PROFILING OF *CENTELLA ASIATICA* (L.) URBAN EXTRACT

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Abstract

Centella Asiatica is one of those phytochemical that has been consume for hundreds years and it is claimed that the plant possess various healing effect and antioxidant properties. For many years, a lot of commercial and medicinal researches have been focusing their resources on this plant. Hence, the profiling of this plant is vital. This study was done to investigate the behaviour of active components in two different accessions commercially grown in Johore Bahru. Research procedures were carried out according to the modified method utilizing TLC and HPLC analysis method. The findings suggested that in different parts of Centella Asiatica contain different amount of phytochemicals. The highest concentration of phytochemicals was found in the second accession that was asiaticoside (2.56 ug/ml), madecasoside (5.30 ug/ml) and asiatic acids (3421.60 ug/ml). Leaves contain a higher concentration of those phytochemicals relative to the petioles and the roots.

Keywords : Profiling, Centella Asiatica , TLC, HPLC

Introduction

Centella asiatica, or locally known as pegaga, is a weekly aromatic smelling herb of the family Umbelliferare. It has been used widely in folk medicine for hundreds of years to treat a wide range of illness [1]. This medicinal plant is widely spread in many Asian countries. It is used in different continents by diverse ancient cultures and tribal groups. In India, it is described under the name of Mandukaparni and used in Ayurveda medicine. It is also listed as one of the Traditional Chinese Medicine (TCM) in China. It has been used as a support for faster healing of small wounds. The plant extract has been incorporated into the Indian pharmacopoeia and recommended not only for wound healing but especially for the treatment of skin diseases such as eczema, leprosy and psoriasis [3]. In addition, it is also used in the treatment of burns, itching and insect bites. In contrast with other medicinal plants, this plant has been subjected to quite extensive experimental clinical investigations due to its ability to heal, relieve and recover human being from various pain and sickness. Of the entire genus Centella, only the asiatica species are in the commercial drug today, which is available in the German market as Centellae asiaticae herba, *Centella asiatica* hom. Generally, the herbal material comprises wild plants that are collected in India, Sri Lanka, Madagascar and South Africa. The areal parts of the plant are used for medicinal purposes. These can be harvested throughout the year, and later are dried under the sun. Then, there are stored in closed containers under cool and dry conditions prior to further commercial processing[1].

Foreseeing its potential, it has been chosen as the main interest in this study. The content of active ingredients in the fresh Centella asiatica, and thus the pharmacological activity, is not always constant. The objective of this study is to investigate the existence and distributions of the three active components, namely, asiaticoside, madecassoside and asiatic acid in the different parts of Centella asiatica accessions grew in Malaysian climate and geographic attribute. The plant parts under investigation are the leaf, petiole and root using TLC and HPLC analysis methods.

Material and methods

Plant Materials

Samples of raw and fresh Centella asiatica with commercial maturity (2-4 months) were collected from wet markets in two districts of Johor Bahru, namely, Skudai and Pontian. Three random samples from two different accessions were obtained from these districts. Communications with the seller was done to ensure the samples were originated from the two districts. The whole samples were rinsed with slow running tap water and then disintegrated into its distinctive parts, leaf, petiole and root ready for extraction. Figure 1 shows the first accession and figure 2 shows the second accession of the sampel.

Figure 1. The first accession : small near smooth round leaves with diameter of 3.75cm

Figure 2. The second accession : big edge leaves with diameter of 7.85cm

Extraction

Disintegrated parts of Centella asiatica were extracted according to the modified method of [2] and [5]. Samples were placed in freezer for 8 hours and then in an oven for 80 hours at 35ºC to dry. Later, Xg of each sample (each with three duplicates), namely, leaves, roots, petioles and whole parts were weighted and labelled. Each sample was then mixed uniformly with methanol and water to the ratio of 9: 1 and stirred with hot plate stirrer for 6 hours at ambient temperature in a conical flask. Then, the extracts were filtered with filter papers 125mm Ø and rotary-evaporated until methanol vaporized and finally labeled accordingly.

TLC

TLC analysis was carried out based the modified method of [6]. 3µL of standard and samples were separately spotted on a silica plate. The solvent system utilised is a combination of ethyl acetate: methanol: water (8: 2: 1). The plat was immersed slowly into a development chamber and allowed to develop in the saturated chromatography chamber. The plate was left in the chamber for about 20-30 minutes for the separation of active ingredients. Then, the plate was rinsed and then allowed to dry using hair dryer Various spots were viewed under ultraviolet (UV) light at 365 nm. Finally, the colour and the distance of the unknown spots were compared with the standards and were identified through R_f values which were calculated using the formula :

$$R_f = \frac{\text{distance solvent of migration of substance}}{\text{distance of migration of solvent front}}$$

HPLC

A Water's 515 HPLC Pump was used. The mobile phase applied was methanol and water to the ratio of 8: 2. The HPLC systems consist of a high pressure constant flow pump, a manual injector (20 µL) and Water's 2487 dual λ absorbance detection. Chromatographic separation was performed with a genesis C18 column model 7971 that has a pore size of 4 µm and UV detection at 220 nm. This method is suitable for estimating the bioactive terpene acids in plant material. It is also suitable for the routine assay of pharmaceutical preparations containing a *Centella asiatica* extract. Figure 3 showed the summarized of work procedure.

Results and discussion

TLC

The TLC results in this study are referred to the standard R_f values and the standard colour (Table 1). The standard references for three main phytochemicals in *Centella Asiatica* are asiaticoside 1 mg/ml, madecassoside 3 mg/ml, asiatic acid 10 mg/ml. The TLC results were obtained in those four groups, which the best result was obtained from the second accession originated in Pontian. However, the result of the others three group were not clear and not well spotted. This is due to the low concentration of targeted phytochemicals in the sample. The findings of [6] suggest that at least 8 g of fresh samples are needed to clearly detect the presence of asiatic acid and madecassic acid. However, for asiaticoside as well as madecassoside, the spotted detected are not so clear due to the concentration of the two glycosides being too minute to be detected using TLC.

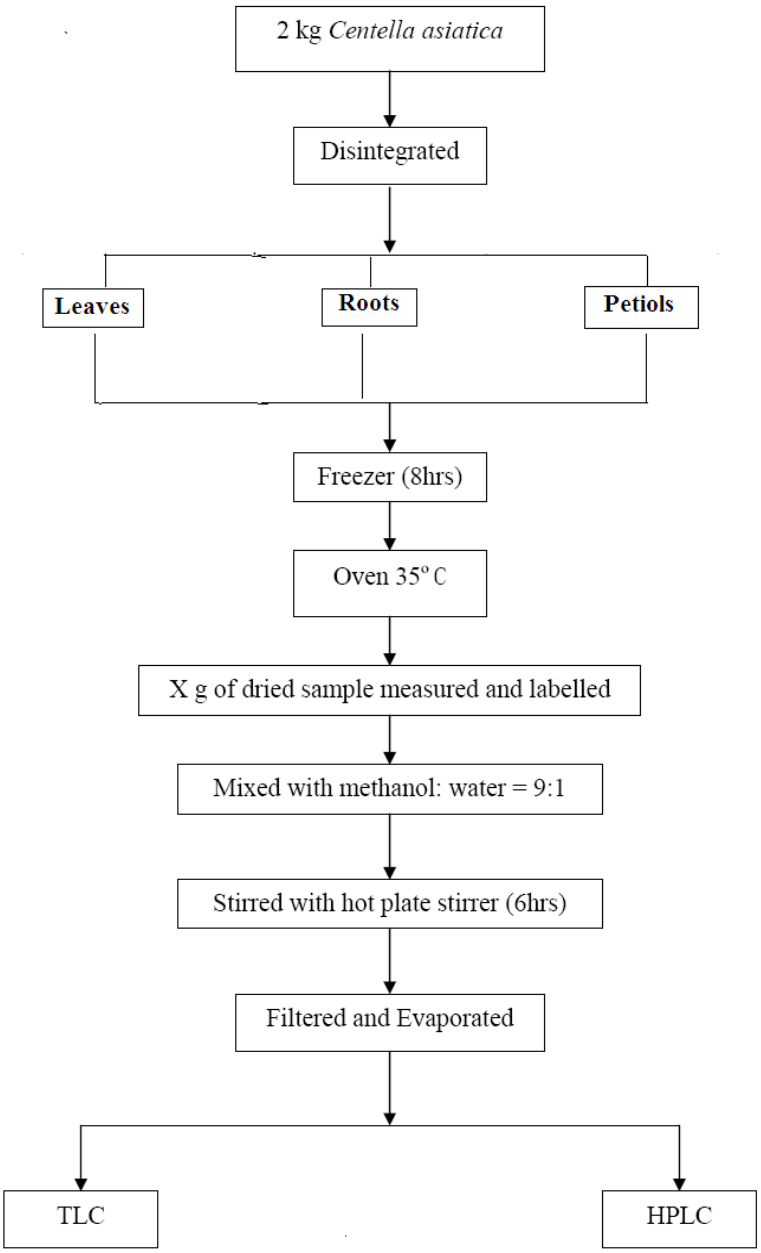

Figure 3. Summarised of work procedure

The three phytochemicals are detected in the fresh sample. No asiaticoside are detected in fresh petioles as well as fresh roots. Both of these are in consistent with the study of [6] and are probably due to the very little amount of spotted volume making them hard to detect.

The findings suggest that the different parts of *Centella asiatica*, contain different amount of the phytochemicals. The reason of high concentration of triterpenoids in the leaf of *Centella asiatica* is in respond to the need for regulation of the pathways for biosynthesis of terpenoids compounds [6].

Table 1. The R_f Values and Colour of Standard Markers of Triterpenoids in Different Plant Parts (second accession: Pontian)

	R_f Values Φ		
	Asiaticoside	**Madecassoside**	**Asiatic acid**
Colour ϕ	Light orange	Dark orange	Blue green
Standard	0.35	0.45	0.59
Plant Parts			
Leaves	0.35	0.45	0.59
Petioles	-	-	0.58
Roots	-	0.45	-

ϕ – visible colour as viewed under UV
Φ – Average R_f Values

TLC analysis alone is not enough to identify the superior accession based on its distribution of bioactive metabolites. Hence, to determine the concentration of each compound present in the distinctive parts of *Centella asiatica,* High Performance Liquid Chromatography (HPLC) method is carried out.

High Performance Liquid Chromatography Analysis
The analysis is preceded with standard calibration curves that are constructed from the chromatograms for the separation of standards asiaticoside, madecassoside and asiatic acid.

Asiaticoside
The leaf extract contained the highest amount of asiaticoside in both of the accessions tested where as the petioles showed the lowest yield as shown in Table 2. Among these, the second accession which is the big edgy leaves from Pontian show the highest yield of asiaticoside. A similar result is reported by [7], who noticed that the leaves of *Centella asiatica* exhibits higher antioxidant activity compared to other plant parts tested. The key compound for antioxidant activity is asiaticoside.

Table 1. Average Concentration of Active Ingredients.

Calculated Average Concentration, C (µg/ml)				
Sample		**Asiaticoside**	**Madecassoside**	**Asiatic acid**
First accession (Skudai)	Leaves	1.14	0.71	0.00
	Petioles	0.17	0.15	0.00
First accession (Pontian)	Leaves	0.39	3.29	0.00
	Petioles	0.00	3.66	571.20
Second accession (Skudai)	Leaves	ND	ND	ND
	Petioles	ND	ND	ND
	Roots	ND	ND	ND
Second accession (Pontian)	Leaves	2.56	5.30	1142.67
	Petioles	0.49	0.00	2390.00
	Roots	0.00	1.57	3421.60

The yield of asiaticoside in the roots of *Centella asiatica* is not significant. This differs from the work of [4], who reported the roots of *Centella asiatica* exhibited higher antioxidative activity than either leaves or petioles. This may be due to the different variety being used, the different determination methods, and possibly, also, due to the synergistic effects of different compounds.

Madecassoside

The yield of madecassoside was vary among the accession and part used (Refer Table 2). It is observed that the petioles of the first accession showed the highest result (3ug/ml). However, the leafs gave the significance existence of madecassoside. It is only absent in the roots of the second accession. The first accession obtained from Skudai showed the lowest yield of madecassoside.

Asiatic Acid

Asiatic acid is the one phytochemical which is present in abundance compared to asiaticoside and madecassoside. However, it is only found in the sample from Pontian in both accession. The highest amount of this phytochemical is found in the roots of the second accession. Root parts showed the highest amount of asiatic acid (Refer Table 2).

Conclusion

There are 15 accessions of *Centella asiatica* in Malaysia, two are used in this study for profiling of *Centella asiatica* using TLC and HPLC for its active compounds, asiaticoside, madecassoside and asiatic acid. Quality and quantification parameters of complex mixtures will eventually lead to developing constitute innovative means of standardizing plant extracts.

TLC analysis managed to identify the presence of compounds in some parts of the plants under investigation. However, targeted compounds are not clearly found due to the concentration of the extracted samples is too minute to be detected using TLC. It is observed that HPLC analysis of active compounds showed variation in the composition from different plant sources. Asiaticoside, the main substance of therapeutic interest are found mostly in the leaf of both accessions. The findings suggested that the second accession was the best in terms of

the concentration of active components. Phytochemicals are not detected in some parts of the plants because the amount of raw material used is too minute.

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