Evaluation of total polyphenol content, total flavonoid content, and antioxidant activity of Centella asiatica

N T C Quyen¹,², N T N Quyen¹,², N N Quy¹,² and P M Quan³,⁴,*

¹NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam; ²Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam ³Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam ⁴Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam

*Corresponding author: pham-minh.quan@inpc.vast.vn

Abstract. Nowadays, the tendency to focus on plant research and their biological activities has increased worldwide. Centella asiatica is a crucial herb widely used in East Asia and is gaining popularity in the West. In Vietnam, besides being used as daily food, it is also an herb that is recommended to treat various skin disorders include leprosy, lupus, varicose ulcers, eczema, female genital diseases and also help reduce anxiety. The aerial parts are also proven to be scientific cash in the biological activities contained in it with broad therapeutic applications in the antibacterial, anti-inflammatory, and so on. This research aimed to assess the quality of phytochemicals, TPC and TFC, and antioxidant activities of C. asiatica (L). Moreover, antioxidant activity was conducted through radical scavenging ABTS and DPPH. Under purified water and 96% ethanol, the phytochemical was collected separately. The findings indicate that C. asiatica (L) produced an extensive range of pharmacologically active substances, including alkaloid, tannins, flavonoids, terpenoids, and reducing sugar. TPC and TFC of ethanol extract in C. asiatica leave achieved 2.14±0.29 mgGAE/g and 23.03±2.89 mgQE/g, respectively. The total polyphenol content of ethanolic and water extraction was different, achieved 2.14 ± 0.29 (mg GAE/g) and 2.82 ± 1.68 (mg GAE/g), respectively. Moreover, the TFC of water extract (30.09 ± 2.67 mg QE/g) was significantly higher than that of ethanolic extract (23.03 ± 2.89 mg QE/g). The antioxidant activity of the C. Asiatica was correlated with total phenolic and flavonoid content with values IC50 achieving 2324.26 µg/ml in aqueous extract, and 1744.77 µg/ml in Ethanolic extract. The results showed that leaves of C. asiatica (L) were a valuable source to exploit metabolic compounds primary and secondary are valid.

1. Introduction
Nowadays, Using plants as food and medicinal products has been receiving a great deal of public attention [1-5]. Depending on the type of tree, they may have different beneficial compounds [6-10]. The benefits of flexible human health have greatly improved the scientific discoveries about other natural compounds from plant organisms [11-15]. Centella asiatica (L) (Hydrocotyle asiatica L), belongs the Umbelliferae family, which was grown in China, India, Indonesia, and Vietnam. It is
fragrant and is used in meals. It is commonly used as an herb in traditional medicine to treat ulcers, lupus, varicose veins, eczema and certain nervous system disorders [16-19].

In Western medicine, Clinical studies have illustrated that an extract of *C. asiatica* (CA) is used to stimulate skin regeneration at the burn site, while also preventing scar tissue formation by inhibiting collagen production at the site of the wound [20],[21]. The crude extract isolated from CA containing glycosides isothankuni and thankuni has been shown to have an anticoagulant effect in rat testing [22]. Previous reports have shown that the CA’s biological activity is useful in combating psoriasis, anticonvulsants, immune stimulants, syrups and anti-cancer. A leaves are eaten in the raw form as salad, the ingredient in rolls, and beverages because of the many essential pharmaceuticals, including triterpenoids and carotenoids[11],[12]. The therapeutic effects of plants are mainly attributed to secondary metabolites such as cardiac glucosides, tannins, flavonoids, and alkaloids. Although this plant has various therapeutic uses, very little is studied regarding its antioxidant activity.

This study focused on a preliminary assessment of plant chemical composition of *C. asiatica* grown in Vietnam and initially determined their phenolic, flavonoid content and antioxidant activity by ethanolic and aqueous extraction.

2. Material and method

2.1. Collection of plant material

*C. asiatica* (L) plants are randomly collected in Go Vap District, Ho Chi Minh City, Vietnam. Completely ripe fresh leaves are preliminarily graded and washed thoroughly under running water, then dried and ground to a fine powder (Figure 1) and stored in an airtight bottle for future use. Chemicals used for analysis such as Folin-Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl, 2,2′-azino-di- [3-ethylbenzthiazoline sulfonate], absolute ethanol and other chemicals are purchased in Merk (Germany) or Xilong (China) based on standards for each experiment.

![Figure 1](image)

**Figure 1.** Raw materials *Centella asiatica* L. a) before grinding and b) after processing and pulverizing

2.2. Preparation of plant extracts

The dry powder of *C. asiatica* (L) is extracted in turn with two solvents, ethanolic and water. CA powder (10g) was mixed with a different solvent. It was then extracted for 1 hour 70°C. The extract is filtered out of the residue and then evaporated into the solvent to obtain a dry portion. The dried herbal portion was stored at 40°C for the following experiments.

2.3. Phytochemical Analysis

Phytochemical analysis of ethanolic and water extract was done for presence/absence of metabolites such as flavonoids, alkaloids, tannin, terpenoids, saponins, anthraquinone, coumarin and reduce sugar [23].
2.4. Total Phenolic Contents (TPC)
The method was conducted based on previous research [24]. Continue with the extraction process to achieve the concentration needed. Afterward, 0.5ml of diluted sample solution was drawn into a test tube. It was then added 2.5ml of 10% Folin-Ciocalteu solution and homogenized using a Vortex machine and left to react for 5 minutes in the dark. Next, add 2ml of 7.5% Na₂CO₃ solution and shake well, put in the dark for 1 hour. Finally, measure the optical absorbance at 756 nm on the UV-Vis spectrophotometer. Use gallic acid as a standard and express polyphenol content in micrograms of gallic acid equivalent per 1 mg of extract (µgGAE/mg extract).

2.5. Total flavonoids contents (TFC)
0.5 ml of the diluted sample solution was put into a test tube. It was then added 0.1 ml of 10% AlCl₃. 0.1 ml of CH₃COOK 1M was mixed with 4.3ml of distilled water. Next, the solution was put at RT for 30 minutes. Then measure the optical absorbance at 415nm on the UV-Vis spectrophotometer. Quercetin is appropriate for usage. The total flavonoids is contained in quercetin equivalent micrograms in 1 mg of extract (µgQE/mg extract) [25].

2.6. Antioxidant Activity
Free radical removal method DPPH (1,1-diphenyl-2-picrylhydrazyl) The extraction obtained from the previous extraction was diluted to a reasonable concentration. 0.5ml of diluted sample was then put into a test tube. Control sample was ethanol extract (99.5%). Then, adding a tube of 1.5 ml DPPH solution (OD517 nm = 1.1 ± 0.02) to a test tube and leave in the dark for 30 minutes. Measure optical absorbance at 517nm on UV-Vis spectrophotometer. Vitamin C (ascorbic acid) is used as the reference standard. The following formula is used to determine DPPH free radical scavenging operation (IC%):

\[
IC(\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100
\]

Inside:
AbsC: Optical absorbance of the control sample
AbsT: Optical absorbance of the sample
The result is reported based on the IC50 value, which is the concentration at which the sample removes 50% of DPPH free radicals.
Free radical removal method ABTS (2,2’-azino-bis) The free radical solution ABTS was prepared by adding 10 ml of ABTS solution of 7.4 mM into 10 ml of K₂S₂O₈ solution of concentration of 2.6 mM and incubating in the dark for 24 h, then diluting with ethanol and then adjusting the absorbance of the solution at a wavelength of 734 nm to 1.1 ± 0.02. Dilute the extract to the appropriate concentration, collecting 0.5 ml of diluted sample extract into a test tube. Control sample was ethanol (99.5%). Afterward, add 1.5ml ABTS solution (OD517 nm = 1.1 ± 0.02) to a test tube and place in the dark for 30 minutes. Measure optical absorbance at 734nm on UV-Vis spectrophotometer. Vitamin The reference standard has been used for C (ascorbic acid). The ABTS (IC%) is carried out by the following formula:

\[
IC(\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100
\]

Inside:
AbsC: Optical absorbance of the control sample
AbsT: Optical absorbance of the sample
The result is stated based on the IC50 value, which is the sample concentration, which removes 50% of the free ABTS radicals.
3. Result and discussion
The results of the phytochemical analysis in leaves of \textit{C. asiatica} (CA) are presented in Table 1. Testing has proved the appearance of most phytochemical components (5/8) such as alkaloids, tannins, flavonoids, terpenoids, and reducing sugar. In general, the saponin content does not appear in both extraction solvents. In contrast, the phenolic and flavonoid components are shown in this assessment, indicating that CA has great promise in exploiting their antioxidant activity. This result is consistent with previous studies on the experimental evaluation of plant chemical composition of CA in the corresponding solvents [26]. Much evidence gathered in previous studies has confirmed that phytochemicals have been identified as biologically active. Several studies have confirmed their contribution to plants' medicinal and physiological properties in the treatment of various diseases. Throughout the treatment of different illnesses, medicinal plants play a significant part.

| Chemical composition group | Alcohol extract | Water extract | Alcohol react picture | Water react picture |
|----------------------------|-----------------|---------------|----------------------|--------------------|
| Alkaloid                   | +               | +             |                      |                    |
| Tannin                     | +               | +             |                      |                    |
| Anthraquinon               | -               | -             | -                    | -                  |
| Flavonoid                  | +               | +             |                      |                    |
| Terpenoid                  | +               | +             |                      |                    |
| Coumarin                   | +               | -             | +                    | -                  |
| Saponin                    | -               | -             | -                    | -                  |
| Reducing sugar             | +               | +             |                      |                    |

Table 1. Phytochemical screening of the \textit{C. asiatica} extraction

In parallel with the phytochemical test, quantitative evaluation of polyphenol, flavonoid components and ability to eliminate free radicals (DPPH and ABTS) was also conducted and obtained results in Table 2. The TPC of ethanol and water extracts was 2.14 ± 0.29 (mg GAE/g) and 2.82 ± 1.68 (mg GAE/g), respectively. The TFC of water extract (30.09 ± 2.67 mg QE/g) was higher than that of ethanol extract (23.03 ± 2.89 mg QE/g). This result is consistent with the previous study of Gunathilake1 in optimizing polyphenol content extracted from CA [27].

The antioxidant activity of CA extract in the DPPH test obtained IC$_{50}$ is 1744.77 µg/mL for ethanolic extract and IC$_{50}$ of water extract is 2324.26 µg/mL. The results showed that \textit{C. asiatica} (L) extract with ethanolic has higher antioxidant activity than that obtained with water. At the same time, in the ABTS assay, extracting CA with water yielded an IC$_{50}$ of 462.38 µg/mL higher than ethanolic extract (1058.10 µg/mL). Previous reports indicate that the majority of the antioxidant potential of plant extracts depends
on its phenolic compounds. Besides, another factor that determines phenolic compounds' antioxidant activity is the stability of aroxy radicals formed in the structure of that compound. However, the mechanisms of specific processes and compounds still need to be studied more carefully [16].

Figure 2. Standard calibration curve of (a) gallic acid and (b) quercetin

Table 2. TPC, TFC and antioxidant activities (IC₅₀ values) of CA extract

| Sample          | Total polyphenol content (mg GAE/g) | Total flavonoid content (mg QE/g) | IC₅₀ value (µg/mL) |
|-----------------|-------------------------------------|-----------------------------------|--------------------|
| Ethanolic extract | 2.14 ± 0.29                         | 23.03 ± 2.89                     | 1744.77            |
| Aqueous extract  | 2.82 ± 1.68                         | 30.09 ± 2.67                     | 2324.26            |
| Ascorbic acid   | -                                   | -                                | 3.05               |

Numbers with identical letters in a column are statistically indifferent (p < 0.05)

4. Conclusion
Current research indicates that C. asiatica (L) extract satisfies and displays most phytochemical components in both extraction tests with ethanolic and water. The findings suggest that there was a significant variation in the leaves of CA in pharmacologically active compounds such as tannins, alkaloids, flavonoids, reducing sugar, and terpenoids. This analysis also shows the TPC, TFC, and antioxidant activity of the leaves of C. asiatica (L). The phenolic content was found 2.82 ± 1.68 µg GAE/mg in aqueous extract. Flavonoid content was 30.09 ± 2.67 µg QE/mg dry weight ethanol extract in C. asiatica. Research has also shown that the antioxidant activity of CA extract is dependent and closely linked to their phenolic composition, achieving 2324.26 µg/ml and 462.38µg/ml in DPPH and ABTS scavenging operation, respectively in Aqueous extract. This study also confirms that C. asiatica (L) has biological activity consistent with previous studies. Therefore, this research could play a part in further studies on the pharmacology of this plant and more applicable as a natural and economical remedy.
Acknowledgment
This work was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

References
[1] Quyen N T C, Ngan T T K, Dao T P and Phuong L T B 2019 Asian J. Chem. 31 2585-2588
[2] Ngan T T K, Muoi N V, Quan P M and Cang M H 2020 Asian J. Chem. 32 1433-1436
[3] Pham T N, Le X T, Nguyen P T N, Tran T H, Dao T P, Nguyen D H, Danh V T and Anh H L T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062005
[4] Nguyen M T, Nguyen V T, Minh L V, Trieu L H, Cang M H, Bui L B, Le X T and Danh V T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062011
[5] Ngan T T K, Huong N C, Le X T and Trieu T A 2019 Asian J. Chem. 31 2759-2762.
[6] Tran T H, Ngo T C Q and Cang M H 2020 IOP Conf. Ser. Mater. Sci. Eng. 736 062040
[7] Dao T P, Tran T H, Nhan N P T, Quyen N T C and H T K Linh 2020 IOP Conf. Ser. Mater. Sci. Eng. 736 022039
[8] Dao T P, Do H T, Le Q K and Cang M H 2020 Asian J. Chem. 32 1399-1403
[9] Dao T P, Tran T H, Nguyen P T N, Linh H T K 2020 IOP Conf. Ser. Mater. Sci. Eng. 736 022038
[10] Thuy D T T, Tuyen T T, Bach L G and Chien N Q 2019 Processes 7 432
[11] Somchit M N 2004 Indian J. Pharmacol 36 377–380
[12] Prakash V 2017 Asian Journal of Pharmaceutical and Clinical Research 10 68–76
[13] Siddiqui B S, Aslam H, Ali S T, Khan S, and Begum S 2007 J. Asian Nat. Prod. Res 9 407–414
[14] Prakash V, Jaiswal N, and Srivastav M 2017 Asian Journal of Pharmaceutical and Clinical Research 10 69–74
[15] Bhattacharya R D, Parmar K M, Itankar P R, and Prasad S K 2017 South African J. Bot 112 237–245
[16] Taylor P, Sultan R A, Bin S, Mahmood Z, and Azhar I 2014 Journal of Herbs, Spices & Medicinal Biological 45 37–41
[17] Chandrika U G and Prasad Kumara P A 2015 Advances in Food and Nutrition Research 76 125–157
[18] Ren L, Cao Q X, Zhai F R, Yang S Q, and Zhang H X 2016 Pharmaceutical Biology 54 2377–2382
[19] Cheng C L, Guo J S, Luk J, and Koo M W L Life Sciences 74 2237–2249
[20] Bhattacharya R D, Parmar K M, Itankar P R, and Prasad S K 2017 South African J. Bot 112 237–245
[21] Orhan I E 2012 Evidence-Based Complement. Altern. Med 1–8
[22] Gohil K J, Patel J A, and Gajjar A K 2010 Indian Journal of Pharmaceutical Sciences 72 546–556
[23] Gul R, Jan S U, Faridullah S, Sherani S, and Jahan N 2017 Scientific World Journal 2017
[24] Thuy N V, Tien N M, Quy N N, Can M Hg, Quan P M, Bui L M, Minh L V 2020 Asian J. Chem. 32 1230–1234
[25] Mahboubi M, Kazempour N, and Boland Nazar A R 2013 Jundishapur J. Nat. Pharm. Prod 8 15–19
[26] Arumugam T, Ayyanar M, Koil Pillai Y J, and Sekar T 2011 Bangladesh J. Pharmacol 6 55–60
[27] Gunathilake K D, Ranaweera K K, and Rupasinghe H P V 2019 Food Sci. Nutr 7 528–536