

## Phytochemical Profile and Antioxidant Activity of *Guazuma ulmifolia* Leaves Extracts Using Different Solvent Extraction

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### ABSTRACT

*Guazuma ulmifolia* is one of the common tropical plants that has long been used as a traditional medicine to reduce body weight as slimming herbs and to lower the cholesterol in the body. The leaves of *G. ulmifolia* contain phenolics compound such as flavonoids and tannin that contributes to its biological activities. In this research, we determined the content of total phenolics, flavonoids, tannins, antioxidant activity, and FTIR spectrum profile from four extracts of *G. ulmifolia* leaves. The extraction method used in this study was stepwise maceration with different solvents *n*-hexane, ethyl acetate, ethanol, and water. The content of total phenolics and flavonoids were found high in ethanol extract of *G. ulmifolia* leaves with 35.42 and 44.85mg QE/g dry powder, respectively. The highest content of total tannins was obtained in ethyl acetate extract of *G. ulmifolia* leaves as 0.55%. Antioxidant activity from four extracts of *G. ulmifolia* leaves was measured using DPPH, CUPRAC, and reducing power method. The highest antioxidant activity for DPPH and CUPRAC method was obtained in ethyl acetate extract with the antioxidant capacity as 55.47 and 98.17µmol trolox/g dry powder, respectively. While using reducing power assay gives the capacity 176.75µmol trolox/g dry powder in the water extract. The pattern of FTIR spectra from four extracts of *G. ulmifolia* leaves gives the distinct characteristics of each spectrum profile. Principal component analysis using FTIR spectrum shows good clustering for each extract with 94% data variability (PC1= 77% and PC2 = 17%). It can be concluded that each extract of *G. ulmifolia* leaves gives a distinct phytochemical profile (phenolics content, flavonoids, tannins, and FTIR spectrum) that contributes to the antioxidant activity. This antioxidant activity mainly influenced by the concentration of phenolic compounds, which is known to have antioxidant properties.

**Keywords:** antioxidant, *Guazuma ulmifolia*, phenolics, flavonoids, tannins

### INTRODUCTION

Traditional medicine derived from plants is one way to utilize natural resources to maintain good health and to treat diseases in the community. One common plant that has been used as traditional medicine is *Guazuma ulmifolia*, known as bastard cedar, and it belongs to the family of Sterculiaceae. The proven pharmacological activity

from this plant involve antiulcer (Berenguer *et al.*, 2007), antihypertensive (Magos *et al.*, 2008), antidiabetic (Adnyana *et al.*, 2013), antioxidant, anticholinesterase, antibiotic-modulating antifungal (Morais *et al.*, 2017), analgesic and anti-inflammatory activities (Damor *et al.*, 2018). The utilization of *G. ulmifolia* as traditional medicine can be linked to the presence of chemical

compounds such as flavonoid, alkaloid, glycosides, phytosterol, and saponin responsible to its particular biological activity. Phytochemical composition and its concentration are greatly affected by some factors, such as genetics, environmental growth conditions, harvest, and post-harvest treatment. Extraction of metabolites using a different method and solvent extraction also play an essential role in the level of metabolites extracted (Rafi *et al.*, 2020). Consistency in the composition and concentration level of bioactive compounds will give consistent biological activity. *G. ulmifolia* rich in phenolic compounds such as phenolic acid, flavonoid, and tannin, and those compounds have been known for their potency as an antioxidant (Pereira *et al.*, 2019). Antioxidant compounds within plant extracts having different molecular structures and polarities. Based on its molecular structure, antioxidant compounds with its chemical characteristics and polarities may or may not be soluble in a solvent. Polar solvents are frequently used for the polyphenol extraction from plant matrices (Altemimi *et al.*, 2017). Ethanol and water as polar solvents are well known for polyphenol extraction and safe for human consumption. Ethyl acetate has a medium polarity and low toxicity that could extract many antioxidant compounds from polar to nonpolar.

From the previous studies, many papers have been reported the composition and concentration of metabolite or compound class present in *G. ulmifolia* with a particular biological activity (Maldini *et al.*, 2013; Morais *et al.*, 2017; Prahastuti *et al.*, 2020). However, there is no reported paper for extracting metabolites in *G. ulmifolia* with different solvent extraction using stepwise maceration for knowing the phytochemical profile and antioxidant activity. In this study, we have investigated the phytochemical profile and antioxidant activity of *G. ulmifolia* using the various polarity of solvents (*n*-hexane, ethyl acetate, ethanol, and water). We also make a correlation between antioxidant activities and phytochemical (phenolics, flavonoid, and tannin) content to show its contribution toward antioxidant activities. By mapping the phytochemical profile and antioxidant activity of different *G. ulmifolia* extracts, we will have better information about each extract's characteristics.

## MATERIAL AND METHODS

*G. ulmifolia* leaves were obtained from Tropical Biopharmaca Research Center (TropBRC) IPB University, Bogor, Indonesia. Mr. Taopik

Ridwan identified the plant, and the voucher specimens were stored in TropBRC, IPB University. Methanol, ethanol, *n*-hexane, ethyl acetate, Folin-Ciocalteu (FC) reagent, sodium carbonate, potassium permanganate, aluminum (III) chloride, potassium acetate, copper (II) chloride, potassium ferricyanide ( $K_3Fe(CN)_6$ ), trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany). Gallic acid, quercetin, trolox, neocuproine, indigo carmine, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

## Sample preparation and extraction

*G. ulmifolia* leaves were dried, pulverized into a powder, and then sieved with 100 mesh particle size. Extraction was performed using a procedure described in Indonesian Herbal Pharmacopoeia 1<sup>st</sup> edition (Ministry of Health Republic of Indonesia, 2008). About 20g of samples were macerated with 200 mL *n*-hexane for 24h at room temperature. After continuous stirring of samples for 6h, samples left for a further 18h without stirring. The filtrate was collected and concentrated with a rotary evaporator at 50°C. The residue from the filtration process with *n*-hexane was then extracted with ethyl acetate, and the obtained filtrate was concentrated. Next, the residue from the previous filtration was then extracted with ethanol until the filtrate was obtained. The residue from previous ethanol filtration was then macerated and extracted with water using the reflux method until obtained the concentrated filtrate. The extraction process using each solvent (*n*-hexane, ethyl acetate, ethanol, and water) was repeated three times. Then the resulted of concentrated filtrate from each solvent was weighed to measure its yield.

## Determination of total phenolics

The total phenolics content (TPC) in each extract of *G. ulmifolia* leaves was determined using the Folin-Ciocalteu method using a procedure described by Rafi *et al.*, (2018). Each of *n*-hexane, ethyl acetate, ethanol, and water extract were pipetted 10 $\mu$ L into 96 well plates. Then approximately 160 $\mu$ L distilled water, 10 $\mu$ L of 10% FC reagent, and 20 $\mu$ L of 7.5%  $Na_2CO_3$  was added subsequently into each extract in the 96 well plates. The mixture was then homogenized and incubated for 30min at room temperature. The maximum absorbance of the mixture was measured at 750nm using a microplate reader (Epoch-BioTek, Winooski, USA). The calibration curve was

prepared by diluting gallic acid in ethanol in 5 different concentrations as 10, 30, 50, 70, 100 (mg/L). TFC of each extract was expressed as milligram gallic acid equivalent per gram dry powder of *G. ulmifolia* (mg GAE/g dry powder).

#### Determination of total flavonoids

The total flavonoids content (TFC) of each extract was investigated using the aluminum chloride colorimetry method following the procedure described by Lee *et al.*, (2011). Each of 10 $\mu$ L extract from *n*-hexane, ethyl acetate, ethanol, and water from *G. ulmifolia* leaves was added with 60 $\mu$ L ethanol, 10 $\mu$ L of 10% AlCl<sub>3</sub>, 10 $\mu$ L of 1M CH<sub>3</sub>COOK, and 120 $\mu$ L distilled water subsequently into 96 well plates. The mixture was homogenized and incubated for 30min at room temperature. Then the absorbance of the mixture was at 415nm using a microplate reader (Epoch-BioTek, Winooski, USA). The calibration curve was prepared by diluting quercetin in ethanol in 7 different concentrations as 20, 40, 60, 80, 100, 120, 140 ( $\mu$ g/mL). TFC was expressed as milligram quercetin equivalent per gram dry powder of *G. ulmifolia* leaves (mg QCE/g dry powder).

#### Determination of total tannins

The total tannins content (TTC) in each extract was determined using the procedure described Indonesian Herbal Pharmacopoeia 1<sup>st</sup> edition (Ministry of Health Republic of Indonesia, 2008). Each of 0.2 g extract from *G. ulmifolia* leaves was added into 250mL Erlenmeyer flask and heated for 30min. Then the mixture left for 10min and filtered until obtained residue. The process was repeated to make sure all the tannins were extracted from the residue. Next, 2.5mL of the mixture was added with 2.5mL of indigo carmine and diluted into 100mL volume with water. Then, the solution was titrated with 0.1N KMnO<sub>4</sub> until obtained the color solution changing into golden yellow. The blank solution was prepared with a similar procedure but without adding the extract. Approximately 1mL of 0.1N KMnO<sub>4</sub> is equivalent to 0.004g tannins. Total tannins can be calculated as follow:

$$\% \text{ total Tannin} = \frac{V \text{ KMnO}_4 \text{ sample} - V \text{ KMnO}_4 \text{ blank (mL)}}{\text{weight extract (g)}} \times \frac{0.004\text{g}}{1 \text{ mL}} \times 100\%$$

#### DPPH radical scavenging activity

The antioxidant activity of the extract was measured with the DPPH method following the procedure described by Salazar-Arandra *et al.*, (2009). Each of 40 $\mu$ L extract was added with 120 $\mu$ L of 125 $\mu$ mol/L freshly prepared DPPH solution into 96 well plates. Then the mixture was incubated in the dark for 30min at room temperature. The decrease in absorbance was measured at 515nm using a microplate reader (Epoch-BioTek, Winooski, USA). Negative control was prepared by mixing 40 $\mu$ L ethanol with 120 $\mu$ L of 125 $\mu$ mol/L freshly prepared DPPH solution. While positive control used trolox and prepared in 9 consecutive concentrations as 50, 75, 100, 150, 200, 250, 300, 350, 400 $\mu$ M. The measurement was repeated three times, and the capacity to scavenge the DPPH radical was expressed in  $\mu$ mol trolox/g dry powder.

#### Cupric ion reducing antioxidant capacity

The cupric ion reducing antioxidant capacity (CUPRAC) of the extracts was determined to the CUPRAC method described by Ozturk *et al.*, (2011). To each well, in a 96 well plate, 40  $\mu$ L of the extracts, 50 $\mu$ L of 10mM CuCl<sub>2</sub>, 50 $\mu$ L of 7.5mM neocuproine, and 60 $\mu$ L NH<sub>4</sub>CH<sub>3</sub>COO buffer (1M, pH 7) solutions were added subsequently. Then the mixture was incubated for 1h at room temperature. After 1h, the absorbance at 450nm was recorded against a reagent blank by using a 96-well microplate reader (Epoch-BioTek, Winooski, USA). The calibration curve was prepared with trolox in 5 different concentrations as 100, 200, 300, 400, 600 ( $\mu$ M). The antioxidant capacity was expressed in  $\mu$ mol trolox/g dry powder.

#### Reducing power assay

The ability of the extracts to reduce ferric ions (Fe<sup>3+</sup>) was assessed with the method described by Benslama and Harrar (2006). Approximately 400 $\mu$ L of the extract was added with 200 $\mu$ L phosphate buffer (0.2M, pH 6.6) and 400 $\mu$ L of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] then the mixture was incubated at 50°C for 20min. Next, about 400 $\mu$ L of trichloroacetic acid was added into the mixture and centrifuged for 10 minutes at 3000 rpm. Finally, 200 $\mu$ L of the supernatant solution was mixed with 400 $\mu$ L of distilled water, and 40 $\mu$ L of 0.1% FeCl<sub>3</sub> and the absorbance was recorded at 700nm using a microplate reader. The calibration curve was prepared using a trolox solution in 5 different concentrations as 200, 400, 600, 800, 1000 ( $\mu$ M).

The antioxidant capacity was expressed in  $\mu\text{mol trolox/g}$  dry powder.

### FTIR Spectra and Classification of *G. ulmifolia* extracts

About 2mg of *G. ulmifolia* leaves extract were mixed with 200mg of KBr and then homogenized and formed into a pellet using a Shimadzu hand press (pressure 8 tons for 10min). The measurement of the FTIR spectrum was performed at the mid-IR area ( $4000\text{-}400\text{ cm}^{-1}$ ) involving a personal computer equipped with OPUS software version 4.2. The IR spectrum was recorded with a scan speed of 32s/min, and a resolution of  $4\text{cm}^{-1}$  with a data display contains 1866 points of absorption. The IR spectra were further used for the classification of *G. ulmifolia* extracts. We used principal component analysis (PCA) to classify the *G. ulmifolia* extracts with The Unscrambler X software version 10.1 (CAMO, Oslo, Norway). Before subjected to the PCA, the IR spectra were preprocessed using standard normal variate (SNV).

### Statistical analysis

The experimental data were done in triplicate measurements and reported as mean  $\pm$  standard deviation. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by Duncan test. A significant difference was defined at the 95% confidence level ( $p < 0.05$ ). Correlation of phytochemical content with antioxidant activity and clustering of the *G. ulmifolia* extracts were performed using Pearson correlation and principal component analysis (PCA), respectively. All the statistical analysis was performed in Unscrambler X version 10.1 (CAMO, Oslo, Norway).

## RESULTS AND DISCUSSION

### Extraction yield

Extraction is the first step to recover and isolate the bioactive phytochemical compounds from the plant materials. The extraction efficiency from plant materials is influenced by several factors, including the chemical nature of phytochemicals, the extraction method used, size of sample particle, and the presence of interfering substances (Stalikas 2007). The extraction yield depends on the pH, temperature, extraction time, solvent polarity, and sample composition. The sample composition and solvent polarity are regarded as the critical parameters under similar time extraction and temperature (Do *et al.*, 2014).

In this work, the extracts of *G. ulmifolia* leaves were obtained by using various range polarity of solvent from nonpolar (*n*-hexane) to polar (water). Extraction yields ranged from 18.24% for ethanol extract to 5.73% for *n*-hexane extract. It is also can be seen that the extraction yield of water (10.94%) is higher than ethyl acetate (9.08%). The average yields of extraction by various solvents decreased in the following order: ethanol > water > ethyl acetate > *n*-hexane. These results show that the extraction yield increases with increasing polarity of the solvent used in the extraction process of *G. ulmifolia* leaves. Compounds such as proteins and carbohydrates may have been extracted in water and ethanol and contribute to higher yield rather than in ethyl acetate and *n*-hexane.

### Total phenolics, total flavonoids and total tannins of *G. ulmifolia* leaves extracts

Total phenolics content from different extracts (*n*-hexane, ethyl acetate, ethanol, and water) of *G. ulmifolia* leaves (Table I). The TPC of different extracts was measured with the FC method using gallic acid as a positive control. The TPC of the extracts range from 13.00 mg GAE/g dry powder for *n*-hexane extract to 35.42 mg GAE/g dry powder for ethanol extract, and they decrease in the following order: ethanol > water > ethyl acetate > *n*-hexane. The TPC of the ethyl acetate extract (16.74 mg GAE/g dry powder) is not significantly higher than that of the water extract (16.97 mg GAE/g dry powder), whereas the TPC of the *n*-hexane extract is significantly less than the other solvents ( $p < 0.05$ ). It can be due to the higher solubility of a non-phenolics compound such as carbohydrate in water extracts rather than in ethyl acetate and *n*-hexane extracts. The TPC of the ethanol extracts gives the highest value due to the possible complex formation of some phenolics compound in the extract that are soluble in this solvent. These phenolics compound may have higher molecular weights solubilized in the ethanol and contain more phenolics group than the phenolics in the water extracts (Do *et al.*, 2014). It can be concluded from the results of TPC from different extract, and ethanol was the best extracting solvent to solubilize the phenolics compound from the extract of *G. ulmifolia* leaves.

Total flavonoids content from different extracts (*n*-hexane, ethyl acetate, ethanol, and water) of *G. ulmifolia* leaves (Table I). The TFC of different extracts was measured using  $\text{AlCl}_3$  reagent and quercetin as a positive control using the UV-Vis spectrophotometry method.

Table I. The total phenolics, flavonoids and tannin content in the four extract of *G. ulmifolia* leaves (n = 3)

Extract	Total phenolics (mg GAE/g dry powder)	Total flavonoids (mg QE/g dry powder)	Total tannin (%)
n-hexane	13.00 <sup>a</sup> ±0.84	21.46 <sup>b</sup> ±0.59	0.14 <sup>a</sup> ±0.06
Ethyl acetate	17.74 <sup>b</sup> ±1.11	31.71 <sup>c</sup> ±0.01	0.55 <sup>c</sup> ±0.06
Ethanol	35.42 <sup>c</sup> ±2.67	44.85 <sup>d</sup> ±3.55	0.45 <sup>bc</sup> ±0.06
Water	16.97 <sup>b</sup> ±2.41	14.15 <sup>a</sup> ±1.10	0.35 <sup>b</sup> ±0.16

\*the letter denotes significance test at p<0.05 Duncan method

Table II. The capacity of antioxidant activity with different methods of DPPH, CUPRAC, and reducing power assay (n = 3)

Extract	Antioxidant capacity (µmol trolox/g dry powder)		
	DPPH	CUPRAC	Reducing power
n-hexane	9.03 <sup>a</sup> ±0.44	21.86 <sup>a</sup> ±1.86	70.90 <sup>ab</sup> ±5.61
Ethyl acetate	55.47 <sup>d</sup> ±1.87	98.17 <sup>d</sup> ±7.89	50.35 <sup>a</sup> ±5.72
Ethanol	21.36 <sup>b</sup> ±0.30	35.52 <sup>b</sup> ±1.68	92.11 <sup>c</sup> ±5.34
Water	37.41 <sup>c</sup> ±3.88	58.79 <sup>c</sup> ±2.81	176.75 <sup>d</sup> ±13.26

The TFC of extracts range from 14.15mg QE/g dry powder for water extract to 44.85mg QE/g dry powder for ethanol extract and they decrease in the following order: ethanol > ethyl acetate > n-hexane > water. The results of TFC in *G. ulmifolia* leaves were entirely synchronous with those obtained from the analysis of total phenolic in different extracts. It was successfully shown that samples with a high phenolics content in the extract of *G. ulmifolia* leaves also contain flavonoids in a considerable amount. The rich-flavonoid plants could be an excellent antioxidant source that would help improve the total antioxidant capacity of an organism and prevent it from the lipid peroxidation (Esmaili *et al.*, 2015).

Total tannins content from the extracts of *G. ulmifolia* leaves in different solvents (Table I). The TTC from the extracts was measured using permanganometric titration with indigo carmine sulphonate as a dye indicator. Total tannins level from different extracts of *G. ulmifolia* leaves have low-level content compared to total phenolics and flavonoids. By comparing the results with various solvents (Table II), we can see that the total phenolics, flavonoids, and tannins content are greatly depending on the solvent polarity. The maceration and extraction of *G. ulmifolia* leaves with ethanol solvent give the highest total phenolics, flavonoids, and tannins content in the extract.

#### Antioxidant Activity of *G. ulmifolia* Leaves Extracts

Antioxidant activity of *G. ulmifolia* extracts was determined using three methods, namely DPPH, CUPRAC, and reducing power assay. In the DPPH method, 2,2-Diphenyl-1-picrylhydrazyl was used as a stable organic free radical reagent with the highest absorption band at 517nm. The absorption of this radical species is lost when an electron or a free radical species is accepted, resulting in a visual discoloration from purple to yellow. This radical species can also combine with many samples in a short time and is sensitive to distinguish active ingredients at low concentrations (Esmaili *et al.*, 2015).

DPPH radical scavenging ability of in vitro from the extracts of *G. ulmifolia* leaves with different extraction solvents (Table II). The n-hexane extract from *G. ulmifolia* leaves showed a reading of the lowest of DPPH radical scavenging (9.03 µmol trolox/g dry powder), while the highest one belonged to the ethyl acetate extract (55.47µmol trolox/g dry powder). Although the DPPH radical scavenging activities from the extracts of different solvents were less than those observed at Trolox as a standard reference compound, this study reveals that *G. ulmifolia* leaves have free radical scavengers or inhibitors possibly acting as a primary antioxidant.

Table III. Pearson correlation phenolic, flavonoids, tannin content and antioxidant activity.

Extract	Variable	Reducing Power	Cuprac	DPPH
<i>n</i> -Hexane	Tannins	0.552	-0.907	-0.927
	Flavonoids	0.986	-0.314	-0.908
	Phenol	-0.978	0.350	0.924
Ethyl Acetat	Tannins	0.306	-0.858	0.914
	Flavonoids	-0.977	-0.015	-0.107
	Phenol	-0.746	-0.818	0.741
Ethanol	Tannins	-0.434	-0.987	0.009
	Flavonoids	-0.979	-0.395	-0.968
	Phenol	0.673	0.992	0.276
Water	Tannins	-0.411	-0.392	-0.925
	Flavonoids	-0.007	0.014	-0.692
	Phenol	0.360	0.341	0.903

Another simple and versatile method to measure the antioxidant capacity from the extract of *G. ulmifolia* leaves is the CUPRAC method using a cupric neocuproine (2,9-dimethyl-1,10-phenanthroline) chelate-abbreviated as (Cu(II)-Nc) as the chromogenic oxidant. This method is based on the redox reaction with antioxidant compounds producing the cuprous-neocuproine chelate-abbreviated as (Cu(I)-Nc)- showing maximum light absorption at 450nm (Apak *et al.*, 2004). The reaction of the chromogenic reagent with *n*-electron reductant (AO) from antioxidant compounds producing a stable orange yellow color within 30min following in the manner (Apak *et al.*, 2008).

Antioxidant compounds that are expected to be found in *G. ulmifolia* leaves, namely phenolics, flavonoids, and tannins were used in standard solutions and assayed using the normal (at room temperature) and incubated against trolox as the standard reference compound. In this study, the antioxidant capacity using the CUPRAC method from the extract of *G. ulmifolia* leaves range from 21.86µmol trolox/g dry powder for *n*-hexane extract to 98.17µmol trolox/g dry powder for ethyl acetate extract. The antioxidant capacity of the extracts assessed by the CUPRAC method decrease in the following order: ethyl acetate > water > ethanol > *n*-hexane. The establishment of the antioxidant capacity of *G. ulmifolia* leaves will promote research on the identification and quantitation of active compounds of this plant, which may help protect consumers against oxidative stress-related diseases.

The reduction power from the extract of *G. ulmifolia* leaves, which potentially serves as a significant antioxidant activity, was calculated

using Fe<sup>3+</sup> to Fe<sup>2+</sup> reduction assay, whereby the initial color was yellow changes to various shades of green and blue depending on the reducing power of the samples. In this assay, the presence of antioxidant compounds leads to Fe<sup>3+</sup>/ferricyanide complex reduced to the Fe<sup>2+</sup> form, and Fe<sup>2+</sup> can be monitored through the measurement of the formation of Perl's Prussian blue at 700nm (Qingming *et al.*, 2010). Antioxidant capacity from the extract of *G. ulmifolia* leaves using reducing power method varied from 50.35µmol trolox/g dry powder for ethyl acetate extract to 176.75µmol trolox/g dry powder for water extract. It can be concluded that the water extract could act as electron donors and convert free radicals to more stable products (Table II). Some of the phenolic compounds, such as flavonoids and phenolic acids in the water extracts exhibited strong antioxidant activity through their reductive capacity in Fe<sup>3+</sup>-Fe<sup>2+</sup> system.

#### Correlation between phytochemical content and antioxidant activity

The correlation between phytochemical levels on the antioxidant activity of four *G. ulmifolia* leaves extracts was carried out using the Pearson correlation. The parameter used was the correlation coefficient (R) between the levels of phytochemical compounds (total phenolics, flavonoids, and tannins) in each extract with antioxidant activity. The higher the R-value, the higher the correlation between variables. Linear regression with the X-axis (levels of phytochemical compounds) and the Y-axis (antioxidant capacity) was used to see the correlation of phytochemical compounds to antioxidant activity in four *G. ulmifolia* extracts (Table III).

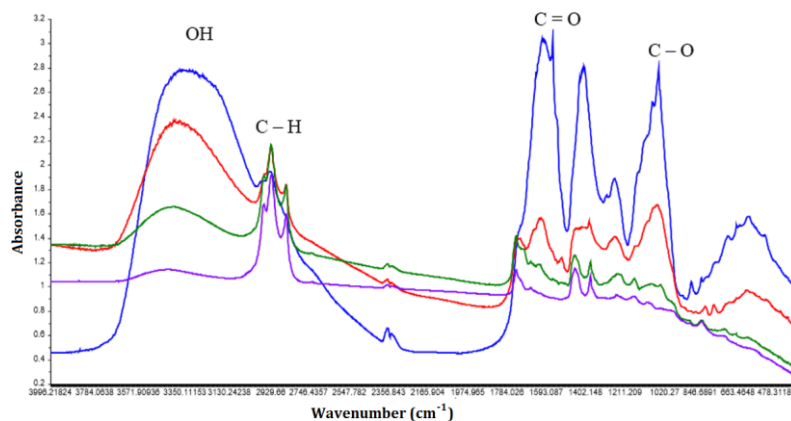


Figure 1. Representative of FTIR spectrum derived from extract of *G. ulmifolia* leaves (– water, – ethanol, – ethyl acetate, – *n*-hexane)

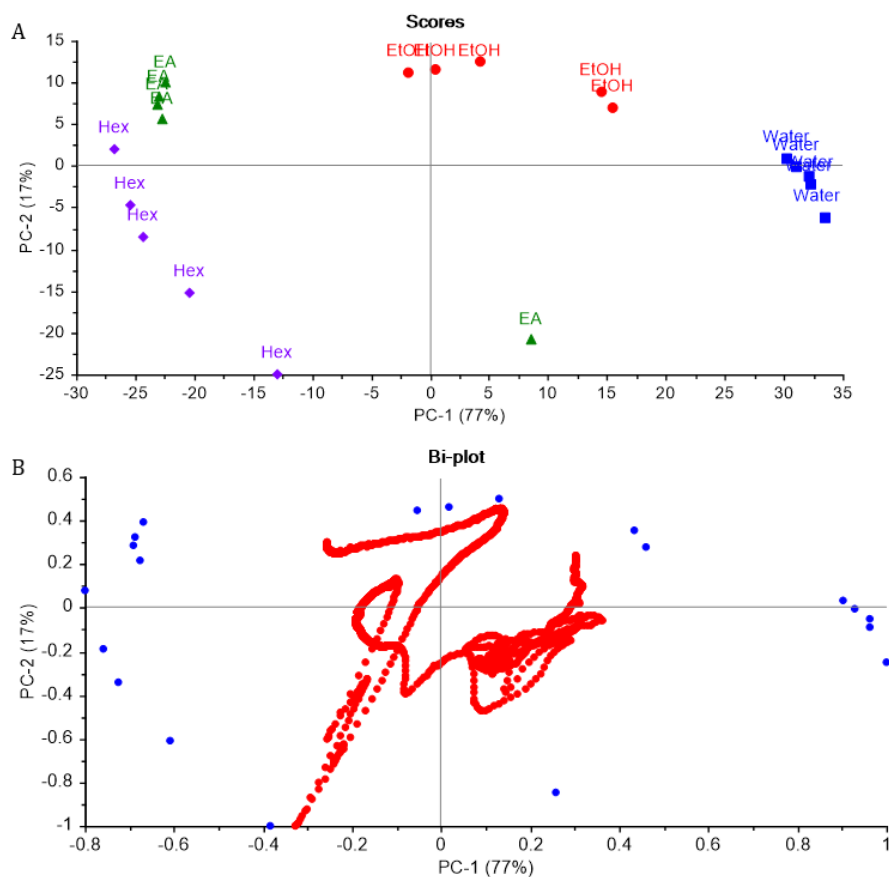


Figure 2. The score plot (A) and loading plot (B) of PCA derived from extract of *G. ulmifolia* leaves using variable mid-IR area (4000-400  $\text{cm}^{-1}$ ) (● = ethanol, ■ = water, ◆ = *n*-hexane, ▲ = ethyl acetate)

Based on the Pearson correlation, a positive correlation was shown by the levels of total flavonoids and phenolics of *n*-hexane extract on the reducing power and DPPH methods' antioxidant capacity. The antioxidant capacity of ethyl acetate extract was influenced by total tannins and phenolics levels with a positive correlation of 0.914 and 0.741, respectively, using the DPPH method. Whereas in ethanol and water extracts, total phenolics content had a positive correlation with antioxidant capacity with the three methods used with the highest values in the CUPRAC and DPPH methods. These results explain that the phenolics group compounds significantly affect the antioxidant activity in ethanol and water extracts compared to other extracts. The role of non-phenolic secondary metabolites can also influence the determination of the antioxidant activity of the teak leaves because the three methods of antioxidant activity that are carried out are not specific to the phenolic group compounds. Also, individual phenolic compounds may have different antioxidant activities, and their interactions with macromolecules such as carbohydrates and proteins may be synergistic or antagonistic (Odabasoglu *et al.*, 2005).

#### Classification of *G. ulmifolia* Leaves Extracts by FTIR Spectra and Chemometrics Analysis

Classification of *G. ulmifolia* leaves extracts with different solvent extraction was done using FTIR spectra combined with chemometrics. Comparison of spectra of different extracts using *n*-hexane, ethyl acetate, ethanol, and water solvents of *G. ulmifolia* leaves (Figure 1). Differences of spectral are observed between different extracts of *G. ulmifolia* leaves samples at 3500-3000, 3000-2800, 1750-1500, and 1200-1000 $\text{cm}^{-1}$  (Figure 1). Peak absorbance at 3500-3000 $\text{cm}^{-1}$  corresponds to hydroxyl absorption, 3000-2800 $\text{cm}^{-1}$  related to methylene C-H asymmetric stretching vibration, 1750-1500 $\text{cm}^{-1}$  is due to the stretching vibration of carbonyl group, and peaks around 1000  $\text{m}^{-1}$  are the vibration peak of C-O an alcohol group.

The principal component analysis (PCA) of the FTIR spectral data with 1866 variables is displayed in a two-dimensional plot using the first two principal components (Figure 2A), which together accounted for PC1 (77%) and PC2 (17%) of the 94% total variation. The PCA score plot indicated that PCA had a relatively high distinguishing capacity between *G. ulmifolia* leaves with four different solvents for the extraction process as a dependent manner. The sample of *G.*

*ulmifolia* leaves can be classified using the loading values for the essential spectral variables (i.e., those with the highest variation on PC1 and PC2 scores). The loading plot shows the *n*-hexane extract of *G. ulmifolia* leaves differentiated from other extracts in the wavenumber at 2900-2950  $\text{cm}^{-1}$ . Ethyl acetate, ethanol, and water extract were differentiated at 2800-2870  $\text{cm}^{-1}$ , 3000-3300  $\text{cm}^{-1}$ , and 1000- 1050  $\text{cm}^{-1}$  (IR fingerprint area). These results indicate that the qualitative and quantitative changes of phytochemical compounds in *G. ulmifolia* leaves corresponding to these spectral regions in FTIR spectra were significant variations related to the choice of solvent extraction.

#### CONCLUSIONS

In this study, the ethanol extract of *G. ulmifolia* leaves have the highest total phenolics and flavonoids content. In contrast, ethyl acetate extract has a higher level of total tannins compared to other extracts used in this study. Moreover, we showed that the highest antioxidant capacity of *G. ulmifolia* leaves found in the ethyl acetate extract assessed by the DPPH and CUPRAC methods. Classification of *G. ulmifolia* leaves extracts using FTIR spectra and PCA showed good clustering with 94% of total variance data between 4 different solvents (*n*-hexane, ethyl acetate, ethanol, and water) for the extraction process. It can be concluded that each extract of *G. ulmifolia* leaves gives a distinct phytochemical profile (total phenolics, total flavonoids, total tannins, and FTIR spectrum) that contributes to the antioxidant activity. This antioxidant activity is mainly influenced by the concentration of phenolics compounds known to have antioxidant activity. Therefore, we suggest that the screening of phytochemical compounds related to the assessment of its bioactivity and combined with chemometric analysis derived from FTIR spectra from the sample extract could give a more comprehensive knowledge of medicinal plant study.

#### ACKNOWLEDGMENT

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