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# Primary metabolites, phenolics content and antioxidant activities of *Hydrocotyle bonariensis* and *Centella asiatica*

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ABSTRACT

*Hydrocotyle bonariensis* or locally known as 'Pegaga Embun', is a creeping herb from the family Araliaceae. *H. bonariensis* is closely related to *Centella asiatica* (Apiaceae), which is commonly known as 'Pegaga Kampung' due to their morphological characteristics. In most studies on phytochemical properties, *C. asiatica* is more popular than *H. bonariensis*. Extensive studies have been conducted on the phytochemical and biological activities of *C. asiatica*; however, a minimal study has been conducted on *H. bonariensis*. Hence, this study was carried out to compare the primary metabolites, phenolics content, and antioxidant activities of the fresh and dry samples of *H. bonariensis* and *C. asiatica*. All the analyses were conducted via *in vitro* assay and were measured using a UV-Vis spectrophotometer. The highest total soluble protein and ascorbic acid content were produced by the fresh sample of *C. asiatica* with 23.28 mg protein $g^{-1}$ FW and 0.35 mg ascorbic acid $g^{-1}$ FW and *H. bonariensis* fresh sample produced 2.80 mg protein $g^{-1}$ FW and 0.34 mg ascorbic acid $g^{-1}$ FW, respectively. Meanwhile, the highest total reducing sugar and hydrolyzed sugar were recorded from the fresh sample of *H. bonariensis* with 1.94 mg reducing sugar $g^{-1}$ FW and 3.84 mg hydrolyzed sugar $g^{-1}$ FW, respectively. In the phenolics content analysis, the highest total phenolics and flavonoids were exhibited by fresh *H. bonariensis* with 0.96 mg GAE $g^{-1}$ and 13.79 mg CE $g^{-1}$ DW (dry weight), respectively. Furthermore, the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition was recorded from the fresh sample of *C. asiatica* with 91%, and the highest ferric reducing antioxidant power (FRAP) value was recorded from the fresh sample of *C. asiatica* with 66.75 mg TE $g^{-1}$ FW. In conclusion, *H. bonariensis* showed high potential in bioactive compound production and as a source of antioxidants. Hence, an extensive study on *H. bonariensis* should be conducted to increase the medicinal values of *H. bonariensis*.

**Keywords:** Primary metabolites, secondary metabolites, antioxidant, *Hydrocotyle bonariensis*, *Centella asiatica*

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## 1 Introduction

Malaysia rainforest is one of the 17 largest mega biodiversity centers in the world. It is one of the most evolved and diverse forests globally that comprises numerous plant species with various medicinal values (von Rintelen et al., 2017). This offers many opportunities for the Malaysian pharmaceutical industry to explore and produce plant-based medicines. One of the most popular medicinal plants is *Centella asiatica* (Apiaceae), better known as pegaga in Malaysia. This plant is not only popular among Malaysian, but it is also famous worldwide. *C. asiatica* based products

are widely available in the market (Maulidiani et al., 2011). Besides *C. asiatica*, *Hydrocotyle bonariensis* (Araliaceae) is another pennywort plant species that has almost the same morphological characteristic as *C. asiatica* (Sumazian et al., 2010). *H. bonariensis* is a hairless and creeping perennial herb that can be found in tropical and subtropical countries including Malaysia, Indonesia, Sri Lanka, and China (Knight and Miller, 2004). Among local community in Malaysia, *H. bonariensis* is commonly known as pegaga air, pegaga embun, and pegaga piring. According to Obaseki et al. (2016), the leaves of *H. bonariensis* contain several phytochemical classes, including alkaloids, tannins, flavonoids, phenolics, and saponins. Besides that, *H. bonariensis* extract has also been found to contain medicinal properties to treat inflammation, rheumatism, arthritis, memory improvement, and high antioxidant properties (Obaseki et al., 2016; Sumazian et al., 2010). Despite having many phytochemicals and medicinal properties in its extracts comparable to *C. asiatica*, research on *H. bonariensis* seems to be sidelined, and only a few studies have been conducted on this plant species. Hence, this study's objective was to compare the primary metabolites, secondary metabolites, and antioxidant activities of fresh and dry samples of *H. bonariensis* and *C. asiatica*.

# 2 Materials and Methods

## 2.1 Plant materials

The *H. bonariensis* plant was obtained from University Agriculture Park, Universiti Putra Malaysia. For *C. asiatica*, the plant was bought from the wet market located in Selangor, Malaysia. The samples were cleaned under running tap water. The samples were divided into two, which is the fresh and dry sample. The fresh sample was used immediately for analysis, and the dry sample was air-dried at room temperature (27 ºC) until the weight remained constant. Then, the dried samples were used for experimentation.

## 2.2 Primary metabolites analysis

### 2.2.1 Total soluble protein content

A procedure explained by Bradford (1976) was used in total protein analysis in this study. The extraction procedure was conducted in the dark at a temperature of 4 ºC. The sample of *H. bonariensis* and *C. asiatica* were homogenized using pre-cooled mortar and pestle and extraction buffer consisting of 1.0 mM of EDTA, 0.1 M of Tris-HCl and 0.1% of β-mercaptoethanol. The extracts were centrifuged at 4 ºC temperature for 20 minutes at 16000 rpm and the supernatant was used for analysis. Then, the Bradford reagent was prepared using Coomassie Brilliant Blue G250 (0.025% $w/v$) which was dissolved in phosphoric acid (42.5% $v/v$) and ethanol (25% $v/v$). A total of 0.1 mL sample extracts were pipetted into test tubes, followed by 5 mL of Bradford reagent. The reaction mixture absorbance was measured at 595 nm. A standard curve of absorbance against different concentrations of bovine serum albumin (BSA) was constructed. The total soluble protein content was expressed as mg protein per gram sample (mg protein $g^{-1}$ sample).

### 2.2.2 Total reducing sugar content

A total of 0.5 g of *H. bonariensis* and *C. asiatica* samples were cut into small pieces and placed into the flasks. Then, 10 mL of distilled water was added and the reaction mixtures were incubated at a temperature of 90 ºC for 15 minutes. After the incubation, the extracts were filtered using Whatman filter paper No. 1 and the supernatant was used for analysis. The total reducing sugar content of *H. bonariensis* and *C. asiatica* samples were analyzed using the Somogyi-Nelson method (Somogyi, 1952; Nelson, 1944). Briefly, 0.1 mL of sample extract and 1 mL of Somogyi-Nelson reagent was pipette into the test tube. The reaction mixture was boiled for 10 minutes in a water bath. The reaction mixture was cooled to room temperature and 1 mL of arsenomolybdate reagent and 6 mL of distilled water were added. The absorbance of the reaction mixture was measured at 520 nm. A standard curve of absorbance against different glucose concentrations was constructed, and the total reducing sugar content was expressed as mg reducing sugar per gram of sample (mg reducing sugar $g^{-1}$ sample).

### 2.2.3 Total hydrolyzed sugar content

A total of 0.5 g of *H. bonariensis* and *C. asiatica* samples were ground with pre-cooled mortar and pestle and homogenized with an extraction buffer (3 ML) was added. The reaction mixture was centrifuged for 30 minutes at 16000 rpm. The supernatant was collected and used for total hydrolyzed sugar content analysis. An Anthrone method by Jermyn (1975) was used for total hydrolyzed sugar content analysis. A total of 0.1 mL sample extract and 0.5 mL of Anthrone reagent was pipette into the test tube. The reaction mixture was incubated for 17 minutes at a temperature of 90 ºC. The reaction mixture was cooled to room temperature and absorbance was measured at 625 nm. The Anthrone reagent was prepared according to the method explained by Handel (1985). A standard curve of absorbance against different concentrations of glucose was constructed. The total hydrolyzed sugar content was expressed as mg hydrolyzed sugar per gram of sample (mg hydrolyzed sugar $g^{-1}$ sample).

#### 2.2.4 Total ascorbic acid content

Total ascorbic content was measured according to the method established by Davies and Masten (1991). The sample was extracted using 1% phosphate citrate buffer at pH 3.5 with chilled mortar and pestle. Then, the homogenates were centrifuged at 11000 rpm for 10 minutes at a temperature of 4 ºC. Lastly, the supernatant was collected and added with 1.72 mM of 2,6-dichloroindophenol (2,6-DCPIP) in 3 mL cuvette and measured at 518 nm immediately after mixing.

### 2.3 Secondary metabolites analysis

#### 2.3.1 Preparation of extract

The extraction for total phenolic acids and flavonoids content of *H. bonariensis* and *C. asiatica* dried leaves was carried out according to the method explained by Marinova (2005). Briefly, a total of 0.5 g of dried leaves was ground with mortar and pestle and homogenized with 50 mL of 80% aqueous methanol. The extraction samples were transferred into the covered flasks and placed in an ultrasonic bath for 20 minutes. Then, the extraction samples were centrifuged for 5 minutes at 14000 rpm. The supernatant was collected and used for the analysis of the secondary metabolites.

#### 2.3.2 Total phenolic acids content

According to Singleton and Rossi (1965), the total phenolic acid content was determined using the Folin-Ciocalteu method. A total of 1 mL of leaf extract was pipetted into a test tube containing 9 mL of distilled water. Then, 1 mL of Folin-Ciocalteu reagent was added, and the mixture was mixed thoroughly using a vortex machine. After 5 minutes, 10 mL of 7% of sodium carbonate was added. The reaction mixture's final volume was adjusted to 25 mL with an addition of 4 mL of distilled water. The reaction mixture was incubated at room temperature for 90 minutes, and absorbance was measured at 750 nm using a spectrophotometer. The standard curve of absorbance against different gallic acid concentrations was constructed, and the total phenolic acid content of the sample was expressed as mg gallic acid equivalent per gram of sample (mg GAE $g^{-1}$).

#### 2.3.3 Total flavonoids content

The total flavonoids content was conducted according to a procedure explained by Marinova (2005) using the aluminium chloride colorimetric method. A total of 1 mL of leaf extract was added with 4 mL of distilled water. After that, 0.3 mL of 5% sodium nitrite was added and incubated for 5 minutes. After 5 minutes, 0.3 mL of 10% aluminium chloride was added. At the sixth minute, 2 mL of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 mL by the addition of 2.4 mL of distilled water. The mixture was mixed thoroughly using a vortex machine, and the absorbance was measured using a spectrophotometer at 510 nm. The standard curve of absorbance against different catechin concentrations was constructed and the total flavonoid content of the sample was expressed as mg catechin equivalent per gram of sample (mg CE $g^{-1}$).

### 2.4 Antioxidant activities analysis

#### 2.4.1 Preparation of extract

The extraction of leaf extract for antioxidant activities analysis was conducted using a procedure explained by Wong et al. (2006). Briefly, a total of 0.5 g of the dried leaf was cut into small pieces and placed in a conical flask. A total volume of 25 mL of distilled water (25 ºC) was added, and the flask was covered with aluminium foil. The flask was placed on an orbital shaker at room temperature and shake for an hour in the dark. The extract was filtered using Whatman filter paper No. 1 and the extract was used in antioxidant activities analysis.

#### 2.4.2 DPPH free radical scavenging activity

DPPH free radical scavenging activity was conducted according to Wong et al. (2006) method. The DPPH at 0.1 mM was prepared in methanol, and the initial absorbance of DPPH was measured immediately using a spectrophotometer at 515 nm. A total of 40 µL of the extract was added with 3 mL of 0.1 mM of methanolic DPPH solution. The mixture was incubated at room temperature for 30 minutes and the change in absorbance was measured at 515 nm. A standard curve of absorbance against the different Trolox concentrations was constructed and the DPPH free radical scavenging activity was expressed as mg Trolox equivalent per gram of sample (mg TE $g^{-1}$).

#### 2.4.3 Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out according to the method explained by Benzie and Strain (1996). Briefly, 200 µL of the extract was added into a test tube containing 3 mL of FRAP reagent that prepared with 300 mM of sodium acetate buffer at pH 3.6, 10 mM of 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution, and 20 mM of $FeCl.6H_2O$ at the ratio of 10:1:1, respectively. The reaction mixture was incubated in a water bath at a temperature of 37 ºC for 30 minutes. After 30 minutes, the reaction mixture was cooled down to room temperature, and absorbance was measured at 593 nm using a spectrophotometer. A standard curve of absorbance against the different concentrations of Trolox concentration was constructed. The FRAP value was expressed as mg Trolox equivalent per gram of sample (mg TE $g^{-1}$).

### 2.5 Statistical analysis

All the experiments were conducted in a completely randomized design (CRD) with three replications. Data were analyzed using analysis of variance (ANOVA), and means separation was conducted using Duncan's Multiple Range Test (DMRT) at P=0.05. The correlation between variables was conducted using Pearson's correlation analysis.

## 3 Results and Discussion

### 3.1 Primary metabolites

Plant metabolites are composed of small molecules with various energy, signaling, structure, and defense mechanisms. Plant primary metabolites are directly involved in plant growth and development, senescence, and stress response (Adedeji and Babalola, 2020). High total soluble protein content was found in *C. asiatica* compared to *H. bonariensis* (Table 1). The highest total soluble protein content was significantly produced by the fresh sample of *C. asiatica* with 23.28 mg protein $g^{-1}$ FW, followed by the dried sample of *C. asiatica* with 14.97 mg protein $g^{-1}$ DW. For *H. bonariensis*, the fresh sample was significantly produced higher total soluble protein content than the dried sample with 2.80 mg protein $g^{-1}$ FW and 0.73 mg protein $g^{-1}$ DW, respectively. The total soluble protein content present in the plant is affected by various factors. Based on the previous study on *Arabidopsis*, the protein content was significantly affected as the photoperiod was changed (Hao et al., 2009). Furthermore, a study by Gibon et al. (2009) found that decrement of photoperiod from 12 hours to 3 hours were significantly reduced the total protein content in *Arabidopsis* by 10 to 15%.

The fresh sample of *H. bonariensis* and *C. asiatica* produced a higher total reducing sugar content than the dried sample (Table 1). The highest total reducing sugar content was significantly accumulated in the fresh sample of *H. bonariensis* with 1.94 mg reducing sugar $g^{-1}$ FW. The lowest total reducing sugar content was produced by the dried sample of *C. asiatica* with 0.18 mg reducing sugar $g^{-1}$ DW, which is five-fold lower than the fresh sample with 1.10 mg reducing sugar $g^{-1}$ FW, respectively. In the previous study conducted on Japanese carrot, the result showed that the total reducing sugar content was affected by seasonal variation. As Japan is a four-season country, changes in season will change the temperature and humidity. As a result, the plant's metabolites, including total reducing sugar content, were also affected. However, in the postharvest study, reducing sugar content remained constant up to 20 days after the carrot was harvested (Sinilal et al., 2011). Another study on the effect of water stress in total reducing sugar content of *Gmelina arborea* showed that reducing sugar content increased as the water supply was decreased. This phenomenon showed that the plant produced higher primary metabolites as an osmoregulatory response to prevent cell dehydration (Raharjo et al., 2010).

The same trend was observed in total hydrolyzed sugar content with a fresh sample of *H. bonariensis* and *C. asiatica* produced higher total hydrolyzed sugar content than the dried sample (Table 1). The fresh sample of *H. bonariensis* was significantly higher than the fresh sample of *C. asiatica* with 3.84 mg hydrolyzed sugar $g^{-1}$ FW and 1.55 mg hydrolyzed sugar $g^{-1}$ FW, respectively. Meanwhile, the dried sample of *C. asiatica* has produced the lowest total hydrolyzed sugar content with 0.94 mg hydrolyzed sugar $g^{-1}$ DW. As compared to total reducing sugar content, the total hydrolyzed sugar content recorded from the *H. bonariensis* and *C. asiatica* contained a higher amount of sugar. This was expected due to the breakdown of the bond between disaccharide and polysaccharide to produce monosaccharide, which can be easily detected and quantified using glucose as standard. A study carried out by Madrid et al. (2012) on *Psoralea glandulosa* found that the extraction solvent's polarity significantly affected the total hydrolyzed sugar content. The methanol extract of *P. glandulosa* produced higher total hydrolyzed sugar content than the other extracts (Madrid et al., 2012)

There were significant differences between the fresh and dried samples of *H. bonariensis* and *C. asiatica* samples on total ascorbic acid content (Table 1). However, no significant difference between the fresh samples of *H. bonariensis* and *C. asiatica*, with total ascorbic acid content recorded, were 0.34 and 0.35 mg ascorbic acid $g^{-1}$ FW, respectively (Table 1). Similarly, the dried sample showed no significant differences between the *H. bonariensis* and *C. asiatica*, with total ascorbic acid content recorded were 0.19 and 0.20 mg ascorbic acid $g^{-1}$ DW, respectively. The total ascorbic content was significantly different, although from the various varieties of the same species. A study on the different seed coat colors of soybean (*Glycine max*) showed that yellow seed coat soybean contained comparatively low ascorbic acid content compared to green and black seed coat soybean varieties (Kumar et al., 2010). Among the yellow, green, and black seed coat soybean, the black seed coat soybean is considered superior compared to other varieties. The yellow seed coat's ascorbic acid content was low than the green seed coat soybean variety (Kumar et al., 2010). The present and previous studies proved that the primary metabolites produced in plants were affected by various factors including genetic, climate, biotic and abiotic stresses, which directly will affect the metabolism and synthesis of primary metabolites in plants.

### 3.2 Secondary metabolites

Plant secondary metabolite is a compound produced by the plants in response to interaction with the environment. Unlike primary metabolites, the plant secondary metabolites produced by the plants are not directly affected by the fundamental life processes. However, secondary metabolites are produced as plant defense mechanisms against biotic and abiotic stresses (Zaynab et al., 2018). In the present study, the analysis of secondary metabolites conducted was total phenolics and flavonoids content. The results in Table 2 showed that *H. bonariensis* samples were significantly produced higher total phenolics and flavonoids content compared to *C. asiatica* samples. Besides that, the fresh samples of *H. bonariensis* and *C. asiatica* were significantly produced higher phenolics and flavonoids content compared to the dried sample. The highest total phenolics content was recorded from the fresh sample of *H. bonariensis* with 0.96 mg GAE $g^{-1}$ (Table 2). The dried sample of *H. bonariensis* was produced two-fold lower total phenolics content with 0.47 mg GAE $g^{-1}$ DW. For *C. asiatica*, the total phenolics content recorded from the fresh and dried samples were 0.58 and 0.42 mg GAE $g^{-1}$, respectively.

*H. bonariensis* produced two-time higher total flavonoid content than *C. asiatica* in both fresh and dried samples (Table 2). The highest total flavonoids content was recorded from the fresh sample of *H. bonariensis* with 13.79 mg CE $g^{-1}$, followed by the fresh sample of *C. asiatica* with 6.44 mg CE $g^{-1}$, respectively. Meanwhile, the lowest total flavonoids content was recorded from the dried sample of *C. asiatica* with 2.41 mg CE $g^{-1}$.

Phenolic is one of the leading groups in plant secondary metabolites. Phenolic can be divided into polyphenol and simple phenol. Polyphenol is a bioactive compound present in the plant which contains more than two aromatic rings, and simple phenol only has a single aromatic ring. The most popular polyphenol is flavonoids, and phenolic acids belong to a simple phenol group (Quideau et al., 2011). Based on the present study, the fresh sample of *H. bonariensis* and *C. asiatica* were significantly produced higher total phenolics and flavonoids content than dried samples. Generally, the drying process is conducted to preserve the samples by removing the water. However, during the drying process, photochemical degradation can occur, which leads to the decrement of bioactive compounds extracted from the plant sample. The dried sample's phenolic content decrement might be due to the attributable degradation of light-sensitive phenolic compounds. During the drying process, the samples were exposed to light for several days, which may be the possible reason for low phenolics and flavonoid content extracted from the sample (Vashisth et al., 2011).

### 3.3 Antioxidant activities

In the antioxidant activities analysis of *H. bonariensis* and *C. asiatica* samples, DPPH free radical scavenging activity and ferric reducing antioxidant power assays were conducted. For the DPPH free radical scavenging activity, the highest percentage of inhibition was significantly exhibited by the fresh sample of *C. asiatica* with 91.00%, followed by the fresh sample of *H. bonariensis* with 45.45% (Table 3). In the dried samples of *H. bonariensis* and *C. asiatica*, the percentage of inhibition recorded was significantly lower than the fresh sample with 20.16 and 34.75%, respectively.

In FRAP assay, results found that the *H. bonariensis* sample in fresh or dried form exhibited higher value than the *C. asiatica* sample (Table 3). The highest FRAP value was recorded from the fresh sample of *C. asiatica* with 66.75 mg TE $g^{-1}$ FW. Meanwhile, the fresh sample of *H. bonariensis* produced 50.27 mg TE $g^{-1}$ FW of antioxidant activities. The lowest FRAP value was produced by the dried sample of *C. asiatica* with 14.75 mg TE $g^{-1}$ DW of antioxidant activities.

Free radicals present in the human body are unstable molecules with high reactivity that can damage human cells and tissues. Most chronic diseases and aging are associated with free radicals. In order to neutralize the free radicals, an antioxidant is needed (Lobo et al., 2010). However, many studies also proved that phenolic compounds could act as an antioxidant due to its abilities in donating their hydrogen atom and single electron to free radicals (Zeb, 2020). In this study, the DPPH free radical scavenging activity and FRAP assay were conducted to measure the antioxidant potential of *H. bonariensis* and *C. asiatica*. In both antioxidant assays, the fresh samples exhibited higher antioxidant potential than the dried sample. According to Vashisth et al. (2011), an endogenous antioxidant present in the sample can be destroyed during the drying process. Thus, the antioxidant potential in the plant sample might be reduced.

### 3.4 Correlation among variables

The correlation between the primary metabolites (total soluble protein, reducing sugar, hydrolyzed sugar, and ascorbic acid contents), secondary metabolites (total phenolics and flavonoids contents), and antioxidant activities (DPPH free radical scavenging activity and FRAP assay) were evaluated using Pearson's correlation analysis (Table 4).

The total soluble protein content was not significantly correlated with all the variables measured based on the results obtained. This showed that the total soluble protein content present in *H. bonariensis* and *C. asiatica* was not influenced by reducing sugars, hydrolyzed sugar content, ascorbic acid, phenolics, and flavonoids content. Besides that, total soluble protein also was not significantly contributed to antiox-

**Table 1.** Primary metabolites analysis of fresh and dry samples of *H. bonariensis* and *C. asiatica*

| Plant species | *Hydrocotyle bonariensis* | | *Centella asiatica* | |
|---|---|---|---|---|
| | Fresh | Dry | Fresh | Dry |
| Soluble protein (mg $g^{-1}$ FW) | 2.80 ± 0.03 c | 0.73 ± 0.03 d | 23.28 ± 0.04a | 14.97 ± 0.03 b |
| Reducing sugar (mg $g^{-1}$ FW) | 1.94 ± 0.04 a | 1.02 ± 0.03 b | 1.10 ± 0.02 b | 0.18 ± 0.02 c |
| Hydrolyzed sugar (mg $g^{-1}$ FW) | 3.84 ± 0.05 a | 1.20 ± 0.02 c | 1.55 ± 0.04 b | 0.94 ± 0.02 d |
| Ascorbic acid (mg $g^{-1}$ FW) | 0.34 ± 0.01 a | 0.19 ± 0.01 b | 0.35 ± 0.02 a | 0.20 ± 0.01 b |

Values are represented as mean ± standard error (n = 3). Means followed by the same letter in the same columns and rows are not significantly different at P=0.05 using Duncan's Multiple Range Test.

**Table 2.** Secondary metabolites analysis of fresh and dry samples of *H. bonariensis* and *C. asiatica*

| Plant species | *Hydrocotyle bonariensis* | | *Centella asiatica* | |
|---|---|---|---|---|
| | Fresh | Dry | Fresh | Dry |
| Total phenolics (mg GAE $g^{-1}$ FW) | 0.96 ± 0.03 a | 0.47 ± 0.02 c | 0.58 ± 0.02 b | 0.42 ± 0.01 c |
| Total flavonoids (mg CE $g^{-1}$ FW) | 13.79 ± 0.22 a | 4.58 ± 0.28 c | 6.44 ± 0.27 b | 2.41 ± 0.08 d |

Values are represented as mean ± standard error (n = 3). Means followed by the same letter in the same columns and rows are not significantly different at P=0.05 using Duncan's Multiple Range Test.

**Table 3.** Antioxidant activities analysis of fresh and dry samples of *H. bonariensis* and *C. asiatica*

| Plant species | *Hydrocotyle bonariensis* | | *Centella asiatica* | |
|---|---|---|---|---|
| | Fresh | Dry | Fresh | Dry |
| DPPH (% inhibition) | 45.45 ± 1.23 b | 20.16 ± 0.77 d | 91.00 ± 1.15 a | 34.75 ± 0.61 c |
| FRAP (mg TE $g^{-1}$ FW) | 50.27 ± 0.54 a | 28.16 ± 0.11 c | 66.75 ± 0.99 b | 14.75 ± 1.04 d |

Values are represented as mean ± standard error (n = 3). Means followed by the same letter in the same columns and rows are not significantly different at P=0.05 using Duncan's Multiple Range Test.

**Table 4.** Correlation analysis between variables

| Variable | TSP | RS | HS | AA | TPC | TFC | DPPH | FRAP |
|---|---|---|---|---|---|---|---|---|
| TSP | 1 | | | | | | | |
| RS | -0.47ns | 1 | | | | | | |
| HS | -0.39ns | 0.89** | 1 | | | | | |
| AA | 0.34ns | 0.68** | 0.65** | 1 | | | | |
| TPC | -0.33ns | 0.90** | 0.98** | 0.72** | 1 | | | |
| TFC | -0.36ns | 0.94** | 0.98** | 0.72** | 0.99** | 1 | | |
| DPPH | 0.51ns | 0.53ns | 0.56ns | 0.95** | 0.63** | 0.62** | 1 | |
| FRAP | -0.10ns | 0.93** | 0.88** | 0.87** | 0.90** | 0.93** | 0.79** | 1 |

**: Significant correlation at P<0.05; ns: Non-significant correlation; TSP: Total soluble protein content; RS: Total reducing sugar content; HS: Total hydrolyzed sugar content; AA: Total ascorbic acid content; TPC: Total phenolics content; TFC: Total flavonoids content; DPPH: DPPH free radical scavenging activity; FRAP: Ferric reducing antioxidant power.

idant activities measured. Besides total soluble protein content, the total reducing sugar and hydrolyzed sugar content were showed a non-significant correlation against DPPH free radical scavenging activity. These results showed that DPPH free radical scavenging activity was not influenced by reducing sugar and hydrolyzed sugar content present in *H. bonariensis* and *C. asiatica*. Meanwhile, the other variable was correlated with each other. The highest correlation was produced between the total phenolics and flavonoids content with P=0.99.

## 4 Conclusion

In the present study, primary metabolites, secondary metabolites and antioxidant activities of the fresh and dry sample of *H. bonariensis* and *C. asiatica* were studied. The study found that both species' fresh sample was more prominent in producing high primary metabolites, secondary metabolites and exhibited high antioxidant activities. In the analysis of the primary metabolites, *C. asiatica* produced higher total soluble protein content. However, in reducing sugar and hydrolyzed sugar content analysis, *H. bonariensis* recorded higher values than *C. asiatica*. No significant difference was found between *H. bonariensis* and *C. asiatica* samples on total ascorbic acid content. In the analysis of the secondary metabolites, *H. bonariensis* significantly accumulated higher total phenolics and flavonoid content than *C. asiatica*. *C. asiatica* produced higher DPPH free radical scavenging activity while *H. bonariensis* exhibited a higher FRAP value for the antioxidant activities. In conclusion, *H. bonariensis* and *C. asiatica* were able to produce higher primary metabolites, secondary metabolites, and antioxidant activities. Hence, instead of focusing on *C. asiatica* for pharmaceutical and nutraceutical industries, the bioactive compounds present in the *H. bonariensis* sample also should be explored and utilized in the industry.

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## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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