

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Effect of Extraction Method and Solvent Type on Total Phenolics Content, Total Flavonoid and Antioxidant Activity of Pegagan Extract (*Centella asiatica* (Linn.) Urban)

Ahyar Riza^{1*}, Syafruddin Ilyas², Endang Sjamsudin³, Delyuzar Delyuzar⁴

¹Doctoral Program, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

³Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Padjajaran, Bandung, Indonesia

⁴Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

ARTICLE INFO

Keywords:

Antioxidant
Centella asiatica
Flavonoid
Phenolic
Solvent

*Corresponding author:

Ahyar Riza

E-mail address:

ahyar.riza@usu.ac.id

All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/bsm.v8i1.902>

ABSTRACT

Background: *Centella asiatica* (CA) or pegagan or horse foot leaves is one of the traditional plants widely used to treat various diseases. This study aims to evaluate the content of total phenolics and total flavonoids, as well as the antioxidant activity of *Centella asiatica* extracts from different methods and solvents. **Methods:** The sample was divided into three groups, (70% ethanol maceration, methanol maceration and water infundation). Total phenolic compounds in the extract were determined by the Follin-Ciocalteu method, total flavonoid content was determined by the aluminum chloride colorimetric assay method, and antioxidant activity was assessed by the immersion DPPH free radicals method. Determination data of IC₅₀ was assessed by regression test and differences between the three extract groups were tested by ANOVA or Kruskal Wallis test. **Results:** The highest total phenolics and total flavonoid were obtained in *Centella asiatica* extract with water solvent, 251.88 ± 0.96 mg/g GAE and 7.26 ± 0.03 mg/g QE, followed by 70% ethanol and methanol. In addition, the best antioxidant activity was also shown in the extract with water, namely 24.09 ± 0.01. The difference test results between all groups showed significant results (p<0.05) in all parameters. **Conclusion:** The best content of total phenolic, total flavonoid, and antioxidant activity was obtained by infundation with water solvent. In the maceration method, the best content of the three parameters measured was obtained in the group with ethanol solvent.

1. Introduction

Indonesia is a country rich in various types of plants that can be used as medicines for various diseases. Indonesia, China, Arabia, Japan, and India are countries that have known medicinal plants for a long time. *Centella asiatica* (CA) or pegagan or horse foot leaves is one of the traditional plants widely used to treat various diseases.^{1,2} *Centella asiatica* has been used by the community for wound healing, treatment of leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, gastric ulcers, fever, amenorrhea, urinary

tract diseases, female genital and also to relieve anxiety and improve cognition in neurodegenerative disorders.^{3,4} There are seven main groups of compounds contained in *Centella asiatica*: saponins, pentacyclic triterpenoids, sterols, sesquiterpenes, eugenol derivatives, caffeoylquinic acid, and flavonoids.⁵ One of the benefits of this plant is that it is a natural antioxidant. The antioxidant function of *Centella asiatica* is very important in preventing free radicals in the body. The presence of excessive free radicals in the body will cause an imbalance, resulting

in oxidative stress.⁶ Oxidative stress is one of the important factors in the development of chronic and degenerative diseases such as atherosclerosis, diabetes, initiating cancer, and also the aging process.⁷

The content of secondary metabolites present in herbs and their activity can be influenced by many factors, both factors originating from the plant itself and in the extraction process.⁷ In the extraction process, many factors affect the secondary metabolite content and activity of a herb,⁸ including the location of the growing place, soil quality, altitude of the growing area, and the selection of the type of solvent.⁹ Solvent selection is very important in solvent extraction procedures. Things that must be considered in the selection of solvents are selectivity, solubility, cost, and safety.^{8,10} Therefore, by knowing the effect of extraction methods and types of solvents, it is hoped that the content of secondary metabolites produced will be more effective and maximized. This study aimed to evaluate the content of secondary metabolites, specifically total phenolics and total flavonoids, as well as the antioxidant activity of *Centella asiatica* extracts from different methods and solvents.

2. Methods

This study planned to examine the effect of solvent type and extraction method on the content of total phenolics, total flavonoids, and antioxidant activity in *Centella asiatica*.¹¹ The weight of *Centella asiatica* used in this study was 10 kilograms. All samples were dried with direct sunlight and a dry cabinet and finally crushed. Extraction of *Centella asiatica* (Linn.) Urban by maceration was done by immersion in a closed vessel for 6 hours at room temperature and let stand for 18 hours while stirring occasionally. The amount of solvent used should be calculated based on the weight of the powder used.

Each extract was then filtered twice to remove the filtrate and the waste. The filtrate was concentrated with a rotary evaporator until a thick extract was obtained. Then, the extract obtained was further evaluated for its content. Storage was carried out at

room temperature and closed glass containers.¹¹

Determination of total phenolic content by the Folin-Ciocalteu method

Preparation of gallic acid solution as standard

Weighed 50 mg of gallic acid and then dissolved with methanol up to 100 mL (concentration of 500 ppm solution) and piped 2.5 mL of each concentration of 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, then dissolved with methanol up to 5 mL. From each concentration, 0.1 mL of solution was pipetted, then 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu, vortexed for approximately 1 minute, and added 1.5 mL of 20% sodium carbonate (Na_2CO_3), then incubated for 90 minutes. There was a color change in the tube, which is blue because phenol undergoes a complex redox reaction with phosphomolybdic acid in the Folin-Cocteau reagent in the alkaline medium, which results in a complex blue color, molybdenum blue. Measured the gallic acid standard solution's absorbance at each concentration and the maximum wavelength at a solution concentration of 500 ppm against the reagent used as a blank by UV-Vis spectrophotometry (400-800 nm). The resulting calibration at 775 nm using the concentration of gallic acid.^{7,10,11}

Preparation of *Centella asiatica* extract test solution

Centella asiatica extract was weighed as much as 50 mg and then dissolved with methanol up to 50 mL (1000 ppm concentration). Taken 0.1 mL of extract solution, added 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu, vortexed for approximately 1 minute, then added 1.5 mL of 20% sodium carbonate (Na_2CO_3), then incubated for 90 minutes. There was a color change in the tube, which is blue because phenol undergoes a complex redox reaction with phosphomolybdic acid in the Folin-Ciocalteu reagent in the alkaline medium, which results in a blue complex, molybdenum blue. The absorbance of the test solution was measured against the gallic acid calibration at 775 nm by UV-Vis spectrophotometry

(400-800 nm). The concentration of phenol in the test solution was calculated from the calibration plot and expressed as mg gallic acid equivalent to mg/g of sample.^{6,7,11,12}

Determination of total flavonoid content by Colorimetric method

Preparation of quercetin solution as standard

Weighed 50 mg of quercetin and then dissolved with methanol up to 50 mL (concentration of 100 ppm solution). Then, 5 mL was pipetted, put into a 50 mL flask, and sufficed with methanol until the marked line (concentration of 100 ppm) was reached. Pipetted 2.5 mL of each concentration of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, then dissolved with methanol up to 5 mL. From each concentration, 2 mL of solution was pipetted, then added 0.1 mL of aluminum chloride (AlCl₃) 10%, 0.1 mL of sodium acetate (CH₃COONa), and 2.8 mL of distilled water, then incubated for 40 minutes. The absorbance of the quercetin standard solution was measured at each concentration, and the maximum wavelength at a solution concentration of 100 ppm against the reagent used as a blank by UV-Vis spectrophotometry (400-800 nm). The resulting calibration at 432 nm using concentration against quercetin.^{6,7}

Preparation of *Centella asiatica* extract test solution

The aluminum chloride method was used to determine the total flavonoid content of *Centella asiatica* extract. *Centella asiatica* extract was weighed as much as 50 mg dissolved with methanol up to 50 mL (1000 ppm solution concentration). Pipetted 2 mL of extract solution and added methanol up to 10 mL (concentration of 300 ppm solution). Take 2 mL of the solution, then add 0.1 mL of aluminum chloride (AlCl₃) 10%, 0.1 mL of sodium acetate (CH₃COONa), and 2.8 mL of distilled water. It was incubated for 40 minutes. The absorbance of the extract solution was measured against the quercetin calibration standard. The calibration standard of the quercetin plot was generated at a wavelength of 432 nm. The flavonoid

concentration in the test sample was calculated from the calibration plot and expressed as a quercetin equivalent mg/g sample.^{6,7}

Antioxidant activity testing

Free radical scavenging activity by the DPPH method

The ability of the test sample to reduce the oxidation process of DPPH (1,1-diphenyl-2-picryl-hydroxyl) as a free radical in methanol solution (thus reducing the purple color of DPPH) with an IC₅₀ value (the concentration of the test sample that can reduce free radicals by 50%) is used as a parameter to determine the antioxidant activity of the test sample.^{6,7,10,11}

0.5 mM DPPH solution

A total of 20 mg of DPPH was weighed, then put into a 100 mL volumetric flask, dissolved in methanol, diluted with methanol to the marked line, and obtained 0.5 mM DPPH solution (200 ppm concentration).¹¹

Determination of maximum absorption wavelength

A DPPH solution of 40 ppm concentration was homogenized, and its absorption was measured at a wavelength of 400-800 nm with a UV-visible spectrophotometer. Then in each flask, 5 mL of 0.5 mM DPPH solution (200 ppm concentration) was added. Then, the volume was filled with methanol until the marked line was allowed to stand for 60 minutes in a dark place and then measured the absorption on a Visible spectrophotometer at a wavelength of 516 nm.^{6,7,11,12}

Determination of percent attenuation

Antioxidant ability is measured as a decrease in the absorbance of DPPH solution (DPPH purple color attenuation) due to the addition of the test solution. The absorbance value of the DPPH solution before and after adding the test solution was calculated as percent attenuation.¹¹

$$\% \text{ Absorbance} = \frac{\Delta_{\text{sample}} - \Delta_{\text{control}}}{\Delta_{\text{control}}} \times 100\%$$

Description:

Δ_{Control} = Absorbance does not contain samples

Δ_{Sample} = Absorbance of the sample

IC₅₀ value analysis

The IC₅₀ value is a number that indicates the concentration of the test sample (µg/mL), which gives 50% DPPH silencing (able to inhibit/reduce the oxidation process by 50%). 0% value means no antioxidant activity, while a 100% value means total silencing. Testing needs to be continued with a dilution of the test solution to see the concentration limit of its activity. The results of the calculation are entered into a regression equation with the extract concentration (µg/mL) as the abscissa (X-axis) and the % silencing (antioxidant) value as the ordinate (Y-axis). Specifically, a compound is said to be a powerful antioxidant if the IC₅₀ value is less than 50 ppm, strong for IC₅₀ worth 50-100 ppm, moderate if IC₅₀ is worth 100-150 ppm, and weak IC₅₀ is worth more than 150 ppm.^{11,12}

Statistical analysis

All measurements were repeated three times, and the results were expressed as mean values and standard deviations. Data were tested for normality using the Shapiro-Wilk test, the determination of IC₅₀ values was done by regression test, and differences between the three extract groups were tested by ANOVA or Kruskal Wallis test (IBM SPSS Ver. 25).

3. Results

This study (Table 1) obtained the average results of total phenolics, total flavonoids, and antioxidant activity in the three groups of *Centella asiatica* extracts with different solvents. In the content of total phenolics, the highest content was obtained in *Centella asiatica* extract with water solvent, which amounted to 251.88 ± 0.96 mg/g GAE, followed by 70% ethanol and methanol solvents respectively 172.43 ± 0.98 mg/g GAE and 95.77 ± 0.95 mg/g GAE.

Then, not much different from the total flavonoid content, the highest content was also obtained in *Centella asiatica* extract with water solvent of 7.26 ± 0.03 mg/g QE followed by 70% ethanol and methanol solvents respectively of 4.93 ± 0.01 mg/g QE and 2.35 ± 0.01 mg/g QE. In addition, the best antioxidant activity in order expressed by the IC₅₀ value is shown in the extract with water, 70% ethanol, and methanol solvents, namely 24.09 ± 0.01, 54.22 ± 0.27, and 113.91 ± 0.01. The difference test results between all groups showed significant results (p<0.05) in all parameters assessed, namely total phenolics, total flavonoids, and antioxidant activity.

The results of the post hoc test in Table 2 between two solvent groups, namely 70% ethanol-methanol, 70% ethanol-water, and methanol-water, showed that there were significant differences in all pairs of solvent groups both in total phenolics, total flavonoids, and even in the antioxidant activity obtained.

Figure 1 shows the graph of the association between antioxidant activity and the total phenolics and total flavonoid content of the three combined groups of extracts with 70% ethanol, methanol, and water solvents. The graph shows the coefficient of determination (R²) of the total phenolic content of the three extracts is 0.961. In addition, the total flavonoid content of the three extracts is 0.975.

4. Discussion

This study assesses total phenolate content using the Folin-Ciocalteu method, which is a specific and sensitive method with phenol compounds. This reagent will form a mixture of dark blue complex solutions when reacted with a solution containing phenolic compounds, and sodium carbonate solution is added. Furthermore, this colored solution will be assessed for absorbance.^{10,11} The results obtained for the total phenolate content were mostly obtained in extracts with water solvents compared to extracts with 70% ethanol and methanol solvents. The results of previous research conducted by Rahmawati A. et al. (2022) showed different results, namely for the highest total phenolate content in *Centella asiatica* extract

with 70% ethanol solvent compared to distilled water 52.128 mg GAE/g sample and 45.654 mg GAE/gr sample.¹⁴ In addition, research conducted by Widayani et al. (2019) also reported the highest total phenolic content in *Centella asiatica* ethanol extract compared to *Centella asiatica* infusion.¹² Phenolic compounds are present in every vegetable, fruit, herb, tea, and juice and are an integral part of the human diet. Several researchers have reported polyphenol content as the most important antioxidant compound in tea extracts.¹³

In addition, the results of the highest total content were shown in the water solvent group compared to the 70% ethanol and methanol solvent groups. Research conducted by Rahman et al. regarding the antioxidant activity of *Centella asiatica* extracts from various solvent polarities obtained the results of flavonoid content in water solvents lower than 50% ethanol solvents and higher than 100% ethanol solvents, which amounted to $35.6 \pm 0.5 \mu\text{g} / \text{mg}$ sample, this study believes that the higher the concentration of ethanol solvents will reduce the content of *Centella asiatica* extract (Rahman M et al. 2013). Research conducted by Khairunnisa S et al. (2022), who used the same method, namely maceration with 70% ethanol solvent, showed flavonoid content levels that were not much different from this study, namely 4.339 mg QE/g [15]. Flavonoids are one of the many ingredients in herbal plants and are one of the phenol compounds that have an effect as antioxidants.⁷ Flavonoid compounds are divided into several types. Each type has a different polarity based on the number and position of hydroxyl groups of each type of flavonoid so it will affect the solubility of flavonoids in solvents.¹⁶

The antioxidant activity of the sample solution was determined using the DPPH method. This method was chosen because it is the most simple, easy, fast, and most sensitive method and only requires a small sample so that it can be widely used to test the ability of compounds that act as electron donors. DPPH is a free radical that is stable at room temperature by giving a blackish violet powder and is quickly oxidized

by sunlight and air and is easily soluble in ethanol. Compounds with antioxidant activity will react with DPPH free radicals through a hydrogen atom donation mechanism that causes a change in DPPH color from purple to yellow. This color change will be assessed for absorbance. Antioxidant activity is expressed by the IC₅₀ value, the concentration of antioxidant compounds that can inhibit 50% of free radicals. Therefore, the smaller the IC₅₀ value, the better the *Centella asiatica* solution.^{10,11} The results of antioxidant activity assessment in this study also obtained the best activity in water solvents with the hot water infused method, compared with 70% ethanol and methanol solvents.

The antioxidant activity obtained in this study, especially in extracts with water solvent and 70% ethanol, can be said to be good or very active. This follows the opinion of Blois. Purgiyanti et al. state that antioxidant activity is very active if it has an IC₅₀ value < 50 $\mu\text{g/ml}$.¹¹ The existence of antioxidant activity in pegagan extract can be based on the content of its secondary metabolites, namely total phenolics and total flavonoids, as seen in the graph of the relationship between total phenolics and total flavonoids to antioxidant activity. In the study, a very high R² value was obtained. This value can be interpreted as the total phenolics and total flavonoid content of the three extracts had an effect of 96.1% and 97.5% on their antioxidant activity, and other factors influenced the rest. The mechanism of phenolics that act as antioxidants can be done through several mechanisms, as described by Urbaniak et al. cit. Hasbullah that the mechanism of action of phenol compounds begins with the dissociation of phenol antioxidant compounds into anion and proton forms. Then the formed ions react with free radicals to form radical compounds from phenol antioxidant compounds and neutral molecules. Phenolic compounds can carry out three mechanisms in free radical inactivation, namely by hydrogen atom transfer (HAT), sequential proton loss and electron transfer (SPLET), and single electron transfer followed by proton transfer (SET-PT).¹⁷ Furthermore, the total

flavonoid content is also explained by Kumar and Pandey (2013) in their review, who explain that the antioxidant activity of flavonoids can occur by the mechanism of free radical capture and the ability to bind metal ions. This can be influenced by configuration, substitution, and the total number of hydroxyl groups. The configuration of the hydroxyl B ring most determines the ability to capture reactive oxygen species (ROS) and reactive nitrogen species (RNS). This is related to its role in contributing hydrogen atoms and electrons to hydroxyl, peroxy, and peroxy nitrite radicals so that they will stabilize until a relatively stable flavonoid radical is formed. Furthermore, the mechanism of action of flavonoid antioxidants consists of three parts: suppressing ROS formation through enzyme inhibition or chelating metal elements involved in free radical formation. Second, ROS capture, and third, improving the performance or protection of the antioxidants that play a role in body protection.¹⁸

The difference in both secondary metabolite content and antioxidant activity of *Centella asiatica* extracts in this study, both in water, 70% ethanol, and methanol solvents, can be said that water solvent is the best solvent and suitable for extracting *Centella asiatica* in this study compared to alcohol solvents, namely 70% ethanol and methanol. The high content obtained can be based on differences in extraction methods, solvent polarity, and *Centella asiatica*.^{8,9} In this study, the water solvent used the hot water-infused extraction or infusion method. This method is similar to the maceration method. Notably, the medicinal material is ground right into a fine powder and then placed in a clean container. The hot or cold extraction solvent is poured over the medicinal material, soaked, and kept for a short time. This approach is appropriate for the extraction of soluble bioactive components. In addition, it is the best method for preparing fresh extracts before use. The solvent-to-sample ratio is typically four:1 or 16:1, depending on the supposed use. As for alcohol solvents, the maceration extraction method is used, an extraction procedure wherein the coarse floor

medicinal material, either leaves or stem bark or root bark, is located in a field; the solvent is poured over it till it wholly covers the medicinal material. The field is then included and kept for at least three days. The contents are stirred periodically and, if positioned in a bottle, need to be shaken sometimes to ensure the whole extraction is. At the end of the extraction, the micelles are separated from the marc by filtration or decantation. Moreover, the micelles are separated from the solvent by evaporation in an oven or over a water bath. This technique is convenient and specifically appropriate for thermolabile plant substances.⁸

Although 70% ethanol solvent did not show the highest results when compared with water solvent, based on the maceration method, the highest and best content was obtained in 70% ethanol solvent.¹⁹ This is in accordance with the results of research by Artanti et al., who reported that the best total phenolate and total flavonoid content was an extract solution using 70% ethanol solvent.⁷ This is due to the selection of the type of solvent related to the polarity of the solvent. In this study, the polarity level from the most polar is water > methanol > 70% ethanol. A compound is said to dissolve in a solvent with the same polarity. In other words, the content of secondary metabolites dissolved depends on the match of the polarity of the type of secondary metabolite and the solvent used.⁶⁻⁸ Total phenolics and total flavonoids in *Centella asiatica* extracts with high water solvents indicate that water solvents have a level of polarity that resembles and is more effective in dissolving *Centella asiatica* phenolic and flavonoid compounds. Water as a solvent can extract polar compounds from a wider range of secondary metabolites because water is the most polar solvent compared to 70% ethanol and methanol solvents, which are relatively cheap, nontoxic, nonflammable, and highly polar. However, even so, this solvent has disadvantages. Namely, it promotes bacterial and mold growth, it may cause hydrolysis, and a large amount of heat is required to concentrate the extract. Therefore, although the water solvent shows the content of total phenolics and total flavonoids and good antioxidant activity, with these

disadvantages, it can have a doubtful antibacterial function. In addition, the study by Peloan et al. reported that herbal infusions with water solvents were easily contaminated by fungi or microbes so that when reacted with the folin-ciocalteu reagent, it could form the same colour as the flavonoids and would cause false positives. In addition, the presence of these properties will also affect the storage time of the extract.²⁰ While alcohol solvents, such as 70% ethanol and methanol, have the advantage, it is also polar, miscible with water, and could extract polar secondary metabolites. Advantages. It is self-preservative at a concentration above 20%. It is nontoxic at low concentrations, and a small amount of heat is required to concentrate the extract. Disadvantages. It does not dissolve fats, gums, and waxes; it is flammable and

volatile.⁸

In addition to the two reasons above, secondary metabolite content and antioxidant activity differences can also be caused by factors originating from *Centella asiatica* itself, such as factors of growing location, type of variety, soil quality, and altitude of the growing area. This factor has been observed by Artanti et al., who reported that the place of growth or type of *Centella asiatica* variety influences the level of secondary metabolite content. Besides that, research conducted by Afrina et al. also reported that the altitude of the growing area of pegagan has an influence on the content of asiaticoside which is a secondary metabolite of *Centella asiatica*.^{7,9}

Table 1. Differences in secondary metabolite content and antioxidant activity in all groups.

Solvent type	Extraction method	Total phenolic (mg/g GAE)	p-value	Total flavonoid (mg/g QE)	p-value	Antioxidant activity (IC ₅₀)	p-value
70% Ethanol	Maceration	172,43 ± 0,98	0,025*	4,93 ± 0,01	0,026*	54,22 ± 0,27	0,027*
Methanol	Maceration	95,77 ± 0,95		2,35 ± 0,01		113,91 ± 0,01	
Water	Infundation	251,88 ± 0,96		7,26 ± 0,03		24,09 ± 0,01	

*Kruskal-Wallis test, significance at p<0,05.

Table 2. Post hoc test of differences in each pair of groups on each parameter tested.

Solvent type comparison		Total phenolic (mg/g GAE)	Total flavonoid (mg/g QE)	Antioxidant activity (IC ₅₀)
70% Ethanol	Methanol	0,043*	0,043*	0,05*
	Water	0,043*	0,046*	0,05*
Methanol	Water	0,043*	0,046*	0,001 ^a

*Mann-Whitney test, significance at p<0,05, ^aIndependent t-test, significance at p<0,05.

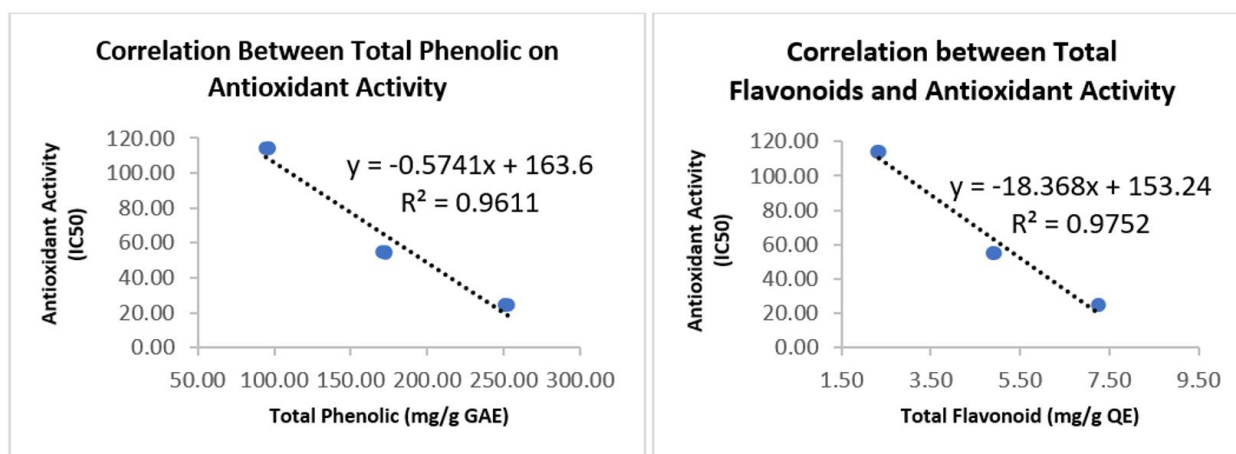


Figure 1. Association of total phenolics and total flavonoids with antioxidant activity of the three solvents.

5. Conclusion

Based on the results obtained, it can be concluded that the highest total phenolic and total flavonoid content is found in extracts with water solvents with hot water infused method of 251.88 ± 0.96 mg/g GAE and 7.26 ± 0.03 mg/g QE followed by 70% ethanol and methanol solvents with maceration method, statistical test results show there are significant differences in the three groups. Then, the best antioxidant activity picture was also shown by the extract with water solvent with IC_{50} value of 24.09 ± 0.01 followed by 70% ethanol and methanol solvents with maceration method. The statistical test results showed significant differences in the three groups, and there was an influence of total phenolic content and total flavonoids on the IC_{50} value, which showed antioxidant activity. Thus, *Centella asiatica* from Wondis chocolate plantation, RT 031/RW 015, Salakmalang, Banjarharjo, Kalibawang, Kulon Progo, Yogyakarta is more suitable to be extracted with water solvent and hot water infused or infundation method.

6. References

1. Nicolas A, Plunkett G. The demise of subfamily Hydrocotyloideae (*Apiaceae*) and the re-alignment of its genera across the entire order Apiales. *Molecular Phylogenetics and Evolution*. 2009; 53(1): 134-51.
2. Gray N, Magana A, Lak P, Wright K, Quinn J, Stevens J, et al. *Centella asiatica* – Phytochemistry and mechanisms of neuroprotection and cognitive enhancement. *Phytochemistry Reviews*. 2018; 17(1): 161-94.
3. Gohil K, Patel J, Gajjar A. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences*. 2010; 72(5): 546–56.
4. Bylka W, Znajdek-Awiżeń P, Studzińska-Sroka E, Brzezińska M. *Centella asiatica* in cosmetology. *Postepy Dermatology Alergologii Journal*. 2013; XXX: 46-9.
5. James J, Dubery I. Identification and quantification of triterpenoid centelloids in *Centella asiatica* (L.) urban by densitometric TLC. *JPC-Journal of Planar Chromatography-Modern TLC*. 2011; 24(1): 82–7.
6. Rahman M, Hossain S, Rahman A, Fatima N, Nahar T, Uddin B, et al. Antioxidant activity of *Centella asiatica* (Linn.) Urban: impact of extraction solvent polarity. *Journal of Pharmacognosy and Phytochemistry*. 2013; 1(6): 27-32.
7. Artanti N, Dewi RT, Maryani F. The effect of location and extraction solvent on phytochemical content and antioxidant activity of pegagan extract (*Centella asiatica* L. Urb). *Jurnal Kimia Terapan Indonesia*. 2014; 16(2): 88-92.
8. Abubakar AR, Haque M. Preparation of medical plants: basic extraction and fractionation procedure for experimental purposes. *Journal of Pharmacy & Bioallied Sciences*. 2020; 12(1): 1-10.
9. Safrina D, Brotojoyo E, Kamila I. Effect of altitude and drying method on organoleptic and asiaticoside content of pegagan (*Centella asiatica* (L) Urb). *Jurnal Teknik Pertanian Lampung*. 2019; 8(3): 208-13.
10. Zulharmitta, Elrika D, Rivai H. Determination of the effect of the type of extraction solvent on the acquisition of phenolic compounds and antioxidant power of miniran herbs (*Phyllanthus niruri* L.). *Jurnal Farmasi Higea* 2010; 1(2): 37-45.
11. Purgiyanti, Purba AV, Winarno H. Determination of total phenol levels and antioxidant activity test of a combination of gotu kola herb extract (*Centella asiatica* L. Urban) and god's crown fruit (*Phaleria macrocarpa* (Scheff.) Boerl.). *Parapemikir Jurnal Ilmiah Farmasi*. 2019;8(2):40-5.
12. Widayani M, Ulfa M, Wirasisya DG. Free radical scavenging effect of infusion and ethanol extract pegagan herb (*Centella asiatica* L. Urb) using dpsh method. *Jurnal Pijar MIPA*. 2019; 14(1): 100-6.

13. Siah WM, Azman MA, Jeeven K, Hayazan MDN, Tahir SM. Effect of infusion condition on total phenolic content and antioxidant activity in *Centella asiatica* tea. *Journal of Tropical Agriculture and Food Science*. 2011; 39(2): 149-56.
14. Rahmawati A, Fachri BA, Safitri RA. Comparison of extraction solvents used for the extraction of total phenolic content from pegagan (*Centella asiatica* L) using microwave-assisted extraction. *7th Int Symposium on Applied Chemistry AIP Publishing*. 2022: 1-9.
15. Khairunnisa S, Hakim AR, Audina M. Comparison of total flavonoid levels based on differences in ethanol solvent concentration from pegagan leaf extract (*Centella asiatica* (L) Urban.). *Journal Pharmaceutical Care and Sciences*. 2022; 3(1): 121-31.
16. Verdiana M, Widarta IWR, Permana IDGM. Influence of solvent type on extraction using ultrasonic waves on antioxidant activity and lemon peel extract (*Citrus limon* (Linn.) Burm F.). *Jurnal Ilmu dan Teknologi Pangan*. 2018; 7(4): 213-22.
17. Hasbullah UHA, Pertiwi RB, Khikmah N, Novita D. Parijoto content, benefits and processing. *Pekalongan: PT. Nasya Expanding Management*. 2018: 21-4.
18. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*. 2013: 1-16.
19. Rattanakom S, Yasurin P. Chemical profiling of *Centella asiatica* under different extraction solvent and its antibacterial activity, antioxidant activity. *Oriental Journal of Chemistry*. 2015; 31(4): 2453-9.
20. Peloan T, Kaempe H. The effect of long storage of red gedi leaf extract on the total flavonoid content. *Pharmacy Medical Journal*. 2020; 3(2): 64-9.