Advances in Biomarker Sciences and Technology 2 (2020) 35–44

Contents lists available at ScienceDirect

Advances in Biomarker Sciences and Technology

journal homepage: http://www.keaipublishing.com/ABST

Chemical fingerprint of *Centella Asiatica*'s bioactive compounds in the ethanolic and aqueous extracts

Siti Nuurul Huda Mohammad Azmin [a,*], Mohd Shukri Mat Nor [b]

[a] *Faculty of Agro-Based Industry, Universiti Malaysia Kelantan Jeli Campus, Locked Bag 100, 17600, Jeli Kelantan, Malaysia*
[b] *Department of Research and Development, Jeli Agricultural Technology, PT7458 Kampung Gemang Baru, 17700, Ayer Lanas, Jeli Kelantan, Malaysia*

ARTICLE INFO

Article history:
Received 29 March 2020
Received in revised form 3 October 2020
Accepted 15 October 2020
Available online 20 October 2020

Keywords:
Centella asiatica
Maceration technique
Model of percentage yield
Bioactive compounds
Ethanolic extracts
Exponential equation model

ABSTRACT

Centella asiatica is a herbal plant that is widely used as medicine due to the benefit of its bioactive compounds such as rutin, kaempferol, quercetin, gallic acid, luteolin and catechin. Typically, the amount of these bioactive compounds are varies depending on the solvent used. Therefore, this study aims to investigate the chemical fingerprint of six *Centella asiatica*'s bioactive compounds (kaempferol, quercetin, luteolin, gallic acid, rutin and catechin) in the ethanolic and aqueous ethanol extracts. Water, ethanol, and 50% aqueous ethanol were used as extracting solvents via maceration (solid-liquid) technique to extract bioactive compounds from *C.asiatica*. Rotary evaporator procedure was performed to concentrate the extracts before these crudes were analysed using HPLC instrument. The percentage yield of crude extract (% w/w) was calculated, and its mathematical model was reported in this study. The exponential equation model was also applied to predict the percentage yield of the *C.asiatica* extract. From the equation, satisfactory results have been obtained, which gave less than 12.21% error with 0.9967 of R^2 value. Besides, the percentage yield of bioactive compounds resulting from HPLC analysis was also explained. HPLC result showed that kaempferol was the highest bioactive compound with 373.2 mg/g dry powder using 100% ethanol as extract solvent. Therefore, the development of this study can be extended to assess this plant potential in the formulation of pharmaceutical and cosmetic products.

Introduction

Centella asiatica L. Urban *sys*. belongs to the genus *Centella* (Syn. *Hydrocotyle*) in the *Apiaceae* family (formerly known as *Umbelliferae*) and subfamily *Mackinlayoideae*.[14,40] This herbaceous perennial herb commonly grows well forming a dense green carpet in moist shady places, damp, marshy and swampy areas such as paddy field.[6] It has various vernacular or common names in other languages based on its geographical origins such as Asian pennywort (English), Rohtosammakonputki (Finnish), tabao en Amhara (African), Byeong pul (Korean), Gotukola (Sinhalese), and Trachiek kranh (Khmer).[16,25] *Centella asiatica* is familiarly known as pegaga by Malaysian and has been used in a folk herbal medicine for centuries[15] such

* Corresponding author.
E-mail address: huda.ma@umk.edu.my (S.N.H. Mohammad Azmin).

https://doi.org/10.1016/j.abst.2020.10.001

as for memory enhancement,[14] antidepressant,[32] wound healing,[42] psoriasis remedy,[35] and in the treatment of other mild and chronic diseases.[13]

Numerous researches have been conducted on the analysis of *C.asiatica*'s bioactive compounds around the world (mainly in Asia such as Malaysia, Indonesia, China, India and Sri Lanka) which have reported many varieties of compounds.[37] The variation in the bioactive compounds was due to differences in geographical location, altitude, location of plants,[12] seasonal changes, harvesting time, physiological factors (such as genetics, plant nutrition, and stage of maturity),[1] part of the plant used, and post-harvesting factors (such as storage conditions and processing treatments.[39] The bioactive compounds that are recorded belonging to *C.asiatica,* including flavonoids, tannins, saponins, alkaloids, terpenoids, phenols, and glycosides.[14,33,43]

The extraction must be employed to obtain the extract yield of the plant as well as these bioactive compounds. There are a lot of extraction techniques can be used, such as solvent extraction, supercritical fluid extraction, solid-phase extraction and microwave-assisted extraction.[11] However, solvent extraction is one of the most extensively studied and most widely used techniques, especially in the extraction of *Centella asiatica* due to its simple method, convenient and rapid to perform. Previously, organic solvents, including ethanol,[3] methanol,[7,31,38] ethanol-water mixture,[21] and methanol-water[20,27] mixture, were used in the extraction of *Centella asiatica.* Generally, holistic extraction procedure starts with careful selection and preparation of plant parts. Then, the most suitable protocols for specific compound groups or plant species will be reviewed comprehensively. Finally, it is crucial to prevent any contamination of the extract, to avoid decomposition of bioactive compounds, and to control solvent impurities during the extraction of raw plant material.

Besides, the chromatographic tests will be carried out to identify and to confirm the presence of the bioactive compounds in the extract yield of the plant. The chromatogram results may be used as a chemical fingerprint by displaying the profile of common bioactive compounds. Chromatography is a useful analytical method used for the qualitative and quantitative analysis of bioactive compounds; these include paper chromatography, thin-layer chromatography, gas chromatography, High-Performance Liquid Chromatography (HPLC), and capillary electrophoresis.[28] Among the chromatographic fingerprinting applied over the past decade as the qualitative and authentication evaluation of plant extracts, HPLC fingerprint arises to be the most widely used due to its efficiency and convenience.[24] Conventionally, the characterisation and fingerprinting of *C.asiatica* plant extract relied on thin-layer chromatography. Still, then, the analysis has been mostly based on High-Performance Liquid Chromatography (HPLC) method as early as 1996.[14] Therefore, the objective of this study is to investigate the chemical fingerprint of six *Centella asiatica*'s bioactive compounds (kaempferol, quercetin, luteolin, gallic acid, rutin and catechin) in the ethanolic and its aqueous extracts. The *Centella asiatica* sample was originated from Jeli Kelantan, which is a region located near to the hills with high humidity (97% at night). A lack of studies focussed on the use of *Centella asiatica* sample planted in the high humidity area has been identified from our literature search. This study used water, ethanol, and 50% ethanol aqueous as extracting solvents via maceration (solid-liquid) technique to extract bioactive compounds from *Centella asiatica*. The crude extracts then were characterised using HPLC method to identify active ingredients in the samples. Besides, the mathematical model for the percentage yield of crude extract was also calculated and presented in this study. The present studies were, therefore, can be extended to assess this plant potential in the formulation of pharmaceutical and cosmetic products.

Materials and methods

Sample

C.asiatica sample was purchased from a local wet market located at Jeli, Kelantan Malaysia. The leaves sample were washed under running tap water to remove all residues and impurities before drying in an electric oven at 40 °C for 48 h. After that, all the dried samples were ground into a fine powder using an electric blender. The powders were kept at 4 °C in an airtight container for further use. This preparation steps followed the procedures mentioned by Rattanakom and Yasurin,[34] with some alteration.

Preparation of crude extract

The preparation of *C.asiatica* crude extract followed the method explained by Rattanakom and Yasurin, (2015) with some modification. *C.asiatica* powder was extracted using three different solvent conditions, which are water, ethanol and 50% aqueous ethanolic solution with 1:10 ratio (g/ml) of raw plant material to solvent. In this study, 70 g of dried *C.asiatica* was immersed in 700 mL of solvent. The mixtures were macerated at 40 °C for 48 h and filtered using Whatman Grade 1 filter paper. The crude extracts were purified using a rotary evaporator (Heidolph Rotary Evaporator, WB 2000 and VV 2000, Heidolph Instruments GmbH & Co KG, Schwabach, Germany) at the temperature of 50 °C with the mixing speed of 120 rpm. Then, the crude extracts were kept at 4 °C before used. The percentage yield (% w/w) of the extract was obtained using Equation (1):

$$\%\ yield = \frac{weight\ of\ extract,\ g}{weight\ of\ plant\ material,\ g}\ X\ 100 \quad (1)$$

The procedure in the extraction of *C.asiatica* crude extract is illustrated in Fig. 1.

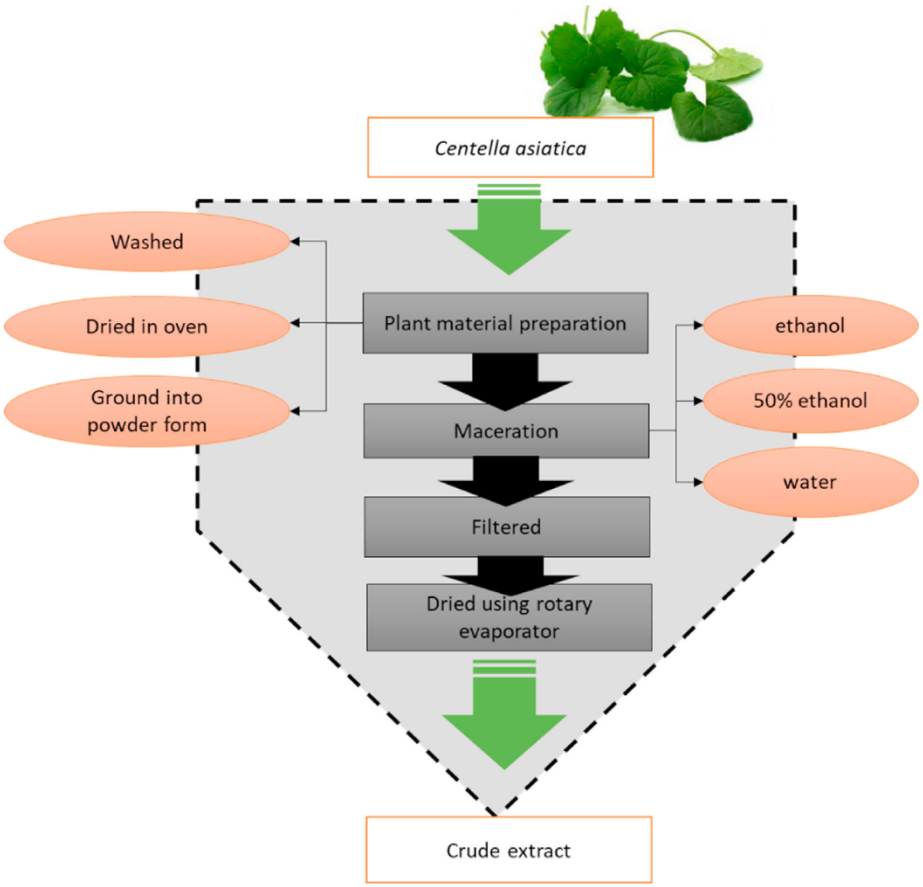

Fig. 1. Procedure for the extraction of *C.asiatica* crude extract.

Statistical analysis

The involved experiments were conducted in triplicate, and the results were expressed as averages. In this study, the statistical analysis was performed using Microsoft Excel 2016. Percent yield of the experimental data versus ethanol ratio in water was plotted to get an actual line. From this actual line, the predicted line was obtained where the most suitable equation that fits well with the line was chosen. The optimum condition was established by plotting the data point with the exponential equation. The model adequacy was evaluated using the absolute percentage error (% Error as stated in Equation (2)), the predicted versus actual plots, and the coefficient of determination (R^2 as expressed in Equation (3)).

$$\%\ Error = \left|\frac{y-\hat{y}}{\hat{y}}\right| X\ 100 \tag{2}$$

$$R^2 = 1 - \frac{\sum (y-\hat{y})^2}{\sum (y-\bar{y})^2} \tag{3}$$

where y is an experimental value, $\hat{y}$ is predicted value and $\bar{y}$ is mean of y values.

Table 1
Gradient elution program.

Run Time (min)	A 0.1% formic acid in water (%)	B 0.1% formic acid in methanol (%)
0	95	5
20	100	0
25	100	0
25.1	95	5
30	95	5

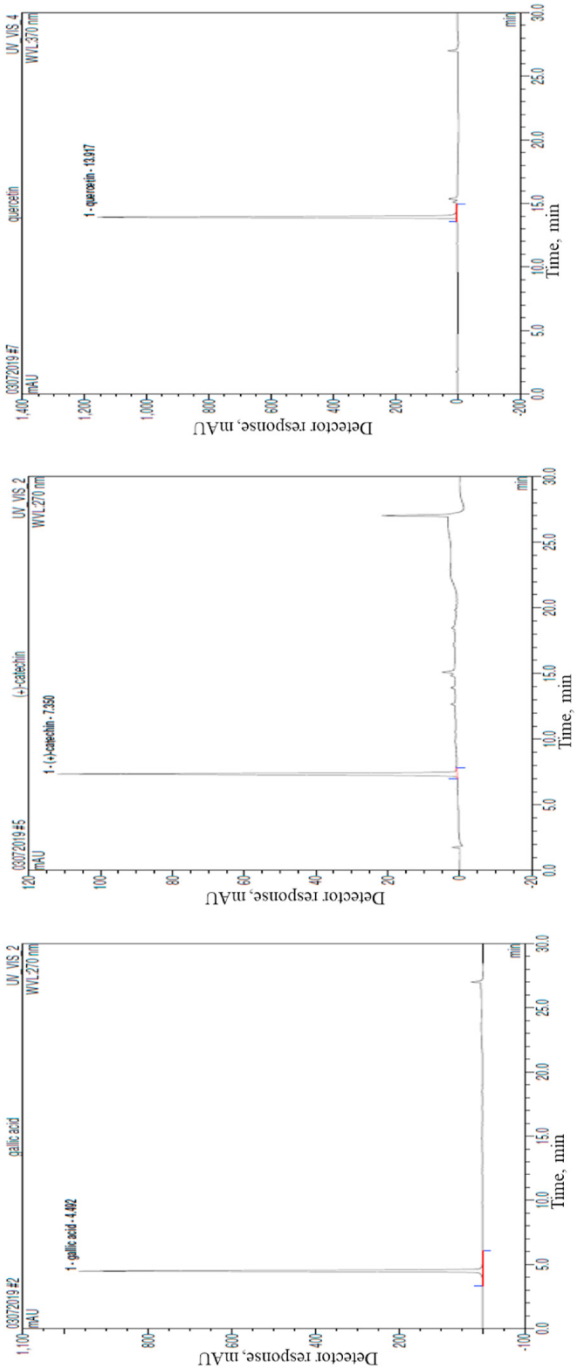

Fig. 2a. HPLC chromatograms for standards of gallic acid and catechin compounds detected by UV at 270 nm; and quercetin compound detected by UV at 370 nm.

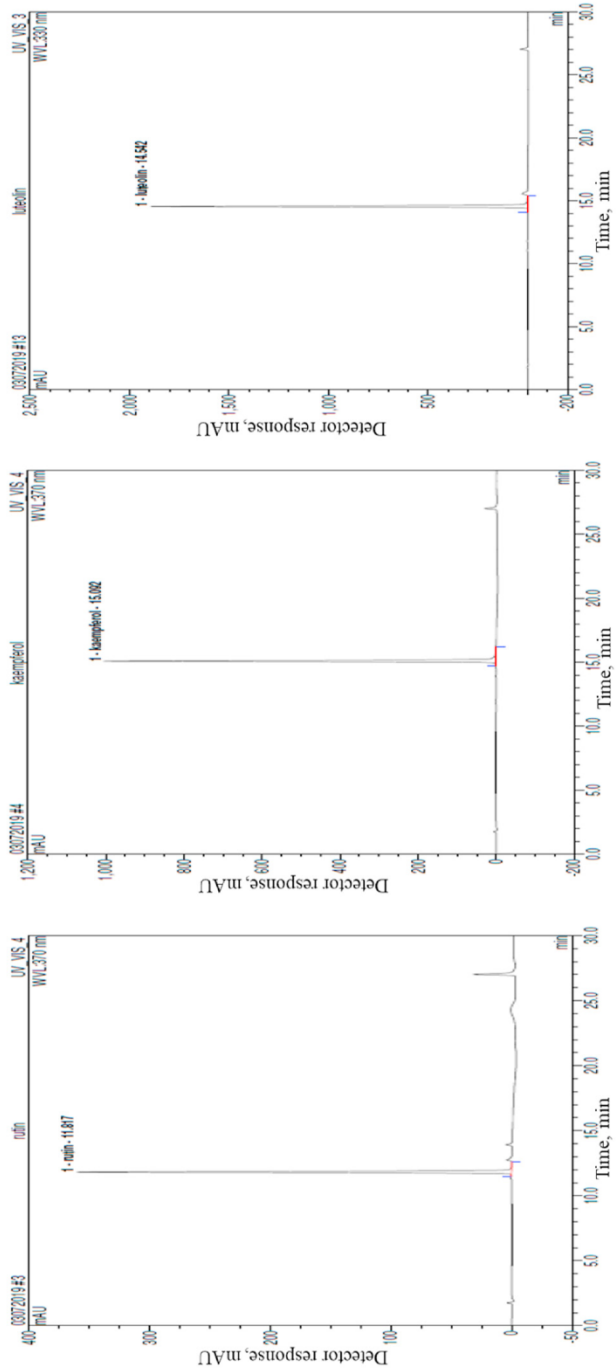

Fig. 2b. HPLC chromatograms for standards of rutin and kaempferol compounds detected by UV at 370 nm; and luteolin compound detected by UV at 330 nm.

Table 2
% yield of ethanolic extract and it's aqueous.

Solvent	Ethanol ratio in water	Weight of crude extract, g	Experimental yield (% w/w)	Prediction yield (% w/w)	Absolute Percentage Error (%)
Water extract	0	8.95	12.78	12.08	5.79
50% ethanolic extract	0.5	2.87	4.09	4.58	10.66
Ethanolic extract	1	1.29	1.84	1.74	5.79
VALIDATION					
Solvent	**Ethanol ratio in water**	**Weight of crude extract, g**	**% yield (Experimental)**	**% yield (Prediction)**	**% Error**
20% ethanolic extract	0.2	6.44	9.20	8.20	12.21

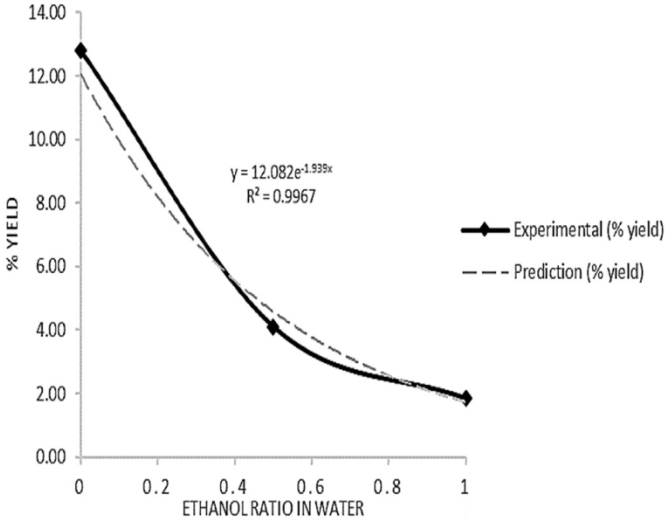

Fig. 3. Experimental and prediction curves of % yield at different ethanol in water ratio.

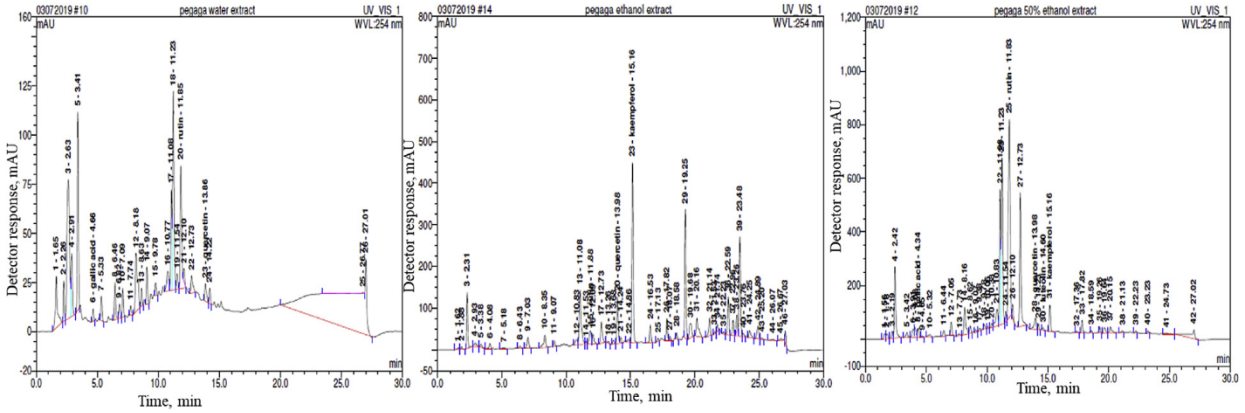

Fig. 4a. HPLC analysis of the *C.asiatica* in water, ethanol and 50% ethanol extracts at 254 nm.

High performance liquid chromatography (HPLC) of C.asiatica crude extract

Six bioactive compound standards which are flavonoids (rutin, quercetin, kaempferol, catechin, luteolin) and phenolic acid (gallic acid) were used in determining the flavonoid content of *C. asiatica*. The selection of standards was based on the bioactive compounds that are commonly found in vegetables and fruits,[4,19,29] and they also play a significant role in

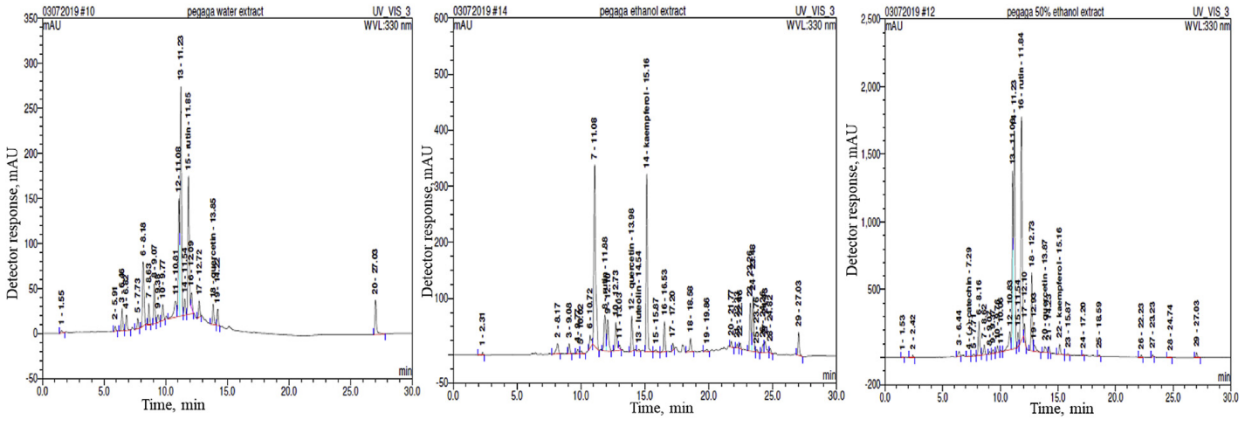

Fig. 4b. HPLC analysis of the *C.asiatica* in water, ethanol and 50% ethanol extracts at 330 nm.

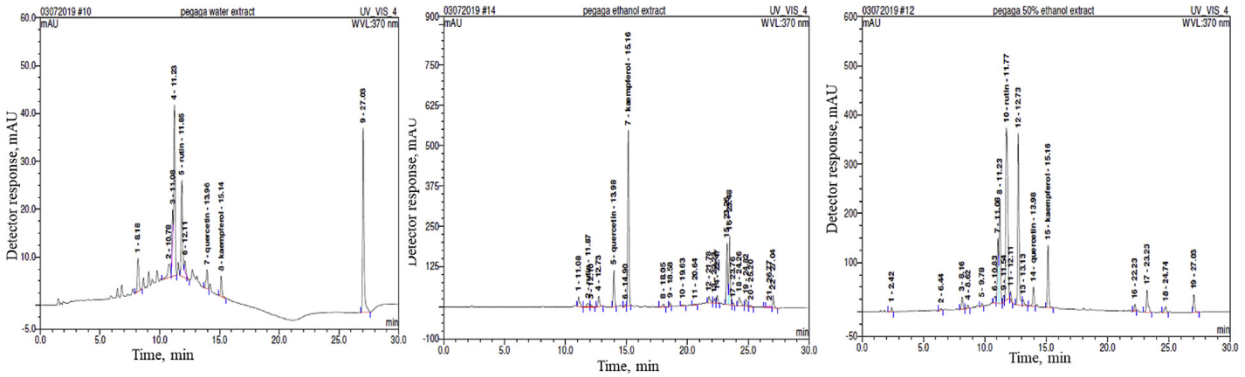

Fig. 4c. HPLC analysis of the *C.asiatica* in water, ethanol and 50% ethanol extracts at 370 nm.

contributing to antioxidant activity.[30] Besides, selected flavonoids represent different groups such as flavonol (rutin, quercetin, kaempferol), catechin and flavones (luteolin).

The HPLC system model Dionex Ultimate 3000 RSLC equipped with a system of DAD and fluorescence detector was used. The separation was performed with a Hypersil ODS C18 column (3.5 μm, 4.6 mm I.D x 150 mm; Thermo Scientific, Waltham, MA, USA) fitted with a Hypersil ODS guard column. The column temperature was maintained at 25 °C. 0.1% formic acid/water (v/v) (solvent A) and 0.1% formic acid/methanol (v/v) (solvent B) extractions were performed in the gradients as shown in Table 1. The injection volume was 10 μL at a flow rate of 1.0 mL/min. This study found that gallic acid and luteolin appeared at the detection of 254 nm while catechin appeared at the detection of 330 nm. In contrast, rutin, quercetin and kaempferol appeared at the detection of 370 nm. Measurements were performed in duplicate.[36]

Chromatographic peaks of selected bioactive compounds (rutin, quercetin, kaempferol, catechin, luteolin, and gallic acid) were identified by comparing the retention times of the HPLC to external bioactive compound standards. The HPLC chromatograms for all six compounds are shown in Fig. 2a and b.

Table 3
Bioactive compounds analysis.

Bioactive compound	Water extract (mg/g dry sample)	Ethanolic extract (mg/g dry sample)	50% Ethanolic extract (mg/g dry sample)
Gallic acid	3.8	0.0	9.3
Rutin	174.7	15.0	322.7
Kaempferol	33.0	373.2	76.1
Catechin	0.0	0.0	0.7
Quercetin	36.3	77.6	25.2
Luteolin	0.0	2.7	1.3

Result and discussion

Extraction yield

Extraction yield refers to the obtained crude extract from a dried plant sample through a solvent extraction procedure for further isolation and utilisation.[29] Table 2 shows the percentage of yield extracts from *C.asiatica* in three different solvents, which are water, ethanol and 50% aqueous ethanol. As can be observed in the table, water demonstrates the highest extraction yield with 12.78% w/w followed by 50% aqueous ethanolic with 4.09% w/w, and ethanol with 1.84% w/w. The higher the percentage of water content in the solvent, the higher the mass of crude extract will be obtained. This phenomenon occurs because water is a better polar solvent as compared to ethanol, and it will attract polar and slightly polar compounds to be part of the crude extract.[22] Most of the bioactive compounds of plant matrices are highly polarisable. It is a result of the presence of aromatic delocalised ∏-electrons in their molecules.[5] Besides, the effectiveness of solid-liquid extraction is also influenced by the solvent type mainly due to the different polarity (different solvent ratio) and physical characteristic of the sample as reported by.[5] Generally, the polarity of the extracting solvent may have a significant impact on the yield of the crude extracts. Water, ethanol, and 50% of ethanol were selected as extracting solvents due to index progression of their polarity. The sequence of the highest to the lowest polarity index is water >50% aqueous ethanolic > ethanol with 9, 7.1 and 5.2, respectively, as reported by.[23] During extraction, solvents diffuse into the solid plant material and solubilise the compounds with similar polarity. These solubilise compounds will diffuse out from the cell as the concentration of the compound in the solvent is less than the concentration in the plant cell. The nature of the used solvent will define the type of bioactive compounds possibly extracted from the plant.[41] Therefore, while the solvent was removed in the crude extract using a rotary evaporator, the compounds that bind to water remained in the crude extract. Besides, the temperature used in rotary evaporator was 50 °C causing some of the water was not fully vaporised as water boiling point is 100 °C. Thus, the final weight of the crude extract may contain a small amount of water. However, this study was conducted to make sure that the optimum amount of water was evaporated, where the weight of crude extract was measured several times until constant weight was obtained. This constant weight of crude extract can be the indicator of the optimum amount of water evaporated.

Fig. 3 shows the % yield of crude extracts with different ratio of ethanol in water. It offers both experimental and prediction results, where the exponential equation for the experimental values is $y = 12.082e^{-1.939x}$ (y is % yield, and x is ethanol ratio in the aqueous mixture) and $R^2 = 0.9967$. The percentage yield curve shows a clear exponential tendency, and an increase in the water to ethanol ratio enhances the extraction yield. From this equation, the absolute percentage error then was calculated using Equation (1).

The % errors were computed for all three % yields, with the error values range from 5.79 to 10.66%. As shown in Fig. 3, the curves of experimental and prediction values of % yield are suited well. The exponential model then was validated with 0.2 ethanol ratio in water (the extraction using this ethanol ratio was conducted in this study), which this ratio is not included in the graph and mathematical fits of the model. Surprisingly, the experimental and prediction values of % yield for this ratio is almost the same, which are 9.20% and 8.20% respectively. The validation of the exponential equation model found that the model fits best with the error of 12.21% as stated in Table 2. The accuracy of this model is satisfactory since the absolute percentage errors are less than 15%. Nevertheless, this exponential equation is only limited to the application of the *C. asiatica* extraction in ethanol and water mixture as a solvent. Thus, this model could be useful to be applied to the variation of ethanol ratio in water in predicting % yield of crude extract of *C.asiatica* in an aqueous ethanol solvent.

Yield determination

The concentration for each bioactive compound was estimated using a peak area for each bioactive compound by referring to the identified peak from HPLC chromatogram. The yield of rutin was measured in mg rutin per g of raw material used for extraction. The measurement of yield for quercetin, kaempferol, catechin, luteolin, and gallic acid was also similar to rutin. All the appeared peaks are shown in Fig. 4a, b and 4c.

HPLC analysis

Table 3 shows the list of bioactive compounds in *C. asiatica* extracts resulting from HPLC analysis. Six bioactive compounds (gallic acid, rutin, kaempferol, catechin, quercetin, and luteolin) are traced with a different concentration in the extracts. In water extract, rutin is found to be the most abundant compound with 174.7 mg/g, followed by quercetin (36.3 mg/g) and kaempferol (33.0 mg/g).

A different trend of bioactive compound concentrations can be witnessed in the case of ethanolic extract, as shown in Table 3. From this table, an ethanolic extract presents the highest value of kaempferol with 373.2 mg/g, pursued by quercetin, rutin and luteolin with 77.6, 15.0, and 2.7 mg/g respectively. However, an ethanolic extract is detected with the absence of gallic acid and catechin.[2] investigated the extraction efficiency of water and 80% methanol on *C. asiatica* herbal tea. The study found that quercetin (282 μg/g in water and 2379 μg/g in 80% methanol extracts), gallic acid (2105 μg/g in water and 3082 μg/g in 80% methanol extracts), rutin (1196 μg/g in water and 4335 μg/g in 80% methanol extracts) and catechin (1498 μg/g in water and 1510 μg/g in 80% methanol extracts) were higher concentration in methanolic extract compared to the aqueous extract. In contrast, kaempferol (4890 μg/g) and luteolin (217 μg/g) compounds were only detected in methanolic extract. These results

showed the same observation with the current study where luteolin appeared in the alcoholic extract (ethanolic and 50% ethanolic extracts). Methanol solvent has been generally found to be more efficient in the extraction of lower molecular weight polyphenols[9] while ethanol solvent has been known as a suitable solvent for polyphenol extraction and is safe for human consumption.[10] The current investigation proved that the ethanol solvent could be applied to extract the polyphenol compounds. The addition of water to the ethanol presented a better performance in extracting all studied compounds even though in a small amount.

It has been established that bioactive compound from flavonoids group was leading in 50% ethanolic extract[26].[26] proved that 50% ethanolic extract gave the highest content of bioactive compounds in *Sambucus nigra L.* flowers. In this study, all of the six bioactive compounds were detected in the 50% ethanolic extract. The list of bioactive compounds from highest to lowest concentrations are ranking as follows: rutin (322.7 mg/g), kaempferol (76.1 mg/g), quercetin (25.2 mg/g), gallic acid (9.3 mg/g), luteolin (1.3 mg/g), and catechin (0.7 mg/g). The reason for the presence of all six bioactive compounds in the solvent might be caused by the addition of a small portion of water that would enhance the extraction efficiency. This case can be observed mainly for catechin as it only appeared in 50% ethanolic extract. The diffusion of extractable bioactive compounds through plant tissues could be improved under the swelling effect of water by increasing the surface area of contact between solvent and solute.[8]

In overall, the highest amount of gallic acid (9.3 mg/g), rutin (322.7 mg/g), and catechin (0.7 mg/g) were detected in 50% ethanolic extract as compared with other two solvents. Interestingly, catechin was only discovered in this solvent extract but with only small concentration, less than 1 mg/g. However, kaempferol (373.2 mg/g), quercetin (77.6 mg/g), and luteolin (2.7) were found the most abundant in the ethanolic extract as opposed to the other solvents. Besides, there are no compounds with the highest concentration detected in water, even though water is exposed to have the highest polarity index among the different studied solvents. This case occurs because not all of the compound can be dissolved in water while the water diffuses in the plant cell. Thus, the less bioactive compound was obtained in the water extract. Among all of these six bioactive compounds in three different extracts, kaempferol, rutin and quercetin are considered as dominant compounds with the amount of more than 15 mg/g, and gallic acid, luteolin and catechin were found in a small amount with less than 10 mg/g. Results of this study showed that *C.asiatica* consist of a similar sequence in term of the highest to the lowest ranking in flavonol groups, starting with kaempferol and followed by rutin, and quercetin same as results reported by.[27] It shows that *C.asiatica* contains a higher amount of quercetin as compared to the vegetables such as broccoli, kale, french beans, celery, onions and cranberries that contain >0.05 mg/g of quercetin17,18.[17] also reported the association between quercetin intake and relative risk of death from coronary heart diseases.

Conclusions

In conclusion, different solvent results in different extraction yield. This study reveals that the exponential equation can be used to calculate the extraction yield of *C.asiatica* extracted in ethanol and its aqueous solvents. The equation gives a small value of percentage error, which is 12.21%. 100% ethanol, as extract solvent showed the best solvent for the extraction of kaempferol, quercetin and luteolin while 50% ethanol was the best solvent in extracting gallic acid, rutin and catechin. From the result, it can be concluded that *C.asiatica* consists of high flavonols and flavanals contents. Therefore, the use of *C.asiatica* as a salad or medicine purposes can give positive side effects as the reported bioactive compounds content play a significant role in contributing to antioxidant activity.

Credit author statement

Both authors (Siti Nuurul Huda Mohammad Azmin & Mohd Shukri Mat Nor) are responsible in conducting experiments related to the project and writing this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by a “Short Term Research Grant Scheme” from Universiti Malaysia Kelantan, Malaysia (R/SGJP/A07.00/01552A/002/2018/000499). The authors would like to thank to Mr. Muhammad Muzakkir bin Abdul Halim, research assistance for this project and Miss Rosliza Ahmad from Centre for Herbal Standardisation, Universiti Sains Malaysia, for her assistance in HPLC analysis.

References

1. Alqahtani A, Tongkao-On W, Li KM, Razmovski-Naumovski V, Chan K, Li GQ. Seasonal variation of triterpenes and phenolic compounds in Australian *Centella asiatica* (L.). *Urb. Phytochem. Anal.* 2015;26:436–443. https://doi.org/10.1002/pca.2578.

2. Ariffin F, Heong Chew S, Bhupinder K, Karim AA, Huda N. Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. *J Sci Food Agric*. 2011;91:2731–2739.
3. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) urban. *J Ethnopharmacol*. 1995;48:53–57.
4. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int J Food Sci Nutr*. 2005;56:287–291.
5. Bessada SMF, Barreira JCM, Barros L, Ferreira ICFR, Oliveira MBPP. Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb. f.: an underexploited and highly disseminated species. *Ind Crop Prod*. 2016;89:45–51.
6. Brinkhaus B, Lindner M, Schuppan D, Hahn EG. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella aslatica*. *Phytomedicine*. 2000;7:427–448.
7. Cheng CL, Koo MWL. Effects of *Centella asiatica* on ethanol induced gastric mucosal lesions in rats. *Life Sci*. 2000;67:2647–2653.
8. Chua LS. A review on plant-based rutin extraction methods and its pharmacological activities. *J Ethnopharmacol*. 2013;150:805–817.
9. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15:7313–7352. https://doi.org/10.3390/molecules15107313.
10. Do QD, Angkawijaya AE, Tran-Nguyen PL, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *J Food Drug Anal*. 2014;22:296–302.
11. Doughari JH. Phytochemicals : extraction methods , basic structures and mode of action as potential chemotherapeutic agents. *Intech*. 2009:1–33.
12. Gbolahan BW, Abiola AI, Kamaldin J, Ahmad MA, Atanassova MS. Accession in *centella asiatica*; Current understanding and future knowledge. *J Pure Appl Microbiol*. 2016;10:2485–2494. https://doi.org/10.22207/JPAM.10.4.02.
13. Gohil KJ, Patel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian J Pharmaceut Sci*. 2010;72:546.
14. Gray NE, Alcazar Magana A, Lak P, et al. *Centella asiatica*: phytochemistry and mechanisms of neuroprotection and cognitive enhancement. *Phytochemistry Rev*. 2018;17:161–194. https://doi.org/10.1007/s11101-017-9528-y.
15. Hashim P, Sidek H, Helan M, Sabery A, Palanisamy UD, Ilham M. Triterpene composition and bioactivities of *Centella asiatica*. *Molecules*. 2011;16:1310–1322.
16. Healthbenefitstimes.com. *Gotu Kola Facts and Health Benefits*. 2019 [WWW Document].
17. Hertog MGL, Hollman PCH, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J Agric Food Chem*. 1992;40:2379–2383.
18. Hollman PCH, Hertog MGL, Katan MB. Role of dietary flavonoids in protection against cancer and coronary heart disease. *Biochem Soc Trans*. 1996;24:785–789.
19. Hussin M, Hamid AA, Mohamad S, Saari N, Bakar F, Dek SP. Modulation of lipid metabolism by *Centella asiatica* in oxidative stress rats. *J Food Sci*. 2009;74:H72–H78.
20. Inamdar PK, Yeole RD, Ghogare AB, De Souza NJ. Determination of biologically active constituents in *Centella asiatica*. *J Chromatogr A*. 1996;742:127–130.
21. Jiang H, Zheng G, Lv J, et al. Identification of *Centella asiatica's* effective ingredients for inducing the neuronal differentiation. Evidence-Based Complement. *Alternative Med*. 2016;2016:1–9. https://doi.org/10.1155/2016/9634750, 9634750.
22. Kolář P, Shen JW, Tsuboi A, Ishikawa T. Solvent selection for pharmaceuticals. *Fluid Phase Equil*. 2002;194–197:771–782. https://doi.org/10.1016/S0378-3812(01)00716-6.
23. Kumoro AC, Hasan M, Singh H. Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves. *Sci Asia*. 2009;35:306–309. https://doi.org/10.2306/scienceasia1513-1874.2009.35.306.
24. Li J, He X, Li M, Zhao W, Liu L, Kong X. Chemical fingerprint and quantitative analysis for quality control of polyphenols extracted from pomegranate peel by HPLC. *Food Chem*. 2015;176:7–11.
25. Lokanathan Y, Omar N, Ahmad Puz NN, Saim A, Hj Idrus R. Recent updates in neuroprotective and neuroregenerative potential of *Centella asiatica*. *Malays J Med Sci*. 2016;23:4–14.
26. Milena V, Tatjana M, Gökhan Z, et al. Advantages of contemporary extraction techniques for the extraction of bioactive constituents from black elderberry (Sambucus nigra L.) flowers. *Ind Crop Prod*. 2019;136:93–101.
27. Mohd Zainol MK, Abdul-Hamid A, Abu Bakar F, Pak Dek S. Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*. *Int. Food Res. J*. 2009;16:531–537.
28. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC Technical Report). *Pure Appl Chem*. 2008;80:2195–2230. https://doi.org/10.1351/pac200880102195.
29. Mustafa RA, Hamid AA, Mohamed S, Bakar FA. Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. *J Food Sci*. 2010;75:C28–C35.
30. Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J Sci Food Agric*. 2000;80:985–1012.
31. Punturee K, Wild CP, Vinitketkumneun U. Thai medicinal plants modulate nitric oxide and tumor necrosis factor-α in J774. 2 mouse macrophages. *J Ethnopharmacol*. 2004;95:183–189.
32. Puttarak P, Dilokthornsakul P, Saokaew S, et al. Effects of *Centella asiatica* (L.) Urb. on cognitive function and mood related outcomes: a Systematic Review and Meta-analysis. *Sci Rep*. 2017;7:10646.
33. Rahman MM, Sayeed MS Bin, Haque MA, Hassan MM, Islam SMA. Phytochemical screening, antioxidant, anti-Alzheimer and anti-diabetic activities of *Centella asiatica*. *J Nat Prod Plant Resour*. 2012;2:504–511.
34. Rattanakom S, Yasurin P. Chemical profiling of *Centella asiatica* under different extraction solvents and its antibacterial activity, antioxidant activity. *Orient J Chem*. 2015;31:2453–2459.
35. Ravi A, Khan S, Suklabaidya M, Sudarsanan PS, Chandrasekar K. Antioxidant activity and in silico analysis of *Centella asiatica* and Indigofera aspalathoides in psoriasis. *Biomed. Pharmacol. J*. 2018;11:1403–1412.
36. Rijstenbil JW, Wijnholds JA. HPLC analysis of nonprotein thiols in planktonic diatoms: pool size, redox state and response to copper and cadmium exposure. *Mar Biol*. 1996;127:45–54.
37. Sabaragamuwa R, Perera CO, Fedrizzi B. *Centella asiatica* (Gotu kola) as a neuroprotectant and its potential role in healthy ageing. *Trends Food Sci Technol*. 2018;79:88–97. https://doi.org/10.1016/j.tifs.2018.07.024.
38. Schaneberg BT, Mikell JR, Bedir E, Khan IA, Nachname V. An improved HPLC method for quantitative determination of six triterpenes in *Centella asiatica* extracts and commercial products. *Die Pharm. Int. J. Pharm. Sci*. 2003;58:381–384.
39. Sharma C, Sharma P, Ghosh S, Bhutia S, Pal P, Mohanty JP. Impact of geographical variation on medicinal plant species. *Univers. J. Pharm. Sci. Res*. 2017;3:1–7.
40. Singh S, Gautam A, Sharma A, Batra A. *Centella asiatica* (L.): a plant with immense medicinal potential but threatened. *Int J Pharmaceut Sci Rev Res*. 2010;4:3.
41. Tiwari P, Kumar B, Mandeep K, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Int Pharm Sci*. 2011;1:98–106.
42. Yao C, Yeh J, Chen Y, Li M, Huang C. Wound-healing effect of electrospun gelatin nanofibres containing *Centella asiatica* extract in a rat model. *J. Tissue Eng. Regen. Med*. 2017;11:905–915.
43. Yasurin P, Sriariyanun M, Phusantisampan T. Review: the bioavailability activity of *Centella asiatica*. *KMUTNB Int. J. Appl. Sci. Technol*. 2016;9:1–9. https://doi.org/10.14416/j.ijast.2015.11.001.