substances can prevent the oxidation of other molecules so that they have the effect of improving health in the prevention of degenerative diseases (Kaneria *et al*., 2009).

Guazuma ulmifolia belongs to the family Sterculiaceae, commonly named as a Guacimo or Mutamba. *G. ulmifolia* is commonly known in Caribbean, South America, Mexico, Central America, and some parts of India. In India, this tree was introduced more than 100 years ago and, today, it thrives along roadsides and in wasteland. Based on many previous studies, *G. ulmifolia* had been used as antiobesity (Iswantini *et al*., 2011), antioxidant (Feltrin *et al*.,2012), photocatalyst, antimicrobial and anticancer agents (Karthika *et al*., 2017), and hepatoprotective drugs (Sharma *et al*., 2013). Some of which are associated with oxidative stress. The fruit was chosen for analysis because of its excellence in treating different ailments (Duraiswamy *et al*., 2018). Blood lipid level was decreased and the occurrence of atherosclerosis in animals with hyperlipidemia was inhibited by *G. Ulmifolia* (Sukandar *et al*., 2012). In case of progression of atherosclerosis, the relevance of apoptosis regulation has been discussed recently (Tabas, 2005).

One of the main compounds for the antioxidant activity of *G. ulmifolia* is flavonoids and its derivative products. At the same time, rutin and quercitrin which are phenolic compounds also relate to the antioxidant effects of *G. ulmifolia* extract (Morais *et al*., 2017). This present study aims to analyze the antioxidant activity, secondary metabolite constituents (qualitatively), and total phenolics and flavonoids of *G. ulmifolia*.

Prahastuti *et al*. (2019a) confirmed that *G. ulmifolia* extract (6.25 µg/mL) decreased reactive oxygen species (ROS) in glucose-induced mesangial cells in chronic kidney disease. After comparing several conventional testing methods of antioxidant activities, Amorati and Valgimigli (2015) identify several limitations in them, namely, specialized instrumentation requirement, cannot differentiate between stoichiometry and kinetics reaction, fluorescent low reactivity against ROO• radicals that generate the yield for best antioxidants, the necessity of multiple chromatographic analysis, and long reaction time for each experiment, lack of initiation reproducibility, and incapability to present specific for the superoxide dismutation (SOD).

Various methods used in this research were to evaluate the antioxidant activities, namely, DPPH (2,2-diphenyl-1-picrylhydrazyl), H_2O_2 (hydroperoxide), and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) free radical scavenging test and FRAP (ferric reducing antioxidant power). Phytochemical investigations can produce valuable information about the different phytoconstituents present in *G. ulmifolia* leaves. The plant defense mechanism for countering ROS and phenol synthesizes phenolic compounds in response to ecological pressures (Duraiswamy *et al*., 2018). This research was purposed to evaluate the antioxidant potential of the ethanol extract of *G. ulmifolia* (EEGU) by phytochemical screening assay, total flavonoid and total phenolic tests, and comparative analysis between the antioxidant activities of EEGU and epicatechin.

MATERIALS AND METHODS

Materials

The bastard cedar (*G. ulmifolia*) leaves were obtained from Bumi Herbal plantation, Dago, Bandung, Province of Jawa Barat, Indonesia and determined at Biology Department, School of Life Science and Technology, Bandung Institute of Technology (the Province of Jawa Barat, Indonesia) by the herbarium staff.

Methods

G. ulmifolia extract preparation

Bastard cedar leaves were crushed and macerated using 70% distilled ethanol for 24 hours, the filtrate was collected and the residue was soaked until colorless filtrate. Then, the collected filtrate was concentrated using an evaporator at a temperature of 50°C to obtain EEGU (Widowati *et al*., 2017; Widowati *et al.*, 2018; Prahastuti *et al*., 2019a).

Qualitative phytochemical screening assay

The *G. ulmifolia* leaves extract (EEGU) phytochemical screening was conducted by using Farnsworth method with a slight modification to identify flavonoids, phenols, alkaloids, saponins, tannins, terpenoids, and steroids/triterpenoids qualitatively (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al.*, 2018). The procedures for each compound detection test are as follows.

Phenols identification

About 10 mg of EEGU was placed on spotting plates and added with 1% $FeCl_3$. The phenols availability was shown by forming of black, red, blue, purple, or green color (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Steroids/triterpenoids identification

About 10 mg of EEGU was placed on spotting plates and fully immersed in acetic acid for 10-15 mins. One mL of absolute H_2SO_4 was added to the mixture. Steroids were detected when blue or green color is formed, while triterpenoids were shown by orange or red sediment formation (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al.*, 2018; Prahastuti *et al*., 2019b).

Tannins identification

In the test tube was added by 10 mg of EEGU and 2 mL of HCl 2N, then heated for 30 mins in water bath. The mixture was chilled and the filtrate was collected. The filtrate was added by pentyl alcohol. If purple color was formed exhibited the sample positive containing tannins (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al.*, 2018; Prahastuti *et al*., 2019b).

Saponins identification

The tube containing water, 10 mg of EEGU was added and boiled for 5 mins. Then it was shaken tremendously. After that, 1 N HCl was dripped into the test tube. The stable foam presence on the surface of the solution indicated saponin content (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Terpenoids identification

EEGU as much as 10 mg was placed on spotting plates and mixed with vanillin and H_2SO_4. The reaction was positive if the color changed into purple (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al.*, 2018 Prahastuti *et al*., 2019b).

Flavonoids identification

In the test tube, EEGU as much as 10 mg was inserted and added with the Mg/Zn powder and HCl 2 N. The mixture was heated for 5 – 10 mins and then allowed to cool and filtered. Afterward, the pentyl alcohol and filtrate were mixed. Red or orange color indicated positive reaction and sample contained flavonoid (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al.*, 2018; Prahastuti *et al*., 2019b).

Alkaloids identification

About 10 mg EEGU was added ammonia 10% then vaporized with chloroform in water bath up to two layers. The base layer collected in first tube and added with 3 drops HCl 1N, the residue as second tube was dissolved in HCl 1N. The residue as upper layer was added with 2 drops Draggendorf solution. The positive reaction if the sample changed to red or yellow (Bera *et al.*, 2015; Adnyana *et al.*, 2016; Widowati *et al.*, 2018; Prahastuti *et al.*, 2019b).

Phenolic content assay

Phenolic content was assayed with minor modification of Folin-Ciocalteu's method. In the sample well, the EEGU as much as 15 µL was inserted and mixed with 10% Folin-Ciocalteu's reagent as much as 75 µL and 7.5% sodium carbonate as much as 60 µL. The blank solution was created by mixing 135 µL of 10% DMSO with 15 µL of EEGU. Then, it was heated for 10 mins at 50˚C. Furthermore, the absorbance was determined using a microplate reader at 760 nm. Total phenolic content was asserted as gallic acid equivalence (GAE) in µg/mg sample. This test was done triplicate (Nurhayati *et al*., 2018; Rusmana *et al*., 2017; Widowati *et al*., 2015; 2017; 2018; Prahastuti *et al*., 2019b).

Flavonoid content assay

Flavonoid content was conducted using the altered previous method (Kalita *et al.*, 2013; Prahastuti *et al.*, 2019b). Briefly, standard solution in 7 concentration level of epicatechin and EEGU at 1000 µg/mL and 2000 µg/mL as much as 15 µL was used in this method. It was mixed with 75 µL $AlCl_3$ 2%. The absorbance was quantified in 415 nm of wavelength. The linear regression equation (y = ax + b) was made based on the standard (quercetin) absorbance value. The analysis of sample flavonoid content was performed based standard linear regression equation. The flavonoid content was presented as quercetin equivalence (QE) in µg/mg. The experiment was done triplicate (Prahastuti *et al.*, 2019b).

DPPH scavenger activity assay

A total of 200 µL of 0.0777 mmol DPPH in methanol was added to the EEGU as much as 50 µL with various concentrations in the 96-well microplate. Then, it was incubated for 30 min at room temperature. After that, the absorbance value was quantified using microplate reader at a wavelength of 517 nm. The negative control was contained 250 µL of DPPH, while the blank solution was contained 50 µL of the sample and 200 µL of DMSO (Widowati *et al.*, 2015; 2016; 2017; 2018). The subsequent formula was used to measure the DPPH scavenging activity.

$$\text{DPPH scavenging activity (\%)} = \frac{A-B}{A} x\ 100$$

A: control solutions absorbance

B: sample absorbance

H_2O_2 scavenger activity assay

The H_2O_2 scavenging was measured according to previous studies (Mukhopadhyay *et al*., 2016; Utami *et al*., 2017) with slight modification. Each sample well was contained 60 µL of EEGU, 12 µL of $FeH_8N_2O_8S_2$ 1 mM and 3 µL of H_2O_2 5 mM. The mixture that containing 12 µL of $FeH_8N_2O_8S_2$ and 63 µL of DMSO were used as the negative control, while the mixture that containing 60 µL of EEGU and 90 µL of DMSO were used as the blank solution. After added with H_2O_2, incubated in the dark and room temperature for 5 min. The 1,10-phenanthrolines as much as 75 µL was added into the sample and control well and incubated again for 10 min in the dark and room temperature. The 510 nm wavelength was used to measure the absorbance. The subsequent formula was used to calculate the H_2O_2 scavenger activity:

H_2O_2 scavenger activity (%) = $\frac{A}{B} x\ 100$

A: control solutions absorbance

B: sample absorbance

ABTS reduction activity assay

Briefly, the EEGU as much as 2 µL at various concentrations was added into the well. Then, it was mixed with 198 µL of ABTS work. As for the control wells, each of them was added with 198 µL of ABTS work and 2 µL of DMSO. Meanwhile, the blank well was added with 198 µL of DMSO and 2 µL of the EEGU. The microplate was closed, then incubated for 6 min at 37˚C. The absorbance value was determined using microplate reader at a wavelength of 745 nm (Widowati *et al*., 2016; 2017; 2018). The ABTS reduction percentage was calculated using the subsequent formula:

ABTS reduction activity (%) = $\frac{A-B}{A} x\ 100$

A: control solutions absorbance

B: sample absorbance

FRAP

The acetate buffer (acetic acid was added to create conformation at pH 3.6) at 300 mM of concentration as much as 10 mL, dissoluble ferric chloride hexahydrate in distilled water at 20 mM of concentration as much as 1 mL, and dissoluble TPTZ in 40 mM HCl at 10 mM of concentration as much as 1 mL was mixed to create the FRAP reagent. A total of 7.5 µL of the sample was mixed with the FRAP reagent as much as 142.5 µL in a 96-well microplate, then mixed and incubated for 30 min at 37ºC. The wavelength at 593 nm was used to measure the absorbance value using the microplate reader. Afterward, a $FeSO_4$ standard curve with varying concentrations, starting from 0.019 to 95 µg/mL was created. The results were presented as µM Fe (II)/µg extract (Widowati *et al*., 2017; 2018).

Statistical analysis

The SPSS program (version 20.0) was used to carry out the statistical analysis. The DPPH scavenger, H_2O_2 scavenger, and ABTS reduction assays results were analyzed using linear regression analysis. Then, the linear regression equation generated in this process was used to determine the Median Inhibitory Concentration 50 (IC_{50}).

RESULTS AND DISCUSSION

There have been previous studies documenting the natural compounds of *G. ulmifolia* extract. Both its fruits and leaves contain tannins, saponins, flavonoids, terpenoids, cardiac glycoside, and alkaloids **(**Patil, 2013)**.** However, EEGU has been reported to have high cardiac glycosides, moderate levels of tannin, phenol, terpenoids, and coumarin, and lower concentrations of sterol, quinone, and acid, but not flavonoids and saponins (Duraiswamy *et al*., 2018).

Duraiswamy *et al.* (2018) have declared that the total phenolic and flavonoid contents of *G. ulmifolia* are 27.2797±0.1756 mg TAE/g DW and 0.5544±0.00018 mg QE/g DW. Meanwhile, the present study yielded higher values, which were 32.24±1.42 µg GAE/mg EEGU and 6.48±0.14 µg QE/mg EEGU.

Phenolic compounds are a very good antioxidant or have a broad number of biochemical activities such as antimutagen, anticarcinogenic and modify gene expression for curing various oxidative stress diseases. A phenolic compounds antioxidant activity reflects its composition, the hydroxyl groups location also number, and the nature of its substitution on the aromatic ring. Flavonoids are excellent biological reaction modifications as the yeomen's function in antihistamine, antimicrobial, memory, and even mood-enhancing properties. The plants thus find

their medicinal values due to the presence of respective phytochemical constituents (Duraiswamy *et al*., 2018). Flavonoids induce mechanism that inhibit tumor invasion, kill the cancer cell, apart from this show anti-inflammatory, antiallergic, anticancer and antimicrobial (Sharma *et al*., 2015).

Phytochemical screening

The *G. ulmifolia* leaves extract was subjected to preliminary phytochemical analysis, and the results are shown in Table I. This research showed that the extract contains flavonoids, phenols, tannins, steroids, terpenoids, and alkaloids while saponins and triterpenoids were not detected.

Table I. The EEGU qualitative phytochemical screening assay

Phytochemical assay	Results
Tannins	+
Saponins	-
Flavonoids	+
Phenols	+
Alkaloids	+
Terpenoids	+
Steroids/Triterpenoids	+/-

(+): present; (-): not detected

Phenolic content

Total phenolics were gauged by a colorimetric method modified from a previous study (Widowati *et al*., 2015; 2016; 2017; 2018). It was based on entangle phenolic compounds reduce Folin-Ciocalteu's reagent with a blue complex generated unanimously. The total polyphenols were measured using the calibration curve for gallic acid. The experiments revealed the presence of a high quantity of phenolics in the extract, which was 32.24 ± 1.42 µg GAE/mg EEGU.

Flavonoid content

The total flavonoid content of EEGU was measured by the aluminum chloride method. Its quantification revealed the presence of a high quantity of flavonoids in EEGU, which was 6.48±0.14 µg QE/mg EEGU.

DPPH scavenger activity

Various assay have been used to test antioxidant activity, but the most commonly used methods are the forming of free radical species and being neutralized with antioxidant compounds. Unpaired electron caused a stable free radical DPPH. DPPH is a stable free radical that centered on nitrogen, usually used to assign the compounds or plant extracts radical scavenging activity (Kedare and Singh, 2011; Sasikumar and Kalaisezhiyen, 2014). In the presence of hydrogen donor, it becomes paired and reduced the absorption at 517 nm (Widowati *et al*., 2015). The extracts reduced stable DPPH radicals to yellow-colored diphenyl picrylhydrazine (DPPH-H) during DPPH test. The radical DPPH is widely used as a substrate for examining antioxidant activity. The IC_{50} value is the concentration allowed by an antioxidant to scavenge 50% of the DPPH free radical; higher antioxidant activity is defined by a smaller IC_{50} value. In this study, the IC_{50} of EEGU was 45.70±0.7 µg/mL, while epicatechin had an IC_{50} of 0.56±0.29 µg/mL. Epicatechin was discovered to be the most active extract in DPPH scavenging activity because it provided the lowest IC_{50} value (Table II) and the highest DPPH scavenging activities (Figure 1), particularly at a concentration of 200 µg/mL.

Table II. The antioxidant activities (IC_{50}) of EEGU and epicatechin

Sample	IC_{50} of DPPH Scavenger Activity (µg/mL)	IC_{50} of H_2O_2 Scavenger Activity (µg/mL)	IC_{50} of ABTS-reducing Activity (µg/mL)	FRAP-reducing highest Activity (µM Fe (II)/µg extract)
EEGU	45.70	162.93	35.96	202.71
Epicatechin	0.56	57.91	16.74	236.33

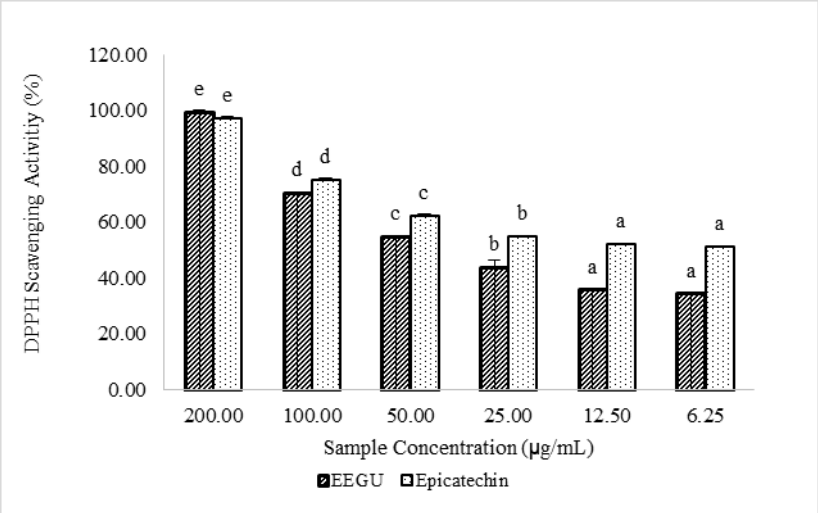


Figure 1. The DPPH scavenging activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 200, 100, 50, 25, 12.50, and 6.25 µg/mL. This experiment was done triplicate for each sample concentration

H_2O_2 scavenger activity

Hydrogen peroxide plays a role in producing energy in various in vivo systems, phagocytosis, intercellular signal transfer, cell growth control, and the synthesis of essential biological compounds (Packer *et al*., 2008). As byproducts of normal aerobic metabolism H_2O_2 is generated and increased during infections, workouts, and stress conditions (Mukhopadhyay *et al*., 2016). The Median Inhibitory Concentrations (IC_{50}) of epicatechin and EEGU in H_2O_2 radical scavenging activity was shown in Table II. The IC_{50} values of EEGU and epicatechin were 162.93 µg/mL and 57.91 µg/mL, respectively. Figure 2 also shows that epicatechin had higher activities than EEGU, particularly at 300 µg/mL.

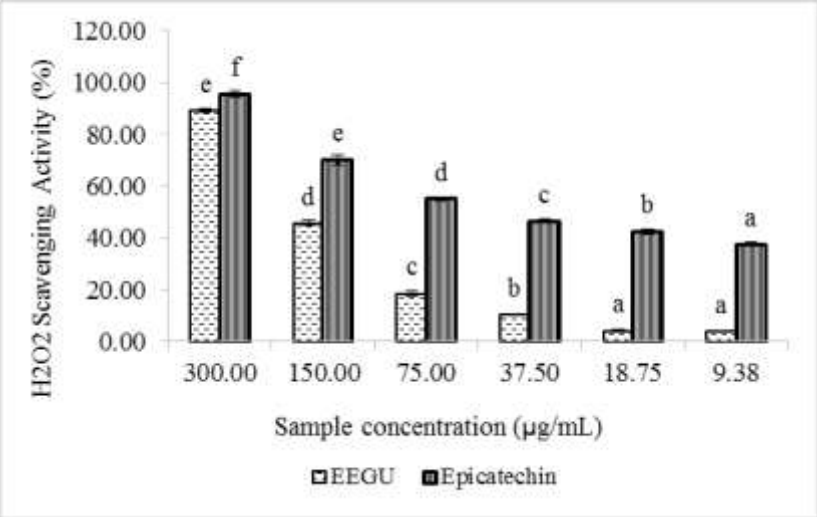


Figure 2. The H_2O_2 scavenging activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 300, 100, 150, 75, 37.5, 18.75, and 9.38 µg/mL This experiment was done triplicate for each sample concentration

ABTS-reducing activity

The activity of ABTS-reducing assay gauge the antioxidant's relative potential to scavenge the ABTS generated. In this study, ABTS was produced by reaction between a powerful oxidizer and ABTS salt. The ABTS radical blue-green-colored solution was reduced by hydrogen-donating antioxidant (Widowati *et al*., 2016) and analyzed at spectrum of a long-wave absorption. The percentages of ABTS-reducing activity of EEGU and epicatechin were compared, with epicatechin having the highest activity at 50 µg/mL, as shown in Figure 3. Also, their IC_{50} values were 35.96 µg/mL and 16.74 µg/mL, respectively (Table II).

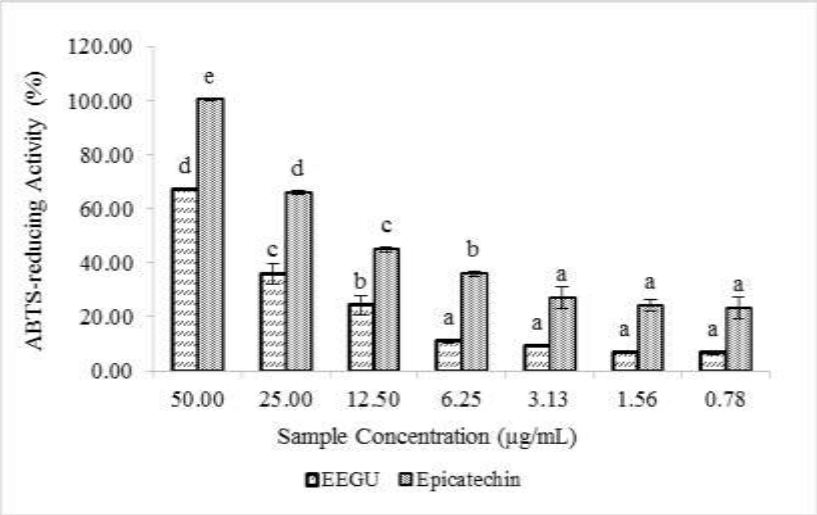


Figure 3. The ABTS-reducing activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to reach final concentrations of 50, 25, 12.50, 62.50, 3.13, 1.56, and 0.78 µg/mL. This experiment was done triplicate for each sample concentration

FRAP

The FRAP method was based on the ferroin analog reduction in acidic medium, the $TPTZ^{3+}$ to the colored Fe^{2+} complex of $Fe(TPTZ)^{2+}$ (greatly blue) by antioxidant (Widowati *et al*., 2018).. A reduction in the corresponding tripyridyltriazine Fe(III) complex at 593 nm results from the absorbance of Fe(II) complex.

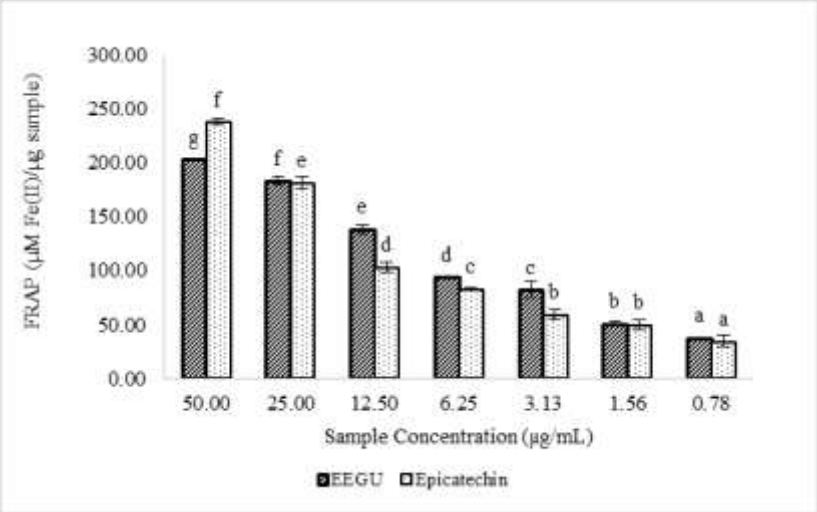


Figure 4. The FRAP of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 50, 25, 12.50, 6.25, 3.13, 1.56, and 0.78 µg/mL. This experiment was done triplicate for each sample concentration

The highest FRAP activities of EEGU and epicatechin were 202.71 µg/mL and 236.33 µg/mL, respectively (Table II, Figure 4). Duraiswamy *et al.* (2018) claim that the most significant inhibition of FRAP activity is 40%, and this is produced by the fruit extract of *G. ulmifolia* at 500 µg/mL. Meanwhile, in this research, the highest FRAP activity was 202.71 µM Fe(II), which was generated by EEGU at 50 µg/mL.

High correlations between antioxidant activities and phytochemical content of polyphenols were observed. The antioxidant activities potentially contribute to the polyphenol contents found in the plant extracts. *G. ulmifolia* stem bark and leaves extracts exhibited DPPH scavenger activity at IC_{50} of 25.2 µg/mL and 39.3 µg/mL (dos Santos *et al*., 2018). There have been some previous studies examining the antioxidant activities of EEGU using different assays, namely, FRAP, DPPH, MDA dosage, human erythrocyte suspension, hemolytic activity, and inhibition of oxidative hemolysis in human erythrocytes by 2,2′-Azobis(2-amidinopropane) dihydrochloride-induced (AAPH) or DOX (Duraiswamy *et al*., 2018; dos Santos *et al*., 2018; Kaneria *et al*., 2009).

CONCLUSIONS

Based on the phytochemical screening, EEGU has been confirmed to contain flavonoids, phenols, tannins, alkaloids, steroids, and terpenoids but not saponins and triterpenoids. The flavonoid contents and total phenolic of EEGU are 32.24 µg extract/mg GAE and 6.48 µg QE/mg extract, respectively. Comparison between EEGU and epicatechin using different tests for antioxidant activities, namely DPPH scavenging, ABTS reduction, H_2O_2 scavenging, and FRAP assays, proves that the IC_{50} values of EEGU are generally higher than those of epicatechin. For DPPH scavenging, H_2O_2 scavenging, and ABTS reduction activities, EEGU can produce IC_{50} values of 45.70 µg/mL, 162.93 µg/mL, and 35.96 µg/mL. Meanwhile, for the same activities, epicatechin only yields IC_{50} values of 0.56 µg/mL, 57.91 µg/mL, and 16.74 µg/mL. At the same time, EEGU has the highest FRAP reduction properties at 50 µg/mL or equal to 202.71 µM Fe

(II)/µg. These findings assert that EEGU and epicatechin have antioxidant activities and that EEGU is an active natural product.

ACKNOWLEDGMENT

The authors would like to acknowledge the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education of the Republic of Indonesia for their financial support through the scheme of Penelitian Terapan Peruguruan Tinggi in 2019. Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia for supporting the metodology and laboratory facilities used in the research and the authors would also like to extend their gratitude to Dwi Surya Artie and Dewani Tediana Yusepany for technical support.

REFERENCES

Adnyana, I. K., Abuzaid, A. S., Iskandar, E. Y. and Kurniati, N. F., 2016, Pancreatic lipase and α-amylase inhibitory potential of mangosteen (*Garcinia mangostana* Linn.) pericarp extract, *International Journal of Medical Research & Health Sciences*, 5(1): 23-28.

Amorati, R. and Valgimigli, L., 2015, Advantages and limitations of common testing methods for antioxidants, *Free Radical Research*, 49(5): 633-649.

Bera, T. K., Chatterjee, K. and Ghosh, D., 2015, In-vitro antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq., *Biomarkers and Genomic Medicine*, 7(1): 18-24.

dos Santos, J. M., Alfredo, T. M., Antunes, K. Á., da Cunha, J. D. S. M., Costa, E. M. A., Lima, E. S., Silva, D. B., Carollo, C. A., Schmitz, W. O., Boleti, A. P. D. A. and dos Santos, E. L., 2018, *Guazuma ulmifolia* Lam. decreases oxidative stress in blood cells and prevents doxorubicin-induced cardiotoxicity, *Oxidative Medicine and Cellular Longevity*, 2018: 1-16.

Duraiswamy, B., Singanan, M. and Varadarajan, V., 2018, Physicochemical, phytochemicals and antioxidant evaluation of *Guazuma ulmifolia* fruit, *International Journal of Pharmacy and Pharmaceutical Science*s, 10(9): 87.

Feltrin, A.C., Boligon, A.A., Janovik, V. and Athayde, M.L., 2012. Antioxidant potential, total phenolic and flavonoid contents from the stem bark of *Guazuma ulmifolia* Lam, *Asian Journal of Biological Sciences*, 5(5): 268-272.

Iswantini, D., Silitonga, R.F., Martatilofa, E. and Darusman, L.K., 2011. Zingiber cassumunar, Guazuma ulmifolia, and Murraya paniculata extracts as antiobesity: in vitro inhibitory effect on pancreatic lipase activity, *Hayati Journal of Biosciences*, 18(1): 6-10.

Kalita, P., Tapan, B. K., Pal, T. K. and Kalita, R., 2013, Estimation of total flavonoids content (TFC) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn., *Journal of Drug Delivery and Therapeutics*, 3(4): 33-37.

Kaneria, M., Baravalia, Y., Vaghasiya, Y. and Chanda, S., 2009, Determination of antibacterial and antioxidant potential of some medicinal plants from saurashtra region, India, *Indian Journal of Pharmaceutical Sciences*, 71(4): 406.

Karthika, V., Arumugam, A., Gopinath, K., Kaleeswarran, P., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M. and Benelli, G., 2017. Guazuma ulmifolia bark-synthesized Ag, Au and Ag/Au alloy nanoparticles: Photocatalytic potential, DNA/protein interactions, anticancer activity and toxicity against 14 species of microbial pathogens, *Journal of Photochemistry and Photobiology B: Biology*, 167: 189-199.

Kedare S. B. and Singh R. P., 2011, Genesis and development of DPPH method of antioxidant assay, *Journal of Food Science and Technology*, 48: 412–422.

Morais, S.M., Calixto-Júnior, J.T., Ribeiro, L.M., Sousa, H.A., Silva, A.A.S., Figueiredo, F.G., Matias, E.F.F., Boligon, A.A., Athayde, M.L., Morais-Braga, M.F.B. and Coutinho, H.D.M., 2017. Phenolic composition and antioxidant, anticholinesterase and antibiotic-modulating

antifungal activities of Guazuma ulmifolia Lam.(Malvaceae) ethanol extract, *South African Journal of Botany*, 110: 251-257.

Mukhopadhyay, D., Dasgupta, P., Roy, D. S., Palchoudhuri, S., Chatterjee, I., Ali, S. and Dastidar, S. G., 2016, A sensitive in vitro spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline, *Free Radicals & Antioxidants*, 6(1): 123-131.

Nurhayati, B., Rahayu, I. G., Rinaldi, S. F., Zaini, W. S., Afifah, E., Arumwardana, S., Kusuma, H. S. W., Rizal, R. and Widowati, W., 2018, The antioxidant and cytotoxic effects of *Cosmos caudatus* ethanolic extract on cervical cancer, *The Indonesian Biomedical Journal*, 10(3): 243-249.

Packer, L., Cadenas, E. and Davies, K.J., 2008, Free radicals and exercise: an introduction, *Free radical biology & medicine*, 44(2): 123-125.

Patil, J. U. and Biradar, S. D., 2013, Pharmacognostic study of *Guazuma ulmifolia*, *International Research Journal of Pharmaceutical*, 4: 130-131.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. and Bitto, A., 2017, Oxidative Stress: Harms and Benefits for Human Health, *Oxidative Medicine and Cellular Longevity*, 2020,1-13.

Prahastuti, S., Hidayat, M., Hasiana, S. T., Widowati, W., Amalia, A., Qodariah, R. L., Rizal, R., Kusuma, H. S. W., and Khoiriyah, Z., 2019a, Ethanol extract of jati belanda (*Guazuma ulmifolia* L.) as Therapy for Chronic Kidney Disease in In Vitro model, *Journal of Reports in Pharmaceutical Sciences*, 8(2): 229.

Prahastuti, S., Hidayat, M., Hasianna, S.T., Widowati, W., Amalia, A., Yusepany, D.T., Rizal, R. and Kusuma, H.S.W., 2019b, Antioxidant potential ethanolic extract of *Glycine max* (l.) Merr. Var. Detam and daidzein, *Journal of Physics: Conference Series*, 1374: 1-12.

Rozqie, R., Diah, M. and Rukmi, W., 2012, The effect of jati belanda (*Guazuma ulmifolia* Lamk) Leaves Extract on Histopathology of Rat's Kidney, *Tropical Medicine Journal*, 2(1): 57-65.

Rusmana, D., Wahyudianingsih, R., Elisabeth, M., Balqis, B., Maesaroh, M. and Widowati, W., 2017, Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin, *The Indonesian Biomedical Journal*, 9(2): 84-90.

Sasikumar, V., and Kalaisezhiyen, P., 2014, Evaluation of free radical scavenging activity of various leaf extracts from Kedrostis foetidissima (Jacq.) Cogn, *Biochemistry and Analytical Biochemistry*, 3(2): 1.

Sharma, M., Chopra, S. and Prasad, S.B., 2015. Guazuma tomentosa: A valuable medicinal plant, *International Journal of Pharmacognosy and Phytochemical Research*, 7(1): 197-200.

Sukandar, E.Y., Nurdewi and Elfahmi, 2012. Antihypercholesterolemic effect of combination of *Guazuma ulmifolia* lamk. leaves and *Curcuma xanthorrhiza* Roxb. rhizomes extract in wistar rats, *International Journal* of *Pharmacology*., 8: 277-282.

Syaefudin, Wahyuni, W. T., Artika, I. M. and Sulistiyani, 2014, Antioxidant activity of flavonoid from *Guazuma ulmifolia* Lamk. leaves and apoptosis induction in yeast cells, *Journal of Biological Sciences*, 14(4): 305.

Tabas, I., 2005. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency, *Arteriosclerosis, thrombosis, and vascular biology*, 25(11): 2255-2264.

Utami, S., Adityaningsari, P., Sosiawan, I., Endrini, S., Sachrowardi, Q. R., Laksono, S. P., Nafik, S., Arrahmani, B. C., Afifah, E. and Widowati, W., 2017, Antioxidants and Anticholinesterase Activities of the Characterized Ethanolic of Ripe Sesoot (*Garcinia picrorrhiza* Miq.) Fruit Extract (GpKar) and Xanthone, *Majalah Obat Tradisional*, 22(3): 160-165.

Widowati, W., Fauziah, N., Herdiman, H., Afni, M., Afifah, E., Kusuma, H. S. W., Nufus, H., Arumwardana, S. and Rihibiha, D. D., 2016. , Antioxidant and Anti-aging Assays of *Oryza sativa* Extracts, Vanillin and Coumaric Acid., *Journal of Natural Remedies*, 16(3): 88-99.

Widowati, W., Herlina, T., Ratnawati, H., Constantia, G., Deva, I. D. G. S. and Maesaroh, M., 2015, Antioxidant potential of black, green and oolong tea methanol extracts., *Biology, Medicine, & Natural Product Chemistry*, 4(2): 35-39.

Widowati, W., Janeva, B. W., Nadya, S., Amalia, A., Arumwardana, S., Kusuma, H. S. W. and Arinta, Y., 2018., Antioxidant and antiaging Antiaging activities Activities of *Jasminum sambac* Extract, and its Compounds,. *Journal of Reports in Pharmaceutical Sciences*, 7(3): 270.

Widowati, W., Rani, A. P., Hamzah, R. A., Arumwardana, S., Afifah, E., Kusuma, H. S. W., Rihibiha, D. D., Nufus, H., and Amalia, A., 2017, Antioxidant and antiaging assays of *Hibiscus sabdariffa* Extract and its Compounds, *Natural Product Sciences*, 23(3): 192-200.

The ethanol extract of the bastard cedar (Guazuma ulmifolia L.) as antioxidants

by Sijani Prahastuti

Submission date: 16-Aug-2021 04:27PM (UTC+0700)
Submission ID: 1631987625
File name: ct_of_the_bastard_cedar_Guazuma_ulmifolia_L._as_antioxidants.pdf (273.53K)
Word count: 5213
Character count: 28286

Pharmaciana
Vol.10, No.1, March 2020, Page. 77-88
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v10i1.13636

The ethanol extract of the bastard cedar (*Guazuma ulmifolia* L.) as antioxidants

Sijani Prahastuti [1], Meilinah Hidayat[1], Stella Tinia Hasiana[1], Wahyu Widowati*[1], Wahyu Setia Widodo[2], Rr. Anisa Siwianti Handayani[2], Rizal Rizal[2], Hanna Sari Widya Kusuma[2]

[1]*Medical Research Center, Faculty of Medicine, Maranatha Christian University*
Jl. Prof. Drg. Surya Sumantri 65, Bandung, West Java, Indonesia
[2]*Aretha Medika Utama, Biomolecular and Biomedical Research Center,*
Jl. Babakan Jeruk II No. 9, Bandung, West Java, Indonesia

Submitted: 19-07-2019 ***Reviewed: 21-11-2019*** ***Accepted: 31-03-2020***

ABSTRACT

Guazuma ulmifolia, or commonly known as the bastard cedar, has many pharmaceutical activities. Therefore, it is claimed as a source of various plant-based medicines. This research was purposed to identify the antioxidant activities of the ethanolic extract of *G. ulmifolia* (EEGU) by phytochemical screening assay, total flavonoid and total phenolic testing, and comparative analysis between the antioxidant activities of EEGU and epicatechin. The qualitative phytochemical screening assay of EEGU detected the availability of phenols, flavonoids, alkaloids, tannins, and terpenoids, but not saponins and triterpenoids. Meanwhile, the total phenolic content was 32.24 μg GAE/mg extract, and the total flavonoid content was 6.48 μg QE/mg extract. The role of antioxidants examined by FRAP, DPPH, H_2O_2, and ABTS assays. These assays are proved that the IC_{50} values of EEGU are higher than epicatechin. For DPPH scavenging, H_2O_2 scavenging, and ABTS reduction activities, EEGU resulted IC_{50} 45.70 µg/mL, 162.93 µg/mL, and 35.96 µg/mL, while epicatechin only yielded IC_{50} 0.56 µg/mL, 57.91 µg/mL, and 16.74 µg/mL respectively. Otherwise, the highest reduction in FRAP activities were shown at 50 µg/mL concentration of epicatechin and EEGU were 236.33 and 202.71 μM Fe (II)/μg respectively. Based on these results, EEGU is concluded as an active natural product because it exhibited antioxidant activities.

Keywords: antioxidant, *Guazuma ulmifolia*, phytochemical, total flavonoid, total phenolic

Corresponding author:
Wahyu Widowati
Medical Research Center, Faculty of Medicine, Maranatha Christian University
Jl Prof. Drg. Surya Sumantri 65, Bandung , West Java, Indonesia
E-mail: wahyu_w60@yahoo.com

INTRODUCTION

Excess of free radicals and oxidants increases oxidative stress, which have a negative impact on a variety of cellular structures, including, proteins, lipids, membranes, and DNA (Pizzino *et al*., 2017). Antioxidants are known to be potential protective agents to reduce oxidative damages to the human body. Most importantly, they are naturally abundant in fruits and can neutralize free radicals by giving an electron and transforming it into harmless molecules. Also, with the reactive free radical quenching properties, these substances can prevent the oxidation of other molecules so that they have the effect of improving health in the prevention of degenerative diseases (Kaneria *et al*., 2009).

Guazuma ulmifolia belongs to the family Sterculiaceae, commonly named as a Guacimo or Mutamba. *G. ulmifolia* is commonly known in Caribbean, South America, Mexico, Central America, and some parts of India. In India, this tree was introduced more than 100 years ago and, today, it thrives along roadsides and in wasteland. Based on many previous studies, *G. ulmifolia* had been used as antiobesity (Iswantini *et al*., 2011), antioxidant (Feltrin *et al*.,2012), photocatalyst, antimicrobial and anticancer agents (Karthika *et al*., 2017), and hepatoprotective drugs (Sharma *et al*., 2013). Some of which are associated with oxidative stress. The fruit was chosen for analysis because of its excellence in treating different ailments (Duraiswamy *et al*., 2018). Blood lipid level was decreased and the occurrence of atherosclerosis in animals with hyperlipidemia was inhibited by *G. Ulmifolia* (Sukandar *et al*., 2012). In case of progression of atherosclerosis, the relevance of apoptosis regulation has been discussed recently (Tabas, 2005).

One of the main compounds for the antioxidant activity of *G. ulmifolia* is flavonoids and its derivative products. At the same time, rutin and quercitrin which are phenolic compounds also relate to the antioxidant effects of *G. ulmifolia* extract (Morais *et al*., 2017). This present study aims to analyze the antioxidant activity, secondary metabolite constituents (qualitatively), and total phenolics and flavonoids of *G. ulmifolia*.

Prahastuti *et al*. (2019a) confirmed that *G. ulmifolia* extract (6.25 μg/mL) decreased reactive oxygen species (ROS) in glucose-induced mesangial cells in chronic kidney disease. After comparing several conventional testing methods of antioxidant activities, Amorati and Valgimigli (2015) identify several limitations in them, namely, specialized instrumentation requirement, cannot differentiate between stoichiometry and kinetics reaction, fluorescent low reactivity against ROO• radicals that generate the yield for best antioxidants, the necessity of multiple chromatographic analysis, and long reaction time for each experiment, lack of initiation reproducibility, and incapability to present specific for the superoxide dismutation (SOD).

Various methods used in this research were to evaluate the antioxidant activities, namely, DPPH (2,2-diphenyl-1-picrylhydrazyl), H_2O_2 (hydroperoxide), and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) free radical scavenging test and FRAP (ferric reducing antioxidant power). Phytochemical investigations can produce valuable information about the different phytoconstituents present in *G. ulmifolia* leaves. The plant defense mechanism for countering ROS and phenol synthesizes phenolic compounds in response to ecological pressures (Duraiswamy *et al*., 2018). This research was purposed to evaluate the antioxidant potential of the ethanol extract of *G. ulmifolia* (EEGU) by phytochemical screening assay, total flavonoid and total phenolic tests, and comparative analysis between the antioxidant activities of EEGU and epicatechin.

MATERIALS AND METHODS

Materials

The bastard cedar (*G. ulmifolia*) leaves were obtained from Bumi Herbal plantation, Dago, Bandung, Province of Jawa Barat, Indonesia and determined at Biology Department, School of Life Science and Technology, Bandung Institute of Technology (the Province of Jawa Barat, Indonesia) by the herbarium staff.

Methods

G. ulmifolia extract preparation

Bastard cedar leaves were crushed and macerated using 70% distilled ethanol for 24 hours, the filtrate was collected and the residue was soaked until colorless filtrate. Then, the collected filtrate was concentrated using an evaporator at a temperature of 50°C to obtain EEGU (Widowati *et al*., 2017; Widowati *et al*., 2018; Prahastuti *et al*., 2019a).

Qualitative phytochemical screening assay

The *G. ulmifolia* leaves extract (EEGU) phytochemical screening was conducted by using Farnsworth method with a slight modification to identify flavonoids, phenols, alkaloids, saponins, tannins, terpenoids, and steroids/triterpenoids qualitatively (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018). The procedures for each compound detection test are as follows.

Phenols identification

About 10 mg of EEGU was placed on spotting plates and added with 1% $FeCl_3$. The phenols availability was shown by forming of black, red, blue, purple, or green color (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Steroids/triterpenoids identification

About 10 mg of EEGU was placed on spotting plates and fully immersed in acetic acid for 10-15 mins. One mL of absolute H_2SO_4 was added to the mixture. Steroids were detected when blue or green color is formed, while triterpenoids were shown by orange or red sediment formation (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Tannins identification

In the test tube was added by 10 mg of EEGU and 2 mL of HCl 2N, then heated for 30 mins in water bath. The mixture was chilled and the filtrate was collected. The filtrate was added by pentyl alcohol. If purple color was formed exhibited the sample positive containing tannins (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Saponins identification

The tube containing water, 10 mg of EEGU was added and boiled for 5 mins. Then it was shaken tremendously. After that, 1 N HCl was dripped into the test tube. The stable foam presence on the surface of the solution indicated saponin content (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Terpenoids identification

EEGU as much as 10 mg was placed on spotting plates and mixed with vanillin and H_2SO_4. The reaction was positive if the color changed into purple (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018 Prahastuti *et al*., 2019b).

Flavonoids identification

In the test tube, EEGU as much as 10 mg was inserted and added with the Mg/Zn powder and HCl 2 N. The mixture was heated for 5 – 10 mins and then allowed to cool and filtered. Afterward, the pentyl alcohol and filtrate were mixed. Red or orange color indicated positive reaction and sample contained flavonoid (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Alkaloids identification

About 10 mg EEGU was added ammonia 10% then vaporized with chloroform in water bath up to two layers. The base layer collected in first tube and added with 3 drops HCl 1N, the residue as second tube was dissolved in HCl 1N. The residue as upper layer was added with 2 drops Draggendorf solution. The positive reaction if the sample changed to red or yellow (Bera *et al*., 2015; Adnyana *et al*., 2016; Widowati *et al*., 2018; Prahastuti *et al*., 2019b).

Phenolic content assay

Phenolic content was assayed with minor modification of Folin-Ciocalteu's method. In the sample well, the EEGU as much as 15 µL was inserted and mixed with 10% Folin-Ciocalteu's reagent as much as 75 µL and 7.5% sodium carbonate as much as 60 µL. The blank solution was treated by mixing 135 μL of 10% DMSO with 15 μL of EEGU. Then, it was heated for 10 mins at 50˚C. Furthermore, the absorbance was determined using a microplate reader at 760 nm. Total phenolic content was asserted as gallic acid equivalence (GAE) in µg/mg sample. This test was done triplicate (Nurhayati *et al*., 2018; Rusmana *et al*., 2017; Widowati *et al*., 2015; 2017; 2018; Prahastuti *et al*., 2019b).

Flavonoid content assay

Flavonoid content was conducted using the altered previous method (Kalita *et al*., 2013; Prahastuti *et al*., 2019b). Briefly, standard solution in 7 concentration level of epicatechin and EEGU at 1000 µg/mL and 2000 µg/mL as much as 15 μL was used in this method. It was mixed with 75 μL $AlCl_3$ 2%. The absorbance was quantified in 415 nm of wavelength. The linear regression equation (y = ax + b) was made based on the standard (quercetin) absorbance value. The analysis of sample flavonoid content was performed based standard linear regression equation. The flavonoid content was presented as quercetin equivalence (QE) in µg/mg. The experiment was done triplicate (Prahastuti *et al*., 2019b).

DPPH scavenger activity assay

A total of 200 μL of 0.0777 mmol DPPH in methanol was added to the EEGU as much as 50 μL with various concentrations in the 96-well microplate. Then, it was incubated for 30 min at room temperature. After that, the absorbance value was quantified using microplate reader at a wavelength of 517 nm. The negative control was contained 250 μL of DPPH, while the blank solution was contained 50 μL of the sample and 200 μL of DMSO (Widowati *et al*., 2015; 2016; 2017; 2018). The subsequent formula was used to measure the DPPH scavenging activity.

DPPH scavenging activity (%) = $\frac{A-B}{A} x\ 100$

A: control solutions absorbance

B: sample absorbance

H_2O_2 scavenger activity assay

The H_2O_2 scavenging was measured according to previous studies (Mukhopadhyay *et al*., 2016; Utami *et al*., 2017) with slight modification. Each sample well was contained 60 μL of EEGU, 12 μL of $FeH_8N_2O_8S_2$ 1 mM and 3 μL of H_2O_2 5 mM. The mixture that containing 12 μL of $FeH_8N_2O_8S_2$ and 63 μL of DMSO were used as the negative control, while the mixture that containing 60 μL of EEGU and 90 μL of DMSO were used as the blank solution. After added with H_2O_2, incubated in the dark and room temperature for 5 min. The 1,10-phenanthrolines as much as 75 μL was added into the sample and control well and incubated again for 10 min in the dark and room temperature. The 510 nm wavelength was used to measure the absorbance. The subsequent formula was used to calculate the H_2O_2 scavenger activity:

H_2O_2 scavenger activity (%) $= \frac{A}{B} x\ 100$

A: control solutions absorbance

B: sample absorbance

ABTS reduction activity assay

Briefly, the EEGU as much as 2 μL at various concentrations was added into the well. Then, it was mixed with 198 μL of ABTS work. As for the control wells, each of them was added with 198 μL of ABTS work and 2 μL of DMSO. Meanwhile, the blank well was added with 198 μL of DMSO and 2 μL of the EEGU. The microplate was closed, then incubated for 6 min at 37˚C. The absorbance value was determined using microplate reader at a wavelength of 745 nm (Widowati *et al*., 2016; 2017; 2018). The ABTS reduction percentage was calculated using the subsequent formula:

ABTS reduction activity (%) $= \frac{A-B}{A} x\ 100$

A: control solutions absorbance

B: sample absorbance

FRAP

The acetate buffer (acetic acid was added to create conformation at pH 3.6) at 300 mM of concentration as much as 10 mL, dissoluble ferric chloride hexahydrate in distilled water at 20 mM of concentration as much as 1 mL, and dissoluble TPTZ in 40 mM HCl at 10 mM of concentration as much as 1 mL was mixed to create the FRAP reagent. A total of 7.5 µL of the sample was mixed with the FRAP reagent as much as 142.5 µL in a 96-well microplate, then mixed and incubated for 30 min at 37ºC. The wavelength at 593 nm was used to measure the absorbance value using the microplate reader. Afterward, a $FeSO_4$ standard curve with varying concentrations, starting from 0.019 to 95 μg/mL was created. The results were presented as µM Fe (II)/µg extract (Widowati *et al*., 2017; 2018).

Statistical analysis

The SPSS program (version 20.0) was used to carry out the statistical analysis. The DPPH scavenger, H_2O_2 scavenger, and ABTS reduction assays results were analyzed using linear regression analysis. Then, the linear regression equation generated in this process was used to determine the Median Inhibitory Concentration 50 (IC_{50}).

RESULTS AND DISCUSSION

There have been previous studies documenting the natural compounds of *G. ulmifolia* extract. Both its fruits and leaves contain tannins, saponins, flavonoids, terpenoids, cardiac glycoside, and alkaloids (Patil, 2013)**.** However, EEGU has been reported to have high cardiac glycosides, moderate levels of tannin, phenol, terpenoids, and coumarin, and lower concentrations of sterol, quinone, and acid, but not flavonoids and saponins (Duraiswamy *et al*., 2018).

Duraiswamy *et al*. (2018) have declared that the total phenolic and flavonoid contents of *G. ulmifolia* are 27.2797±0.1756 mg TAE/g DW and 0.5544±0.00018 mg QE/g DW. Meanwhile, the present study yielded higher values, which were 32.24±1.42 μg GAE/mg EEGU and 6.48±0.14 μg QE/mg EEGU.

Phenolic compounds are a very good antioxidant or have a broad number of biochemical activities such as antimutagen, anticarcinogenic and modify gene expression for curing various oxidative stress diseases. A phenolic compounds antioxidant activity reflects its composition, the hydroxyl groups location also number, and the nature of its substitution on the aromatic ring. Flavonoids are excellent biological reaction modifications as the yeomen's function in antihistamine, antimicrobial, memory, and even mood-enhancing properties. The plants thus find

their medicinal values due to the presence of respective phytochemical constituents (Duraiswamy *et al*., 2018). Flavonoids induce mechanism that inhibit tumor invasion, kill the cancer cell, apart from this show anti-inflammatory, antiallergic, anticancer and antimicrobial (Sharma *et al*., 2015).

Phytochemical screening

The *G. ulmifolia* leaves extract was subjected to preliminary phytochemical analysis, and the results are shown in Table I. This research showed that the extract contains flavonoids, phenols, tannins, steroids, terpenoids, and alkaloids while saponins and triterpenoids were not detected.

Table I. The EEGU qualitative phytochemical screening assay

Phytochemical assay	Results
Tannins	+
Saponins	-
Flavonoids	+
Phenols	+
Alkaloids	+
Terpenoids	+
Steroids/Triterpenoids	+/-

(+): present; (-): not detected

Phenolic content

Total phenolics were gauged by a colorimetric method modified from a previous study (Widowati *et al*., 2015; 2016; 2017; 2018). It was based on entangle phenolic compounds reduce Folin-Ciocalteu's reagent with a blue complex generated unanimously. The total polyphenols were measured using the calibration curve for gallic acid. The experiments revealed the presence of a high quantity of phenolics in the extract, which was 32.24 ± 1.42 μg GAE/mg EEGU.

Flavonoid content

The total flavonoid content of EEGU was measured by the aluminum chloride method. Its quantification revealed the presence of a high quantity of flavonoids in EEGU, which was 6.48±0.14 μg QE/mg EEGU.

DPPH scavenger activity

Various assay have been used to test antioxidant activity, but the most commonly used methods are the forming of free radical species and being neutralized with antioxidant compounds. Unpaired electron caused a stable free radical DPPH. DPPH is a stable free radical that centered on nitrogen, usually used to assign the compounds or plant extracts radical scavenging activity (Kedare and Singh, 2011; Sasikumar and Kalaisezhiyen, 2014). In the presence of hydrogen donor, it becomes paired and reduced the absorption at 517 nm (Widowati *et al*., 2015). The extracts reduced stable DPPH radicals to yellow-colored diphenyl picrylhydrazine (DPPH-H) during DPPH test. The radical DPPH is widely used as a substrate for examining antioxidant activity. The IC_{50} value is the concentration allowed by an antioxidant to scavenge 50% of the DPPH free radical; higher antioxidant activity is defined by a smaller IC_{50} value. In this study, the IC_{50} of EEGU was 45.70±0.7 μg/mL, while epicatechin had an IC_{50} of 0.56±0.29 μg/mL. Epicatechin was discovered to be the most active extract in DPPH scavenging activity because it provided the lowest IC_{50} value (Table II) and the highest DPPH scavenging activities (Figure 1), particularly at a concentration of 200 μg/mL.

Table II. The antioxidant activities (IC_{50}) of EEGU and epicatechin

Sample	IC_{50} of DPPH Scavenger Activity (μg/mL)	IC_{50} of H_2O_2 Scavenger Activity (μg/mL)	IC_{50} of ABTS-reducing Activity (μg/mL)	FRAP-reducing highest Activity (μM Fe (II)/μg extract)
EEGU	45.70	162.93	35.96	202.71
Epicatechin	0.56	57.91	16.74	236.33

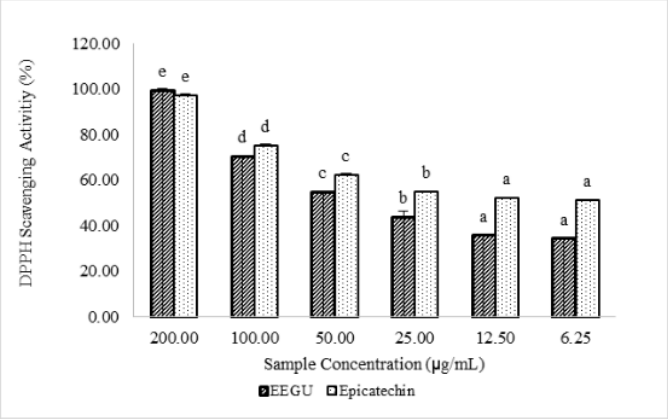


Figure 1. The DPPH scavenging activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 200, 100, 50, 25, 12.50, and 6.25 μg/mL. This experiment was done triplicate for each sample concentration

H_2O_2 scavenger activity

Hydrogen peroxide plays a role in producing energy in various in vivo systems, phagocytosis, intercellular signal transfer, cell growth control, and the synthesis of essential biological compounds (Packer *et al*., 2008). As byproducts of normal aerobic metabolism H_2O_2 is generated and increased during infections, workouts, and stress conditions (Mukhopadhyay *et al*., 2016). The Median Inhibitory Concentrations (IC_{50}) of epicatechin and EEGU in H_2O_2 radical scavenging activity was shown in Table II. The IC_{50} values of EEGU and epicatechin were 162.93 μg/mL and 57.91 μg/mL, respectively. Figure 2 also shows that epicatechin had higher activities than EEGU, particularly at 300 μg/mL.

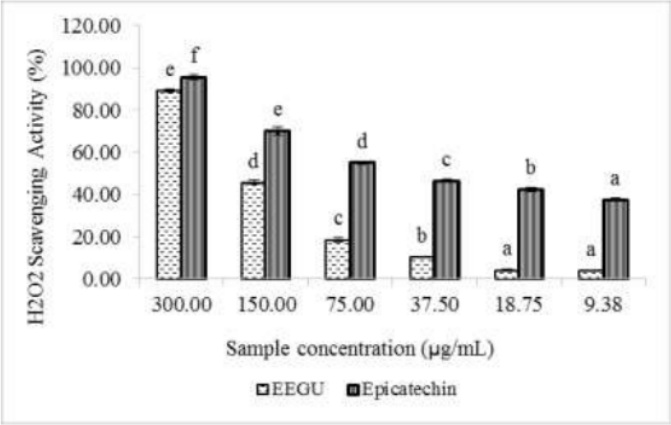


Figure 2. The H_2O_2 scavenging activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 300, 100, 150, 75, 37.5, 18.75, and 9.38 μg/mL This experiment was done triplicate for each sample concentration

ABTS-reducing activity

The activity of ABTS-reducing assay gauge the antioxidant's relative potential to scavenge the ABTS generated. In this study, ABTS was produced by reaction between a powerful oxidizer and ABTS salt. The ABTS radical blue-green-colored solution was reduced by hydrogen-donating antioxidant (Widowati *et al*., 2016) and analyzed at spectrum of a long-wave absorption. The percentages of ABTS-reducing activity of EEGU and epicatechin were compared, with epicatechin having the highest activity at 50 μg/mL, as shown in Figure 3. Also, their IC_{50} values were 35.96 μg/mL and 16.74 μg/mL, respectively (Table II).

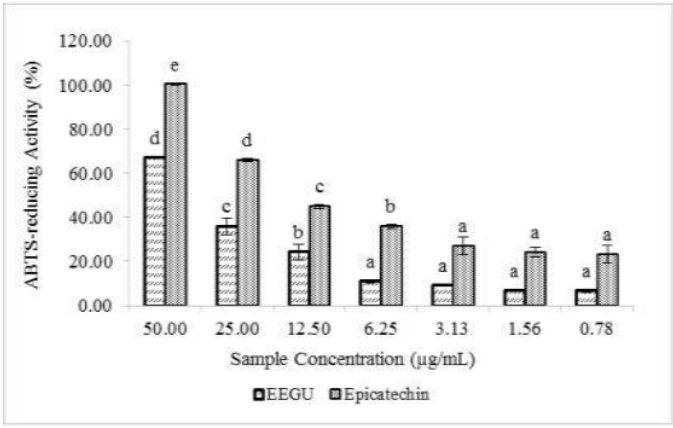


Figure 3. The ABTS-reducing activities of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to reach final concentrations of 50, 25, 12.50, 62.50, 3.13, 1.56, and 0.78 μg/mL. This experiment was done triplicate for each sample concentration

FRAP

The FRAP method was based on the ferroin analog reduction in acidic medium, the $TPTZ^{3+}$ to the colored Fe^{2+} complex of $Fe(TPTZ)^{2+}$ (greatly blue) by antioxidant (Widowati *et al*., 2018).. A reduction in the corresponding tripyridyltriazine Fe(III) complex at 593 nm results from the absorbance of Fe(II) complex.

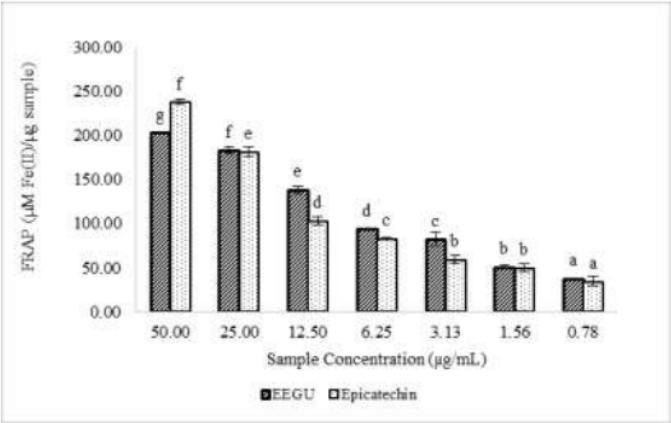


Figure 4. The FRAP of EEGU and epicatechin

*Each sample was diluted in 10% DMSO to create the final concentrations: 50, 25, 12.50, 6.25, 3.13, 1.56, and 0.78 µg/mL. This experiment was done triplicate for each sample concentration

The highest FRAP activities of EEGU and epicatechin were 202.71 µg/mL and 236.33 µg/mL, respectively (Table II, Figure 4). Duraiswamy *et al*. (2018) claim that the most significant inhibition of FRAP activity is 40%, and this is produced by the fruit extract of *G. ulmifolia* at 500 µg/mL. Meanwhile, in this research, the highest FRAP activity was 202.71 µM Fe(II), which was generated by EEGU at 50 µg/mL.

High correlations between antioxidant activities and phytochemical content of polyphenols were observed. The antioxidant activities potentially contribute to the polyphenol contents found in the plant extracts. *G. ulmifolia* stem bark and leaves extracts exhibited DPPH scavenger activity at IC_{50} of 25.2 µg/mL and 39.3 µg/mL (dos Santos *et al*., 2018). There have been some previous studies examining the antioxidant activities of EEGU using different assays, namely, FRAP, DPPH, MDA dosage, human erythrocyte suspension, hemolytic activity, and inhibition of oxidative hemolysis in human erythrocytes by 2,2′-Azobis(2-amidinopropane) dihydrochloride-induced (AAPH) or DOX (Duraiswamy *et al*., 2018; dos Santos *et al*., 2018; Kaneria *et al*., 2009).

CONCLUSIONS

Based on the phytochemical screening, EEGU has been confirmed to contain flavonoids, phenols, tannins, alkaloids, steroids, and terpenoids but not saponins and triterpenoids. The flavonoid contents and total phenolic of EEGU are 32.24 µg extract/mg GAE and 6.48 µg QE/mg extract, respectively. Comparison between EEGU and epicatechin using different tests for antioxidant activities, namely DPPH scavenging, ABTS reduction, H_2O_2 scavenging, and FRAP assays, proves that the IC_{50} values of EEGU are generally higher than those of epicatechin. For DPPH scavenging, H_2O_2 scavenging, and ABTS reduction activities, EEGU can produce IC_{50} values of 45.70 µg/mL, 162.93 µg/mL, and 35.96 µg/mL. Meanwhile, for the same activities, epicatechin only yields IC_{50} values of 0.56 µg/mL, 57.91 µg/mL, and 16.74 µg/mL. At the same time, EEGU has the highest FRAP reduction properties at 50 µg/mL or equal to 202.71 µM Fe

(II)/μg. These findings assert that EEGU and epicatechin have antioxidant activities and that EEGU is an active natural product.

ACKNOWLEDGMENT

The authors would like to acknowledge the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education of the Republic of Indonesia for their financial support through the scheme of Penelitian Terapan Peruguruan Tinggi in 2019. Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia for supporting the metodology and laboratory facilities used in the research and the authors would also like to extend their gratitude to Dwi Surya Artie and Dewani Tediana Yusepany for technical support.

REFERENCES

Adnyana, I. K., Abuzaid, A. S., Iskandar, E. Y. and Kurniati, N. F., 2016, Pancreatic lipase and α-amylase inhibitory potential of mangosteen (*Garcinia mangostana* Linn.) pericarp extract, *International Journal of Medical Research & Health Sciences*, 5(1): 23-28.

Amorati, R. and Valgimigli, L., 2015, Advantages and limitations of common testing methods for antioxidants, *Free Radical Research*, 49(5): 633-649.

Bera, T. K., Chatterjee, K. and Ghosh, D., 2015, In-vitro antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq., *Biomarkers and Genomic Medicine*, 7(1): 18-24.

dos Santos, J. M., Alfredo, T. M., Antunes, K. Á., da Cunha, J. D. S. M., Costa, E. M. A., Lima, E. S., Silva, D. B., Carollo, C. A., Schmitz, W. O., Boleti, A. P. D. A. and dos Santos, E. L., 2018, *Guazuma ulmifolia* Lam. decreases oxidative stress in blood cells and prevents doxorubicin-induced cardiotoxicity, *Oxidative Medicine and Cellular Longevity*, 2018: 1-16.

Duraiswamy, B., Singanan, M. and Varadarajan, V., 2018, Physicochemical, phytochemicals and antioxidant evaluation of *Guazuma ulmifolia* fruit, *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(9): 87.

Feltrin, A.C., Boligon, A.A., Janovik, V. and Athayde, M.L., 2012. Antioxidant potential, total phenolic and flavonoid contents from the stem bark of *Guazuma ulmifolia* Lam, *Asian Journal of Biological Sciences*, 5(5): 268-272.

Iswantini, D., Silitonga, R.F., Martatilofa, E. and Darusman, L.K., 2011. Zingiber cassumunar, Guazuma ulmifolia, and Murraya paniculata extracts as antiobesity: in vitro inhibitory effect on pancreatic lipase activity, *Hayati Journal of Biosciences*, 18(1): 6-10.

Kalita, P., Tapan, B. K., Pal, T. K. and Kalita, R., 2013, Estimation of total flavonoids content (TFC) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn., *Journal of Drug Delivery and Therapeutics*, 3(4): 33-37.

Kaneria, M., Baravalia, Y., Vaghasiya, Y. and Chanda, S., 2009, Determination of antibacterial and antioxidant potential of some medicinal plants from saurashtra region, India, *Indian Journal of Pharmaceutical Sciences*, 71(4): 406.

Karthika, V., Arumugam, A., Gopinath, K., Kaleeswarran, P., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M. and Benelli, G., 2017. Guazuma ulmifolia bark-synthesized Ag, Au and Ag/Au alloy nanoparticles: Photocatalytic potential, DNA/protein interactions, anticancer activity and toxicity against 14 species of microbial pathogens, *Journal of Photochemistry and Photobiology B: Biology*, 167: 189-199.

Kedare S. B. and Singh R. P., 2011, Genesis and development of DPPH method of antioxidant assay, *Journal of Food Science and Technology*, 48: 412–422.

Morais, S.M., Calixto-Júnior, J.T., Ribeiro, L.M., Sousa, H.A., Silva, A.A.S., Figueiredo, F.G., Matias, E.F.F., Boligon, A.A., Athayde, M.L., Morais-Braga, M.F.B. and Coutinho, H.D.M., 2017. Phenolic composition and antioxidant, anticholinesterase and antibiotic-modulating

antifungal activities of Guazuma ulmifolia Lam.(Malvaceae) ethanol extract, *South African Journal of Botany*, 110: 251-257.

Mukhopadhyay, D., Dasgupta, P., Roy, D. S., Palchoudhuri, S., Chatterjee, I., Ali, S. and Dastidar, S. G., 2016, A sensitive in vitro spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline, *Free Radicals & Antioxidants*, 6(1): 123-131.

Nurhayati, B., Rahayu, I. G., Rinaldi, S. F., Zaini, W. S., Afifah, E., Arumwardana, S., Kusuma, H. S. W., Rizal, R. and Widowati, W., 2018, The antioxidant and cytotoxic effects of *Cosmos caudatus* ethanolic extract on cervical cancer, *The Indonesian Biomedical Journal*, 10(3): 243-249.

Packer, L., Cadenas, E. and Davies, K.J., 2008, Free radicals and exercise: an introduction, *Free radical biology & medicine*, 44(2): 123-125.

Patil, J. U. and Biradar, S. D., 2013, Pharmacognostic study of *Guazuma ulmifolia*, *International Research Journal of Pharmaceutical*, 4: 130-131.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. and Bitto, A., 2017, Oxidative Stress: Harms and Benefits for Human Health, *Oxidative Medicine and Cellular Longevity*, 2020,1-13.

Prahastuti, S., Hidayat, M., Hasiana, S. T., Widowati, W., Amalia, A., Qodariah, R. L., Rizal, R., Kusuma, H. S. W., and Khoiriyah, Z., 2019a, Ethanol extract of jati belanda (*Guazuma ulmifolia* L.) as Therapy for Chronic Kidney Disease in In Vitro model, *Journal of Reports in Pharmaceutical Sciences*, 8(2): 229.

Prahastuti, S., Hidayat, M., Hasianna, S.T., Widowati, W., Amalia, A., Yusepany, D.T., Rizal, R. and Kusuma, H.S.W., 2019b, Antioxidant potential ethanolic extract of *Glycine max* (l.) Merr. Var. Detam and daidzein, *Journal of Physics: Conference Series*, 1374: 1-12.

Rozqie, R., Diah, M. and Rukmi, W., 2012, The effect of jati belanda (*Guazuma ulmifolia* Lamk) Leaves Extract on Histopathology of Rat's Kidney, *Tropical Medicine Journal*, 2(1): 57-65.

Rusmana, D., Wahyudianingsih, R., Elisabeth, M., Balqis, B., Maesaroh, M. and Widowati, W., 2017, Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin, *The Indonesian Biomedical Journal*, 9(2): 84-90.

Sasikumar, V., and Kalaisezhiyen, P., 2014, Evaluation of free radical scavenging activity of various leaf extracts from Kedrostis foetidissima (Jacq.) Cogn, *Biochemistry and Analytical Biochemistry*, 3(2): 1.

Sharma, M., Chopra, S. and Prasad, S.B., 2015. Guazuma tomentosa: A valuable medicinal plant, *International Journal of Pharmacognosy and Phytochemical Research*, 7(1): 197-200.

Sukandar, E.Y., Nurdewi and Elfahmi, 2012. Antihypercholesterolemic effect of combination of *Guazuma ulmifolia* lamk. leaves and *Curcuma xanthorrhiza* Roxb. rhizomes extract in wistar rats, *International Journal* of *Pharmacology*., 8: 277-282.

Syaefudin, Wahyuni, W. T., Artika, I. M. and Sulistiyani, 2014, Antioxidant activity of flavonoid from *Guazuma ulmifolia* Lamk. leaves and apoptosis induction in yeast cells, *Journal of Biological Sciences*, 14(4): 305.

Tabas, I., 2005. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency, *Arteriosclerosis, thrombosis, and vascular biology*, 25(11): 2255-2264.

Utami, S., Adityaningsari, P., Sosiawan, I., Endrini, S., Sachrowardi, Q. R., Laksono, S. P., Nafik, S., Arrahmani, B. C., Afifah, E. and Widowati, W., 2017, Antioxidants and Anticholinesterase Activities of the Characterized Ethanolic of Ripe Sesoot (*Garcinia picrorrhiza* Miq.) Fruit Extract (GpKar) and Xanthone, *Majalah Obat Tradisional*, 22(3): 160-165.

Widowati, W., Fauziah, N., Herdiman, H., Afni, M., Afifah, E., Kusuma, H. S. W., Nufus, H., Arumwardana, S. and Rihibiha, D. D., 2016. , Antioxidant and Anti-aging Assays of *Oryza sativa* Extracts, Vanillin and Coumaric Acid., *Journal of Natural Remedies*, 16(3): 88-99.

Widowati, W., Herlina, T., Ratnawati, H., Constantia, G., Deva, I. D. G. S. and Maesaroh, M., 2015, Antioxidant potential of black, green and oolong tea methanol extracts., *Biology, Medicine, & Natural Product Chemistry*, 4(2): 35-39.

Widowati, W., Janeva, B. W., Nadya, S., Amalia, A., Arumwardana, S., Kusuma, H. S. W. and Arinta, Y., 2018., Antioxidant and antiaging Antiaging activities Activities of *Jasminum sambac* Extract, and its Compounds,. *Journal of Reports in Pharmaceutical Sciences*, 7(3): 270.

Widowati, W., Rani, A. P., Hamzah, R. A., Arumwardana, S., Afifah, E., Kusuma, H. S. W., Rihibiha, D. D., Nufus, H., and Amalia, A., 2017, Antioxidant and antiaging assays of *Hibiscus sabdariffa* Extract and its Compounds, *Natural Product Sciences*, 23(3): 192-200.

The ethanol extract of the bastard cedar (Guazuma ulmifolia L.) as antioxidants

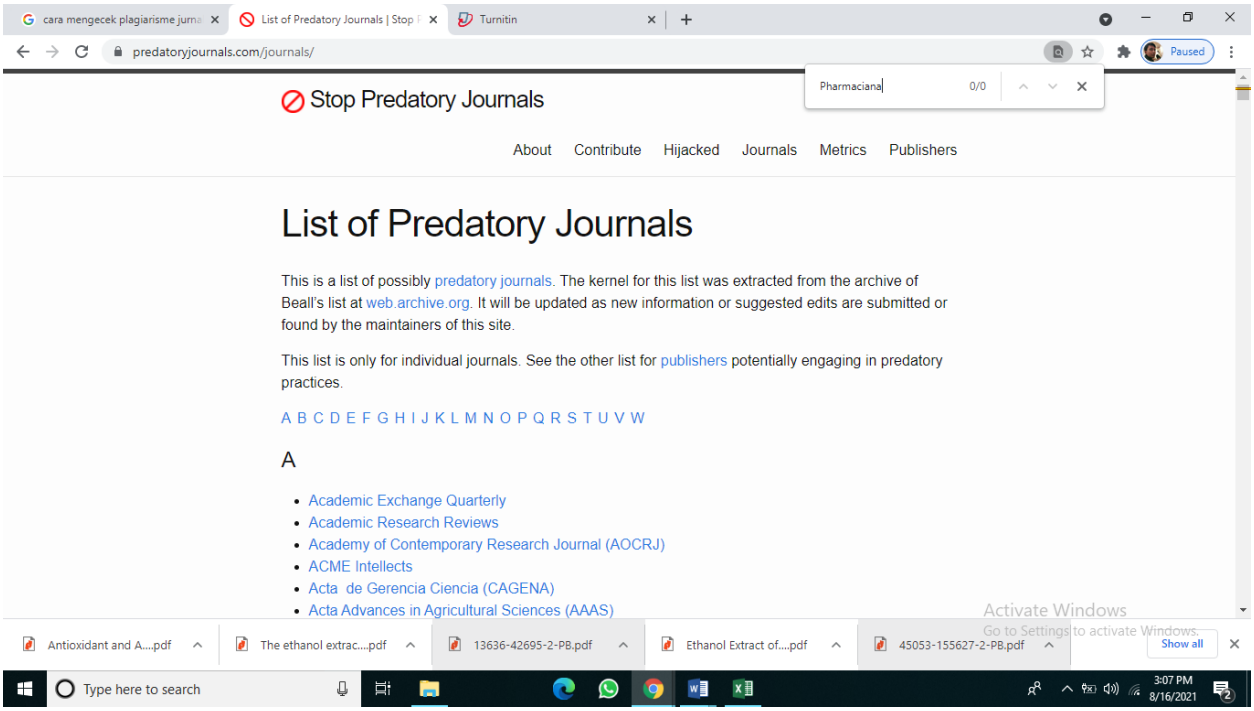
ORIGINALITY REPORT

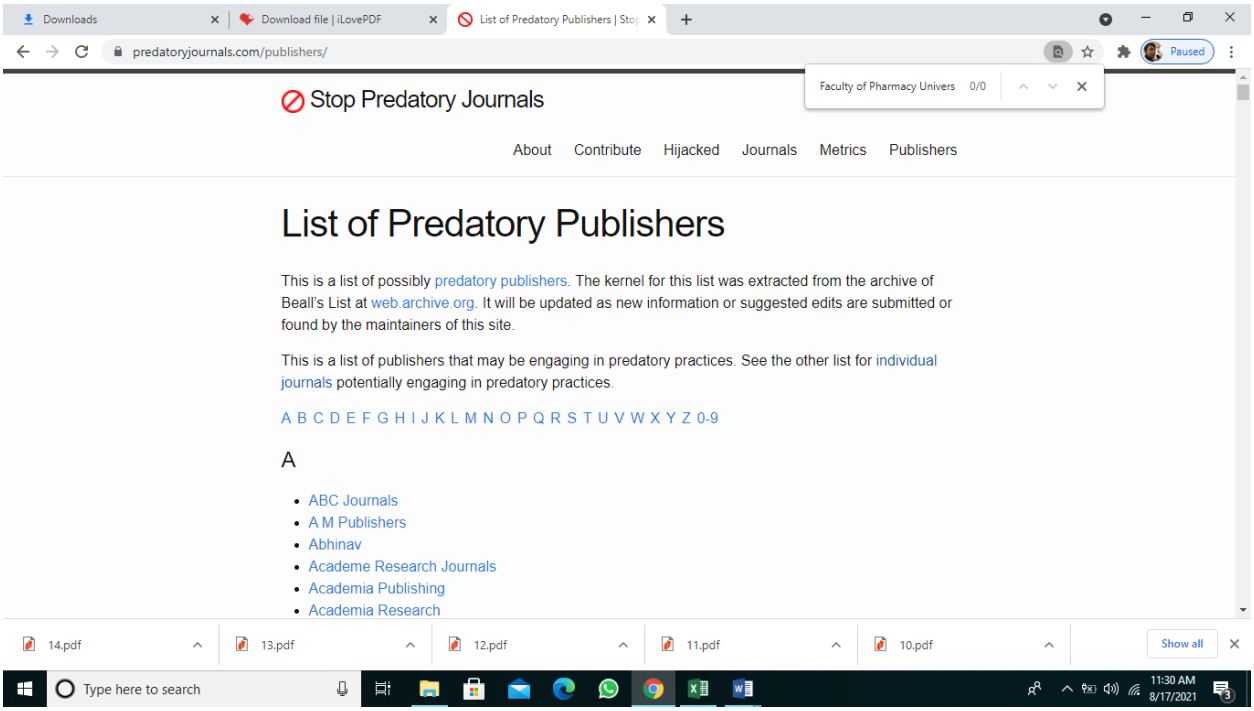
7%	7%	4%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	journal.uad.ac.id Internet Source	4%
2	Tatiane Matarazzo Cantero Campos, Pedro Ismael da Silva Junior, Giuseppina Negri, Roberto Manuel do Nascimento et al. "Antimicrobial activity of flavonoids glycosides and pyrrolizidine alkaloids from propolis of Scaptotrigona aff. postica.", Cold Spring Harbor Laboratory, 2021 Publication	2%
3	Submitted to Sriwijaya University Student Paper	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		


cara mengecek plagiarisme jurna
List of Predatory Journals | Stop
Turnitin
predatoryjournals.com/journals/
Paused
Pharmaciana 0/0
Stop Predatory Journals
About Contribute Hijacked Journals Metrics Publishers
List of Predatory Journals
This is a list of possibly predatory journals. The kernel for this list was extracted from the archive of Beall's list at web.archive.org. It will be updated as new information or suggested edits are submitted or found by the maintainers of this site.
This list is only for individual journals. See the other list for publishers potentially engaging in predatory practices.
A B C D E F G H I J K L M N O P Q R S T U V W
A
Academic Exchange Quarterly
Academic Research Reviews
Academy of Contemporary Research Journal (AOCRJ)
ACME Intellects
Acta de Gerencia Ciencia (CAGENA)
Acta Advances in Agricultural Sciences (AAAS)
Activate Windows
Go to Settings to activate Windows.
Antioxidant and A....pdf
The ethanol extrac....pdf
13636-42695-2-PB.pdf
Ethanol Extract of....pdf
45053-155627-2-PB.pdf
Show all
Type here to search
3:07 PM
8/16/2021


Downloads
Download file | iLovePDF
List of Predatory Publishers | Sto
predatoryjournals.com/publishers/
Paused
Faculty of Pharmacy Univers 0/0
Stop Predatory Journals
About Contribute Hijacked Journals Metrics Publishers
List of Predatory Publishers
This is a list of possibly predatory publishers. The kernel for this list was extracted from the archive of Beall's List at web.archive.org. It will be updated as new information or suggested edits are submitted or found by the maintainers of this site.
This is a list of publishers that may be engaging in predatory practices. See the other list for individual journals potentially engaging in predatory practices.
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z 0-9
A
ABC Journals
A M Publishers
Abhinav
Academe Research Journals
Academia Publishing
Academia Research
14.pdf
13.pdf
12.pdf
11.pdf
10.pdf
Show all
Type here to search
11:30 AM
8/17/2021