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Original Article

Evaluation of nutritional and chemical compositions and morphology of horny little devil (*Smilax myosotiflora*) from different regions in Malaysia

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Abstract

Smilax myosotiflora is a wild creeping plant that was scientifically proven to possess antioxidant, aphrodisiac and synergistic effects. However, the composition of the plant can be varied by locations and this will affect the efficacy of the plant bioactivities. Therefore, this study aimed to determine the morphology, and the compositions of nutritional and chemical in the plant from different regions in Malaysia. *S. myosotiflora* was collected from Kelantan, Perak, and Pahang rainforests. The morphology and nutritional composition of the plant were determined through SEM-EDX and proximate analysis accordingly. Total phenolic compounds (TPC) and total flavonoid compounds (TFC) were determined while the GC-MS analyses were run on the plant for its chemical profile. It was found that the morphologies of *S. myosotiflora* tubers were comparable among different areas. Carbon, oxygen and potassium were the main elements with only a low intensity of calcium detected on the surfaces of the Kelantan tubers. *S. myosotiflora* were significantly different in nutritional compounds but not in TPC and TFC between the samples. There were 15 identical compounds detected in the chloroform extract of *S. myosotiflora* samples, where 2-methyl-7-phenylindole was the most abundant. Considering the highest TPC, TFC and the most ingredients obtained through the GC-MS, Perak is the best location to harvest and promote the cultivation of *S. myosotiflora* plant. However, more studies should be performed on *S. myosotiflora* to explore more benefits for the pharmaceutical and agricultural sectors.

Keywords: *Smilax myosotiflora*, morphology, nutritional profile, chemical profile

1. Introduction

Smilax myosotiflora or the horny little devil is a herbaceous creeping plant that grows wildly in the forests of peninsular Malaysia, southern Thailand, Java, Burma, and throughout tropical regions in Southeast Asia (SEA). It has been known in vernacular as 'ubi jaga', 'ubi besi', 'akar tanding', 'akar restong' or 'itah besi' in Malaysia and Indonesia, or as 'Khao-yen bai bang' and 'Lek thong daeng' in Thailand (Rosdi, Sul'ain, Darnis, & Ishak, 2022). The species originated from the monocotyledon family of Smilacaceae, the second largest family in the Liliales order.

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The hooked thorns allow *S. myosotiflora* to hang onto and grow over soils and surfaces up to 10 m height. Its leaves are light green, smooth and broadly elliptic 4-17 cm long, while the tubers are dark brown with rough surface, very hard, have an irregular round shape and are covered with the hairy roots. *S. myosotiflora* grows best in moist soil with pH 5-6, rich in humus and nutrients, sheltering under the bigger trees in lowland and hilly areas with good drainage (Jones & German, 1993).

The *S. myosotiflora* tubers have been documented as the most functional part of the plant, and were used by locals and the aboriginal people in Malaysia and the southern part of Thailand for various therapeutic effects (Mohammad, Milow, & Ong, 2012; Rosdi *et al.*, 2022). Over the generations, *S. myosotiflora* was pronounced as an aphrodisiac, a lumbago reliever, an energy booster, restoring vitality and libido, and has been used to treat rheumatism and syphilis traditionally (Nurul Ayuni *et al.*, 2018; Ong, & Azliza, 2015 & Ahmed, Fatimah, Siti Zaiton, & Parveen, 2015; Zaki, Gandaseca, Mohd Rashidi, & Ismail, 2019). In numerous scientific studies, it was proven to possess the aphrodisiac, synergistic, antioxidant and anthelmintic effects, thus having potential to treat several critical medicinal problems (Chyang, Mustapa, & Ambia, 2018; Dasuki, Khaizil, Emylia, Noor Izani, & Mohsin, 2012; Mustaffar Bakri, 2013; Rahman, Fatt, & Sulaiman, 2010; Wan, Ahmad, & Sul'ain, 2013). However, the composition of the plant may vary by area and this could impact its efficacy of the functional activities.

Though the distribution of wild *S. myosotiflora* plants in Malaysia shares similar climate, studies have shown that other factors such as local microclimates, temperature, light intensity, soil composition, and plant collection period influence the morphology and composition of the plant (Alcántara-Ayala *et al.*, 2020; Bouba *et al.*, 2012; Kosanic, Anderson, Harrison, Turkington, & Bennie, 2018; Zhang *et al.*, 2018). The distinctive effects on the plant in response to those variations could lead to an inconsistency in the bioactive compounds of *S. myosotiflora* and its pharmacological activities. Therefore, this study was performed to evaluate the profiles of *S. myosotiflora* from three locations in Malaysia by investigating its morphology as well as nutritional and chemical compositions. These data are essential for theoretical foundations, knowledge and technical support in medicinal plant quality control, which will contribute to the benefit of pharmaceutical and agricultural sectors.

2. Materials and Methods

2.1 Plant materials and sample preparation

S. myosotiflora were harvested from the forests of Kelantan (4°50'55.3"N 102°03'11.5"E), Perak (5°29'31.6"N, 101°26'26.6"E) and Pahang (4°41'14.2"N, 102°06'33.8"E) as shown in Figure 1, with the help of the aboriginal people and local villagers. The *S. myosotiflora* tubers were cleaned under the running tap water to remove any surface pollutants, and the hairs were discarded concurrently. *S. myosotiflora* tuber samples were prepared following a previous study (Wan, Ahmad, & Sul'ain, 2016). Cleaned samples were dried in a ventilated drying oven (Memmert, Germany) at 40-50°C for a few days. Then a power grinder (Golden Bull, Malaysia) was applied to obtain powdered samples. Prior to that, the dried

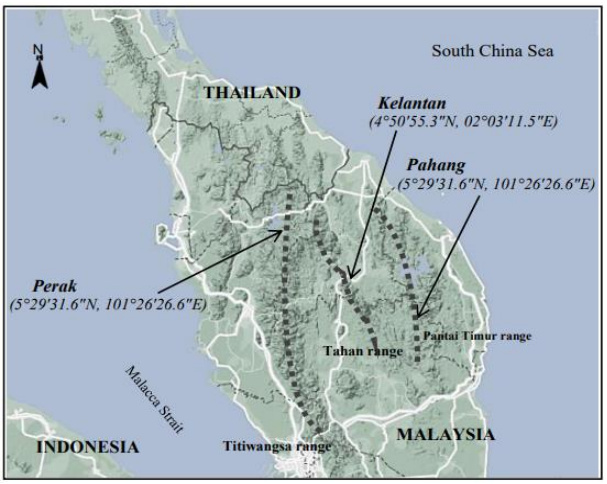

Figure 1. *S. myosotiflora* harvesting locations in Kelantan, Perak & Pahang, in Malaysia

tubers were broken down into smaller pieces using a mortar and a pestle. The ground tubers were passed through a 200-mesh sieve to obtain the fine powder. The powder samples were kept in tightly sealed containers at 4ºC until use.

2.2 Morphological evaluation

Scanning electron microscope (SEM) analysis was carried out to investigate the morphology of *S. myosotiflora*. The powder samples were mounted on carbon stubs and sputter coated with gold (Leica, Germany) for 15 min. The coated *S. myosotiflora* samples were viewed at 20 kV with field emission SEM (Fei, Switzerland). Energy-dispersive X-ray (EDX) spectroscopy was done for quantitative analysis of the elemental composition of *S. myosotiflora* tubers at 20kV on an EDX spectrometer (Quanta, US) equipped with an X-flash detector.

2.3 Nutritional analysis

Proximate analysis was commenced to determine the total ash, calories, carbohydrates, crude fat, moisture, and crude protein of *S. myosotiflora* in accordance to Ng *et al.* (2020) and the official standard methods of the Association of Official Analytical Chemists (AOAC, 2005) with slight modifications. The total ash content was assayed by incinerating the powder in a muffle furnace at 550°C for three hours (AOAC 930.05). For the calorie measurement, a bomb-calorimeter (IKA-WERKE, Germany) system and software were used to determine the calorific values of the samples. The carbohydrate contents of *S. myosotiflora* tubers were estimated by difference of crude protein, crude fat, moisture and total ash (Method of Analysis of Nutrition Labelling AOAC). Soxhlet extraction with petroleum ether as its solvent was conducted to determine the amount of crude fat in the samples (AOAC 936.15). *S. myosotiflora* moisture was determined gravimetrically by drying the sample overnight in a hot air oven at 105°C (AOAC 931.04). For crude protein quantification, semi-micro Kjeldahl method was applied using a Kjeldahl analyzer (FOSS, Denmark) with the nitrogen conversion factor of 6.25 (AOAC 991.20).

2.4 Total phenolic and flavonoid content (TPC and TFC) assays

Fine ground *S. myosotiflora* tuber sample of 10 mg was stirred in 10 mL distilled water (dH_2O) at room temperature for three hours before filtering through Whatman no. 1. Then, the filtrate was used to determine the total phenolic and flavonoid contents in the *S. myosotiflora* quantitatively. The use of water in this study as the extraction solvent was intended to imitate a common way on how the plant is prepared and used in folk medicine as reported by Rosdi *et al.* (2022).

The TPC of the plant was determined using a 96-well microplate with Folin–Ciocalteu method from Mangao *et al.*, 2020 with slight modifications. In brief, 20µL of *S. myosotiflora* (1mg/mL) or gallic acid and 100µL of 10% Folin–Ciocalteu reagent was aliquoted into the 96-well plate. After 5 min, 80µL of 700mM sodium carbonate (Na_2CO_3) was added to the same wells, followed by two hours of incubation in the dark. The absorbances of samples were measured at 765nm. The *S. myosotiflora* TPC was expressed in mg GAE/g DW (mg gallic acid equivalent per gram of dry weight used) based on the calibration curve for gallic acid (12.5-400mg/L with R^2=0.99). The gallic acid dilutions of 10-400 mg/mL were used as calibration standards.

Meanwhile, the TFC of *S. myosotiflora* was quantified using aluminium chloride colorimetry in 96-well plate as described by Sembiring, Elya, and Sauriasari (2018) with slight modifications. The dH_2O, 100µL and 10µL sodium nitrate (50mg/mL) were added to the well followed by 25µL *S. myosotiflora* filtrate (1mg/mL) or quercetin (10–200 mg/L) as the standard. After 5 min, 15µL aluminium chloride (100mg/mL) and 50µL of 1M sodium hydroxide were added. The absorbance of the sample mixture was determined at 510 nm against a blank, and the TFC value was expressed in mg QE/g DW (mg quercetin equivalent per gram of dry weight sample used), based on the calibration curve for quercetin (12.5-400mg/L with R^2=0.99). TPC and TFC assays were run in triplicates and the readings were done using a multimode microplate reader (Thermo Scientific, US).

2.5 GC-MS analysis

S. myosotiflora fine powder was macerated in chloroform overnight to form the *S. myosotiflora* chloroform extract (SMCE). The SMCE was dissolved in methanol and dichloromethane (50:50) before injecting into the GC-MS system equipped with a 5975 Mass Selective Detector (Agilent, USA) and a HP-5 MS capillary column (30 m length x 0.25 mm internal diameter with 0.25 µm film thickness). Samples were run simultaneously using a modified method based on Zubair *et al.* (2017) where the carrier gas was 99.9 % helium at a constant flow rate 1.5 mL/min. The temperature of the injector was initially 300 °C prior to the injection of a 1.0 µL sample. The temperature program was as follows: initial temperature 150°C held for 1 min, then ramping at a rate of 10 °C/min up to 290 °C held for 5 min. The temperature of mass spectra determination (MSD) transfer line was 300°C. The MSD was operated in electron ionization (EI) mode, with an ionization energy of 70eV, and the mass range scanned was 3–500 *m*/*z*. The temperature of ion source was 230 °C while the MS quadrupole was at 150 °C. The identification of separated volatile compounds was based on the comparison of their retention time (RT) with those in NIST17 mass spectral library. The relative amount of each component was calculated by comparing its average peak area to the total area (%).

2.6 Statistical analysis

The mean values and standard deviations of nutritional contents, TPC and TFC were calculated according to the duplicate or triplicate readings from three independent experiments. Data were first assessed for normality using the Kolmogorov-Smirnov test. The determination of significant differences was done using one-way ANOVA or Kruskal-Wallis test, followed by Mann-Whitney when not normally distributed. All statistical analyses were performed using GraphPad PRISM Version 6.0 by Dotmatics, California. *P*-value < 0.05 was required to call a significant difference between groups.

3. Results and Discussion

3.1 S. myosotiflora sample preparation

The preparation of *S. myosotiflora* samples in the study was done according to Wan *et al.*, (2016). Figure 3 showcases the original powder of tuber samples and after the total ash evaluation in nutritional analysis. Figure 3(A) exhibits the *S. myosotiflora* powder samples after finely ground and passed through 200-mesh sieve. Even though the samples were prepared through a standard method, the pulverized tubers of *S. myosotiflora* rendered perceivable color nature where *S. myosotiflora* from Kelantan appeared to be more brownish while Perak's was more whitish. All the samples had been collected from their actual habitats, with Kelantan's sample obtained from the forest of Tahan range, while Perak's from Titiwangsa range, and Pahang's from Pantai Timur range (Figure 1). Therefore, it can be claimed that the *S. myosotiflora* plants grew in wild and visibly away from the industrial contamination or waste. However, the variations of the tuber granule's phenotype and physiology are yet attributable by the origins of the geographical harvesting place, climate change, and the divergent collection periods, which may alter the biochemical composition of the soil and affect the yield of the plant (Kosanic *et al.*, 2018; Wang, Tang, Fu, Huang, & Zhang, 2016). Other external factors, for instance the surrounding temperature, sample storage, and handling techniques in the laboratory, also contribute to variability of physicochemical properties of the plant (Kaur, Singh, Ezekiel, & Sodhi, 2009).

Meanwhile, Figure 3(B) displays the residues of the samples after an overnight incineration when determining the total ash content. As can be seen, the incinerated tuber samples appeared in different colors than theirs before. Residues of *S. myosotiflora* from Kelantan were white ash, while the whitish Perak had transformed to firebrick color, and Pahang's to cantaloupe color. Total ash represents the inorganic residues consisted of minerals that remain after the combustion of carbon and fibers, evaporation of moisture, and so forth, from the samples (Chanda, 2014). Hence the distinctive colors of ashes from *S. myosotiflora* as seen in the figure may indicate the presence of various incombustible

minerals such as sodium, aluminum, nickel, calcium, magnesium, silicon or iron in the plants which variably gained from the soils of their origin places. These minerals are essential nutrients contributing to sustainability of growth and yield in the plant (Veeresham, 2012). The present study established that variations in the minerals exist in the same plant species, dependent on the harvesting area.

3.2 Morphological evaluation

The SEM-EDX analysis was applied to the tubers of *S. myosotiflora* to compare the morphologies and elemental compositions among the samples. According to the SEM images in Figure 2, the granules of *S. myosotiflora* tubers were relatively irregular in shape and size. However, there was no distinct observation on the micromorphology of the granules in the samples, except for Pahang's sample which appeared slightly more aggregated than Kelantan's or Perak's (Figure 2: Panels A, B and C). Overall, the individual *S. myosotiflora* tuber granules were polygonal, spherical to angular in shape at the size range of 5-50 µm and possessed roughish irregularities and fragmented surfaces (Figure 2: Panels D, E and F). Figure 2 also shows the elemental compositions on the surfaces of *S. myosotiflora* ground tubers from the EDX analysis. The components of carbon (C), oxygen (O) and potassium (K) were primarily present in all samples at comparable intensities, with only a small amount of calcium (Ca) solely presence in the *S. myosotiflora* from Kelantan.

From these findings, the wild growing plant of honey little devil from three different locations possessed similar micromorphology and chemical constituents except for a low percentage of Ca in Kelantan sample. Though this *Smilax* species signified rather little discrepancy between the samples, many have reported that the environmental factors, for example climate, temperature and soil composition, have high correlation with morphology and chemical composition in the plant (Abdelsalam *et al.*, 2019; Alcántara-Ayala *et al.*, 2020; Backouchi, Aouida, Khemiri, & Jebara, 2015; Yusuf *et al.*, 2020). Those factors can modify the mineral contents in the soil, which eventually affects the nutrient intake of the plants and alters their morphology and biochemical composition (Kosanic *et al.*, 2018). Therefore, even though the variability were less existed in the tubers of the study, the morphology and biochemical contents in other parts, for instances leaves, flowers or buds, should be further investigated for further clarification.

Figure 3. *S. myosotiflora* dried tubers in powder form (A) and after ashing at 550°C overnight for the total ash determination (B)

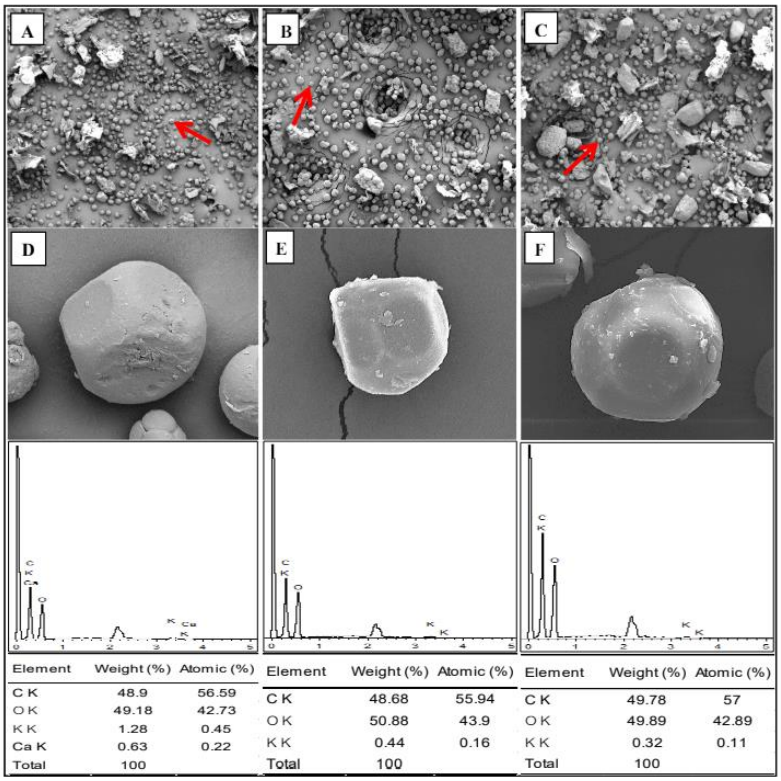

Figure 2. SEM-DEX analysis of *S. myosotiflora* tubers at magnifications 250x and 5000x: Kelantan (A and D), Perak (B and E) and Pahang (C and F), C K - carbon-K, O K - oxygen-K, K K - potassium-K, Ca K - calcium-K

3.3 Proximate analysis

Proximate analysis of total ash, crude fat, crude fiber, protein, moisture, carbohydrate, and protein was performed on the *S. myosotiflora* finely ground samples. Figure 4 outlines the nutritional compositions of the tubers sampled from three states in Malaysia: Kelantan, Perak, and Pahang. According to the graphs, the *S. myosotiflora* tubers exhibited significant differences in several nutritional components between the regions except the TDF and calories. The sample of Kelantan was significantly different with Perak in the percentages of total ash (1.71 ± 0.02 vs 1.31 ± 0.23), crude fat (0.18 ± 0.04 vs 0.38 ± 0.10), moisture (8.65 ± 1.61 vs 5.39 ± 0.62) and carbohydrates (82.57 ± 1.72 vs 87.23 ± 1.33). The percentages of total ash (1.71 ± 0.02 vs 1.04 ± 0.03), crude fiber (15.27 ± 1.38 vs 10.92 ± 0.81), protein (6.58 ± 0.78 vs 3.92 ± 0.12), moisture (8.65 ± 1.61 vs 5.26 ± 0.66) and carbohydrates (82.57 ± 1.72 vs 89.50 ± 0.65) were significantly different between Kelantan and Pahang. The *S. myosotiflora* samples of Perak and Pahang were significantly different only in carbohydrate contents of 87.23 ± 1.33 % vs 89.50 ± 0.65 %. The calories seemed comparable with Perak's sample showing the highest 389.3 ± 28.16kcal/(100g) followed by Kelantan's 387.9 ± 147.20kcal/(100g) and Pahang's 385.4 ± 81.96kcal/(100g).

Based on this study, the variations in nutritional composition also exist in plants growing wild, such as in *S. myosotiflora,* even though their growth was only influenced by the natural factors. According to previous studies, type of soil, moisture, and exposure to environmental factors could lead to modifications in the physicochemical and phytochemical properties, which contribute to the nutritional composition in a plant species (Chanda, 2014; Ogundola, Bvenura, & Afolayan, 2018; Veeresham, 2012). Therefore, for the purposes of phytopharmaceutical development, clinical research, or manufacturing, consistency on the harvesting area is critical as divergence could impact the effectiveness of compounds and their bioactivities. The data manifested that *S. myosotiflora* is a 'high calorie, low fat' plant, which not only can be used for medicinal purposes but also has potential to supplement the diet.

3.4 TPC and TFC assays

Phenolic and flavonoid compounds are among the most functional bioactive ingredients from plants with significant health benefits and play crucial role in the antioxidant, anticancer and aphrodisiac activities (Dasuki *et al.*, 2012; Chittasupho, Manthaisong, Okonogi, Tadtong, & Samee, 2022; Sembiring *et al.*, 2018; Zubair *et al.*, 2017). The TPC and TFC in the *S. myosotiflora* samples were determined using Folin–Ciocalteu and aluminium chloride colorimetry methods. In Table 1, *S. myosotiflora* manifested no significant differences in TPC and TFC levels despite having been harvested from different places and times. Even so, the *S. myosotiflora* from Pahang had the lowest levels of TPC (3.64 ± 0.26 mg GAE/g DW) and TFC (23.43 ± 1.05 mg QE/g DW) among the cases. Meanwhile, Perak's sample possessed the highest TPC and TFC levels at 5.15 ± 0.57mg (GAE/g DW) and 27.48 ± 0.44 mg (QE/g DW).

Based on the data from the three examined *S. myosotiflora* samples, ironically, geographical variations and other environmental factors were imperceptible to impacts the phenolic and flavonoid contents in the tubers, as the levels were fairly similar. Previous studies have evidenced that the contents of phenolics and flavonoids in a plant were mainly influenced by geographical, climate, and other environmental factors such as length of day, temperature, light intensity, and water content in the soil (Danladi *et al.*, 2015; Morreeuw *et al.*, 2021; Zhang *et al.*, 2018). Studies done on the onion (Bibi *et al.*, 2022), mulberry (Zhang *et al.*, 2018), millet (Kumari, Madhujith, & Chandrasekara, 2017), *Melastoma malabathricum* L. (Danladi *et al.*, 2015) and *Moringa oleifera* Lam. (Iqbal & Bhanger, 2006) have demonstrated significant

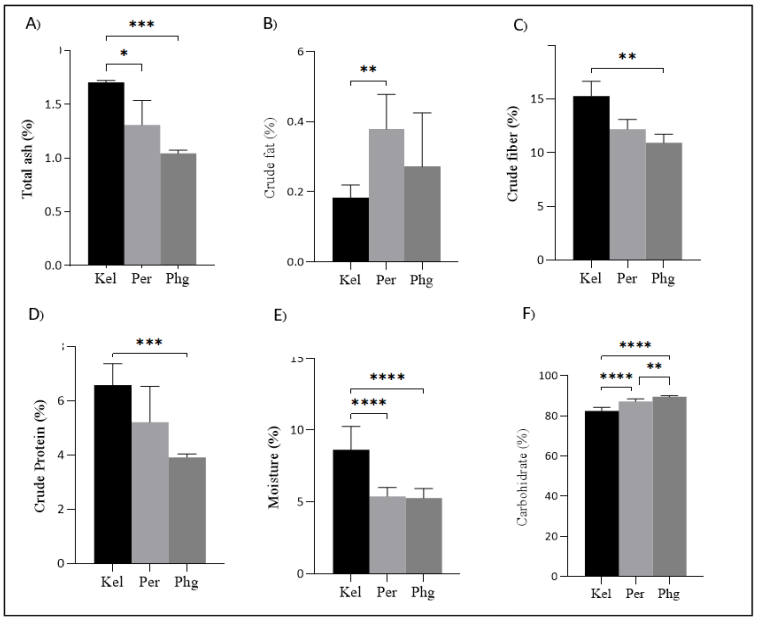

Figure 4. Percentages of the elements in proximate analyses of *S. myosotiflora* sampled from three places: Kelantan (Kel), Perak (Per) and Pahang (Phg). Abbreviations: * - *P*-value < 0.05, ** - *P*-value < 0.005, *** - *P*-value ≤ 0.005, **** - *P*-value < 0.0001

Table 1. TPC and TFC in *S. Myosotiflora*

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
SM Kel	5.13 ± 0.13	24.46 ± 0.31
SM Perak	5.15 ± 0.57	27.48 ± 0.44
SM Phg	3.64 ± 0.26	23.43 ± 1.05

The values are given as mean ± SD. Abbreviations: mg GAE/g DW; mg gallic acid equivalent per gram of dry weight, mg QE/g DW; mg quercetin equivalent per gram of dry weight sample used, SM Kel; *S. myosotiflora* of Kelantan, SM Perak; *S. myosotiflora* of Perak, SM Phg; *S. myosotiflora* of Pahang.

variations in their TPC and TFC induced by the aforementioned factors. Therefore, the findings from this study are in some contrast with those prior studies. Nevertheless, before any conclusion could be made, it is recommended to perform more research on these secondary metabolite contents in the *S. myosotiflora* to confirm the findings.

Meanwhile, Dasuki *et al.* (2012) reported that the TPC of *S. myosotiflora* in methanol extract was 6.55mg GAE/g DW, which was higher than the content in this study. The use of a different extraction solvent to determine the TPC and TFC also explains the variability of phytochemical levels in the plant. An organic solvent with high polarity, for example methanol or ethanol, may enhance the solubility and extraction of complex and high molecular weight compounds, such as the polyphenols. However, for reasons of simplicity and applicability, water has been widely used in traditional medicine as the extraction agent. Its ability to extract high contents of other compounds such as carbohydrates, proteins, and organic acids could interfere the quantification of phenolics and flavonoids comtents in the plant. More research should be conducted using the organic solvent to explore the biochemical compounds in the plant qualitatively.

3.5 Volatile compounds from GC-MS

The chemical constituents in the SMCEs were investigated qualitatively and quantitatively using the GC-MS analysis. According to the data on three examined *S. myosotiflora* samples, 15 constituents were detected and tentatively identified in the SMCEs (Table 2). Although the SMCEs compounds revealed considerably different percentages, overall, 2-methyl-7-phenylindole (RT: 19.772) was the major compound accounting for 3.63 to 15.17% of the total compound contents. In the SMCE Kelantan, it was noticeable that there were a few compounds detected at greater levels than in Perak or Pahang SMCEs, for instance eicosane (RT: 6.257), pentacosane (RT: 8.130), docosane (RT: 9.918), tetracosane (RT: 11.587), 2-methylpentacosane (RT: 13.139) and heneicosane, 3-methyl- (RT: 14.583). Other than that, 1-octadecane (RT: 8.901), nonadecyl trifluoroacetate (RT: 9.659), 1-nonadecene (RT: 10.751), tris(tert-butyldimethyl silyloxy)arsane (RT: 18.986) and 2-methyl-7-phenylindole (RT: 19.772) were found to be comparatively abundant in SMCE Perak, while benzene, (1-methylundecyl)- (RT: 7.357) and bis(2-ethylhexyl) phthalate (RT: 12.799) was the highest in SMCE Pahang. Meanwhile, butyl 9,12-octadecadienoate (RT: 12.186) and octadecane, 3-ethyl-5-(2-ethylbutyl)- (RT: 16.043) only presented in the SMCEs from Perak and Kelantan.

The chemical constituents of *S. myosotiflora* traced using a non-polar solvent, chloroform, displayed almost identical constituents, which mostly were volatile straight chain compounds: alkanes, alkenes, fatty acid esters, and methyl esters. The secondary metabolites of 2-methyl-7-phenylindole from lactone group, which was discovered to be the most prominent compound in the SMCEs, has been reported to have strong correlation with antimicrobial and antiparasitic activities of plants (Bloch, Vijay, Singh, Minna, & Sougata, 2021; Norouzi, Hejazy, Shafaghat, & Shafaghat, 2021; Raj, Vijayakumari, Jebarubi, & Kavitha, 2022). Meanwhile, eicosane, pentacosane, docosane and tetracosane are compounds from alkanes, was the biggest group of phytochemicals detected in the SMCEs. The group is a series of long chain saturated hydrocarbons with single covalent bonds. In the agricultural sector, alkanes are synthesized as part of the epicuticular leaf wax for terrestrial plants, and as a plant chemotaxonomy biomarker (Bush & McInerney, 2013). Therefore, the alkane compounds established in the SMCE

Table 2. List of identified compounds in SMCE from the three regions

No.	RT	Compound name	Formula	Peak area (%)		
				Kel	Per	Phg
1	6.257	Eicosane	$C_{20}H_{42}$	1.04	0.18	0.20
2	7.357	Benzene, (1-methylundecyl)-	$C_{18}H_{30}$	-	0.33	1.05
3	8.130	Pentacosane	$C_{25}H_{52}$	1.56	0.65	0.84
4	8.901	1-Octadecene	$C_{18}H_{36}$	-	1.10	0.47
5	9.659	Nonadecyl trifluoroacetate	$C_{21}H_{39}F_3O_2$	0.48	0.79	-
6	9.918	Docosane	$C_{22}H_{46}$	2.37	0.41	0.68
7	10.751	1-Nonadecene	$C_{19}H_{38}$	0.94	4.17	1.12
8	11.587	Tetracosane	$C_{24}H_{50}$	2.66	0.74	0.95
9	12.186	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	-	1.48	-
10	12.799	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	1.24	1.64	2.58
11	13.139	2-Methylpentacosane	$C_{26}H_{54}$	2.02	0.56	0.72
12	14.583	Heneicosane, 3-methyl-	$C_{22}H_{46}$	1.56	0.31	0.29
13	16.043	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	1.22	-	-
14	18.986	Tris(tert-butyldimethyl silyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	1.07	3.60	1.77
15	19.772	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	3.63	15.17	8.56

Abbreviations: RT; retention time, Kel; SMCE of Kelantan, Per; SMCE of Perak, Phg; SMCE of Pahang, -; not detected

could be developed as a chemotaxonomy profile for *S. myosotiflora* plant. Further investigations are suggested to determine the chemical compositions of mid- and polar compounds in the *S. myosotiflora* plant to solidify the findings.

4. Conclusions

The present study established the qualitative and quantitative morphology, nutritional and chemical compositions including TPC and TFC levels of *S. myosotiflora* samples from Kelantan, Perak and Pahang states in Malaysia. Some significant variations were found in the primary metabolite compounds (nutritional contents) among the samples, for instance in carbohydrates, crude fiber, crude fat, total ash and moisture. In the secondary metabolites, no distinct variations were detected either through EDX spectroscopy, TPC, TFC or GC-MS investigations. On the contrary, even though the micromorphology of the tubers seemed almost identical, the features of the ground powder of *S. myosotiflora* from the three locations were utterly different showing that variability exists in this plant. Despite all, the Titiwangsa range in Perak had the most potential for harvesting *S. myosotiflora* plant due to the highest TPC and TFC, and the most compounds detected in the GC-MS analysis. It is essential to understand the criteria, correlations and deviations of the medical plant contents, for instance in *S. myosotiflora,* to avoid inconsistent quality in pharmaceutical development. Since this is the first study to report on the variability of the promising honey little devil, future studies are strongly suggested in order to optimize the utilization of the plant.

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