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Morphological and Biochemical Characteristics on Different Accession of Pegaga

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Abstract

Objectives: To examine morphological and biochemical characteristics of commonly available accessions of *Centellaasiatica* in Malaysia. **Methods/Statistical Analysis:** Four accessions of Pegaga designated as UPM01, UPM02, UPM03 and UPM04 were collected throughout Malaysia and their morphological characteristics and biochemical contents were investigated. Flavonoid profiling was carried out using High Performance Liquid Chromatography (HPLC). **Findings:** Accession UPM02 had highest total soluble glucose content with 75.4 mg/g, where lowest in UPM03 39.1 mg/g. Among the four accessions, β-carotene content ranged from 2.11 mg/g to 3.3 mg/g. For flavonoid content, UPM03 had the highest concentration of 8.99 mg/g. The flavonoids present in UPM03 were quercetin, kaempherol, luteolin and rutin while others only had rutin or/and kaempherol. **Application/Improvements:** The results from the present studies could be a valuable information not only for researchers but also the farmers across the country.

Keywords: *Centellaasiatica*, Flavonoid, Leaf Morphology, Secondary Metabolites

1. Introduction

Pegaga or scientifically known as *Centellaasiatica* (L.) urban is a small herbaceous plant growing predominantly in almost all parts of the world. It prefer to grow in shady, damp and wet places This slender creeping plant is commonly found in many parts of India, Asia and the Middle East.

In contrast to other medicinal plants, Pegaga has been subjected to quite extensive experimental and clinical investigation. Particularly, the use of Pegaga extract in the treatment of leprosy and wound healing has shown encouraging results[1]. Herbal preparations made from various parts of the plant including roots and leaves are used throughout the world for treatment of various ailments. Pegaga is used as a tonic for brain by the Indians[2]. Besides, the whole plant of Pegaga is beneficial in improving the memory and general mental ability of intellectually disabled children[3].

In Malaysia, leaves of Pegaga have been used for wound healing, kidney problem and urethritis[4] and grown commercially. Past studies on the morphology of the Pegaga were confined on the varieties of them and specific to the interested chemical constituents only[5,6]. Up to now, two morphologically distinct accessions of Pegaga have been identified in Malaysia, which heavily fringed and smooth leaf margins which are focused on made cassoside and asiaticoside contents in the leaf of pegaga[7]. Most of the researches are done on one variety only[8,9], hardly any research has been done on species varieties and their variation factor such as morphological, genetic and chemical characteristics. Such information is needed and useful in selecting superior accessions, in developing new cultivars and the standardization of raw materials in preparation for pharmaceutical industry. The many accessions of Pegaga which are found in Malaysia cause difficulties among the farmers to identify the elite plant use for plantation since different accessions are expected to contain different beneficial values. The objective of this study is to examine morphological and biochemical characteristics of commonly available accessions of *Centellaasiatica* in Malaysia. This study represents the

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first systematic analysis of the morphology, biochemical constituent and flavonoid compound of Pegaga leaves.

2. Materials and Methods

Four accessions of *C. asiatica* of different morphological characteristics were collected from different geographical localities in Malaysia. These four accessions denoted as UPM01, UPM02, UPM03 and UPM04. Matured plants (one month old) were examined and measured based on their morphological characteristics. The morphological characteristics measured were colour, leaf margin, length and width. The length of petiole and stolon were also measured. All the parameters were recorded in five replicates.

Leaves of individual plant were cut into small portions and divided into two equal parts. The first portion was ground with cold mortar and pestle. Extraction was carried out by homogenizing the powder in 3 ml of cold extraction buffer containing 100 mMTris-HCl (pH 7.8), 1 mM EDTA and 0.1% β-mercaptoethanol. The homogenates were then centrifuged at 10000 rpm for 30 min and the supernatant was used to determine total soluble protein and total soluble glucose content. For the second parts of the leaves, a portion was used to determine β-carotene content while other for chlorophyll determination. For flavonoid content, dry leaves were used. Each assay was conducted twice in an individual plant, with five plants for each accession.

Determination of total soluble protein content was based on Bradford method (1976). Total soluble glucose content was quantified as glucose used as standard via Anthrone method. The β-carotene content was determined based on the method described[10]. The amount of the β-carotene was determined as mg of β-carotene equivalent by using an equation obtained from the standard curve of β-carotene. The chlorophyll content was determined using Jeffrey and Humphrey (1975) method.

In determination of total flavonoid content, the sample was extracted and hydrolysed. For HPLC analysis, samples were analysed using a Water (Milford, MA, USA) liquid chromatograph comprising an Empower chromatography manager, a 717 plus auto injector, 501 HPLC pumps and a 486 tunable absorbance detector.

The data were analyzed by one-way ANOVA. Mean values were compared by Duncan's multiple range test at 5% ($p = 0.05$) significance level, using software SPSS version 11.5 (SPSS Inc. USA).

3. Results and Discussion

Morphological characteristics based on the leaf colour showed that three shades of colours were observed among the four accessions in Table 1. Both accessions of UPM01 and UPM03 are light green in colour while UPM04 is dark green and UPM02 is green. Basically, the leaf margins of *C. asiatica* can be divided into three different groups. There are crenulate (having wavy edge), crenate (having a surface with rounded projection) with crenulate base and crenate with dentate base (edged with tooth-shaped projection) been detected in the four accessions as shown in Table 1. Accession UPM02 was found having crenate with crenulate base based leaf margin while crenate with dentate based leaf margin was determined in accessions UPM04. However, both of the accessions UPM01 and UPM03 are having the crenulate margin.

In terms of length of leaf expressed in centimetres, *C. asiatica* accession UPM03 has the longest leaf length followed by UPM01, UPM04, and UPM02 as shown in Table 1. The width of the leaves overall is narrower than the length of the leaves. The widest leaf was also found in UPM03while the narrowest was identified in UPM02. A comparison of *C. asiatica* showed that there were statistically significant differences in quantitative morphological traits among the specimens originate from different places. Meanwhile, UPM03 also exhibited the longest petiole and stolon with 18.78 ± 1.26 cm and 15.70 ± 1.40 cm, respectively. The length of the petiole between UPM02 and UPM04 did not show any significant difference. Apparently, the leaves length varied significantly are mostly related to the location. There are 16 accessions of *C. asiatica* collected from several geographical locations in India were found to show wide distinction in morphology[11].

Table 1. Morphological characteristics based on colour, margin, length and width of leaf as well as length of petiole and stolon of four accessions of *C. asiatica*.

Accession	Leaf Length (cm)	Leaf Width (cm)	Petiole (cm)	Stolon (cm)
UPM 01 	4.88 ±0.21 [b]	4.74 ±0.36[b]	9.32 ±0.61[b]	11.14 ±0.68[b]

UPM 02 	1.24 ±0.11[d]	1.32 ±0.08[d]	2.90 ±0.61[c]	2.68 ±0.53[d]
UPM 03 	7.28 ±0.73[a]	5.94 ±0.67[a]	18.78 ±1.26[a]	15.7 ±1.40[a]
UPM 04 	2.56 ±0.34[c]	1.88 ±0.30[c]	3.01 ±0.38[c]	6.82 ±0.61[c]

Different letters indicate the values are significantly different ($p \leq 0.05$).

The soluble protein content of one month old leaf extracts was determined. Notably, there was no significant difference among them of soluble protein content (data not shown).The amount of total soluble protein detected in *C. asiatica* accession UPM01 contained 9.03 ± 0.03 mg/g Fresh Weight (FW) higher than 0.52 mg/g FW that was detected in Bangladesh *C. asiatica*[8].

Biochemical differentiation based on the total soluble glucose content revealed that leaf tissues of UPM02 contain the highest amount of total soluble glucose, i.e. 75.40 ± 2.80 mg/g FW, which was around two-fold higher than other accessions Figure 1 followed by UPM04 with 49.38 ± 0.22 mg/g FW and UPM01 with 40.30 ± 3.10 mg/g FW. The least total soluble glucose content was observed in UPM03 (39.10 ± 0.83 mg/g FW).The variation in the protein content of *C. asiatica* might be due to more rapid synthesis or less rapid degradation. The increase rate of respiration combined with the lower rate of carbohydrate accumulation suggested that there is a greater energy requirement in plants. Some physical factors such as seasonal, geographical, light density and quality, photoperiod and soil condition is correlated to affect the nutrient levels in plants[12].

Figure 2A showed significant differences in chlorophyll content detected among the leaf tissues of the four accessions. UPM01 was found having higher chlorophyll a (0.08 ± 0.00 mg/g FW) than chlorophyll b (0.05 ± 0.00 mg/g FW). A similar pattern was observed on UPM03. UPM03 was detected having the highest chlorophyll a (0.14 ± 0.01 mg/g FW) and chlorophyll b (0.09 ± 0.01 mg/g FW) while UPM04 having the least amount chlorophyll a (0.01 ± 0.00 mg/g FW) and chlorophyll b (0.02 ± 0.00 mg/g FW). Both UMP 01 and UMP 03 have higher chlorophyll a than b might be due to the reason that both of them had the same leaf margin which is crenulate. Besides, more photosynthetic pigment is needed for the bigger leaf to gain more energy as chlorophyll is an indicator of photosynthetic activity and is also believed to take part in the process of organoganesis[13].

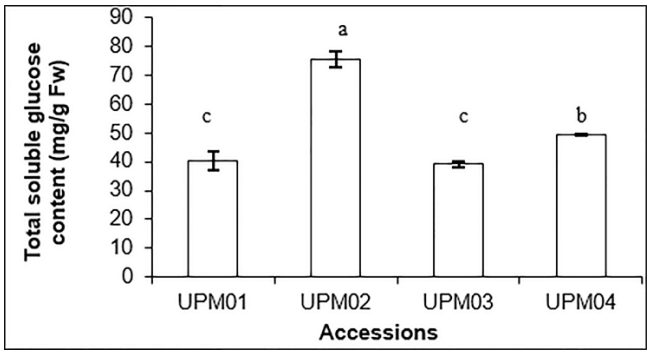
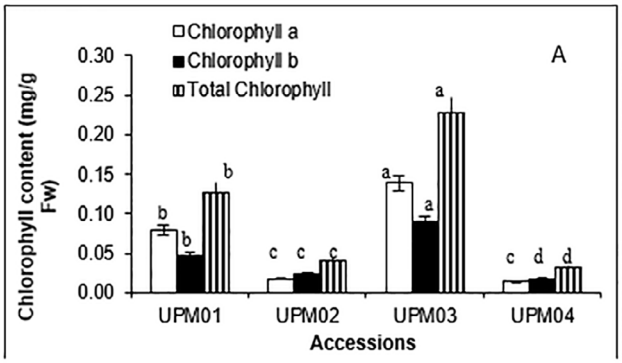


Figure 1. Total soluble glucose content in one month old leaf of four accessions of C. asiatica. Bar indicates the standard error of mean (n = 3). Different letters indicate the values are significantly different ($p \leq 0.05$).

There was significant difference of β-carotene content within the four accessions of *C. asiatica*. Among the four accessions, UPM01 have the highest β-carotene content (3.03 ± 0.02 mg/g FW), followed by UPM02 with 2.33 ± 0.02 mg/g FW and UPM03 with 2.26 ± 0.02 mg/g FW of β-carotene content in Figure 2B. The lowest amount of β-carotene was found in UPM04 with 2.11 ± 0.06 mg/g FW. β-carotene is commonly found in yellow, orange and green leafy vegetables. It is a provitamin A and potent antioxidant. Deficiency in vitamin A will cause serious eye sight problem and become blind eventually.



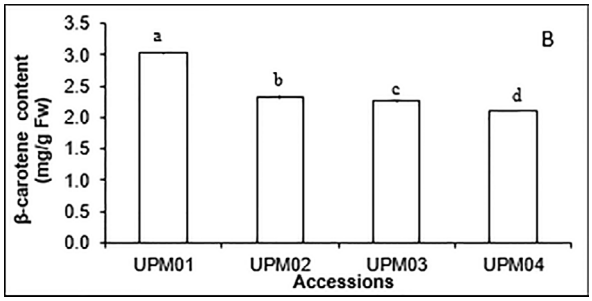


Figure 2. Chlorophyll a, chlorophyll b and total chlorophyll content (A) and β- carotene content (B) in one month old leaf from four accessions of C. asiatica. Bar indicates the standard error of mean (n = 3). Different letters indicate the values are significantly different ($p \leq 0.05$).

The results for total flavonoids content in the studies of *C. asiatica* was presented in Figure 3A. Highest hydrolysed flavonoid content was found in UPM03 (9.0 ± 0.35 mg/g Dry Weight [DW]) as well as the unhydrolysed flavonoid content with 4.13 ± 0.07 mg/g DW. The unhydrolysed flavonoid content detected in the leaf tissues of the *C. asiatica* range from 1.00 ± 0.13 to 4.13 ± 0.07 mg/g DW.

Significant differences were found in the quantitative proportions of the main compounds Figure 3B. Leaf tissue of UPM03 had four flavonoid compounds namely; kaempherol, quercetin, luteolin and rutin with 25.06 ± 2.00 mg/g DW, 12.58 ± 0.60 mg/g DW, 2.04 ± 0.10 mg/g DW and 0.70 ± 0.10 mg/g DW, respectively. Meanwhile, UPM01 and UPM04 contained two types of flavonoids, rutin and kaempherol. There were about 25.32 ± 2.00 mg/g DW of kaempherol and 9.06 ± 1.10 mg/g DW of rutin presented in accession UPM01. Kaempherol in accession UPM04 was about 1.5-fold higher than that in UPM01. However, only rutin was present in UPM02 with 1.79 ± 0.20 mg/g DW. Rutin was presented in all the accessions, although in hydrolyzed samples this can be explained that rutin's glycoside chains were not been completely segregated to form quercetin[14]. Seemingly, no quercetin was detected in some of the accessions. Generally, it was observed that the hydrolysed flavonoid content was about two-fold higher than the unhydrolysed flavonoid content among all the four accessions of *C. asiatica*. The results suggested that there are two single phenylbenzopyran rings which combined together as unhydrolysed flavonoid present in the *C. asiatica*. The variability in the content of evaluated compounds among the populations may be attributed to the different environmental conditions of sampling sites. Besides that, the variety of flavonoids content among the four accessions might also be caused by repetitive or unique sequences that regulated the transcriptional production through enzyme action. The interaction between the extra-chromosomal inheritances in combination with environmental factors might also play some role on secondary metabolites of *C. asiatica*[15]. The yield of made cassoside was vary about 78% between the two accessions of *C. asiatica* collected from Johor Bharu, namely, Skudai and Potian[4].

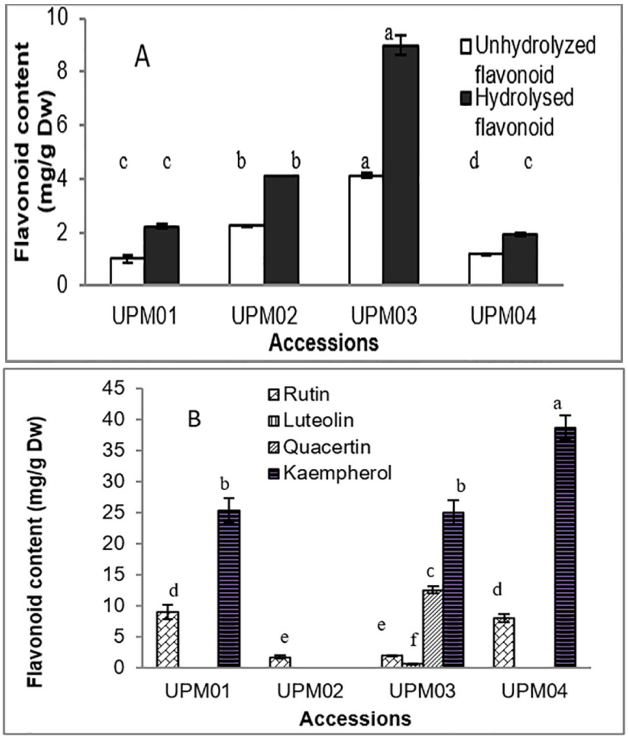


Figure 3 . Unhydrolysed flavonoid and hydrolysed flavonoid content (A) and flavonoids profiles (B) in one month old leaf of four accessions of C. asiatica. Bar indicates the standard error of mean (n = 3). Different letters indicate the values are significantly different ($p \leq 0.05$).

4. Conclusion

It is concluded that there was a significant difference among the *C. asiatica* accessions in term of total soluble glucose, chlorophyll, β-carotene and flavonoid content as well as individual flavonoid compounds. UPM03 has shown a low quantity of soluble glucose and high content in chlorophylls and flavonoid. With the large leaf, UMP03 is found to be a potential accession that can be recommended to the farmer in order to gain the highest benefit

from eating or drinking the juice of Pegaga. It also has great potential to be developed herbal products, applicable to food and nutraceutical industries.

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