Original Article

Morphology, nutritional and chemical compositions evaluations on horny little devil (*Smilax myosotiflora*) from different regions in Malaysia

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Abstract

Smilax myostiflora is a wild creeping plant which scientifically proved to possess antioxidant, aphrodisiac and synergistic effects. However, the compositions of the plant from different locations might vary and affect the efficacy of the plant bioactivities. Therefore, this study aims to determine the morphology, nutritional and chemical compositions of the plant from different regions in Malaysia. *S. myosotiflora* was collected from the Kelantan, Perak and Pahang rainforests. The morphology and nutritional compositions of the plant was determined through the SEM-EDX and proximate analyses accordingly. The total phenolic compound (TPC), total flavonoid

compound (TFC) and GC-MS analyses were performed to determine the plant chemical profile. It was found that the morphologies of *S. myosotiflora* tubers from different areas were comparable. Carbon, oxygen and potassium were the main elements with only low intensity of calcium detected on the surfaces of Kelantan tubers. *S. myosotiflora* were significantly different in nutritional compounds but not in TPC and TFC among 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 most ingredients obtained through GC-MS, Perak is the best location to harvest and promote cultivation of the *S. myosotiflora* plant. However, more studies should be performed on *S. myosotiflora* to profound the findings for the benefits of pharmaceutical and agricultural sectors.

Keywords: *Smilax myosotiflora*; morphology; nutritional; chemical profile.

1. Introduction

Smilax myostiflora or the horny little devil is a herbaceous creeping plant that wildly grows in the forests of Peninsula Malaysia, southern Thailand, Jawa, Burma and throughout tropical climate regions in the Southeast Asia (SEA). It has been known through other vernacular names such as ‘ubi jaga’, ‘ubi besi’, ‘akar tanding’, ‘akar restong’ or ‘itah besi’ in Malaysia and Indonesia or ‘Khao-yen bai bang’ and ‘Lek thong daeng’ in Thailand (Rosdi, Sul'ain, Darnis, & Ishak, 2022). The species is originated from the monocotyledon family of Smilacaceae, the second largest family in the Liliales order. The hooked thorns allow *S. myosotiflora* to hang onto and grow over soils and surfaces up to 10m high. Its leaves are light green, smooth and broadly elliptic from 4-

17cm long while the tubers are dark brown rough surface, very hard and have irregular round shape covered with the hairy roots. In order to become a mature plant, *S. myosotiflora* grows the best in moist soil with pH 5-6, sheltering under the bigger trees, rich in humus and nutrients, as well as good drainages in lowland and hilly areas (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 Southern part of Thailand for many therapeutic effects (Mohammad, Milow, & Ong, 2012; Rosdi *et al*., 2022). Over the generations, *S. myosotiflora* is pronounced as an aphrodisiac, a lumbago reliever, an energy booster, helps to restore vitality and libido and to treat rheumatism and syphilis traditionally (Zaki, Gandaseca, Mohd Rashidi, & Ismail, 2019; Nurul Ayuni *et al*., 2018; Ong, & Azliza, 2015 & Ahmed, Fatimah, Siti Zaiton, & Parveen, 2015). After numerous scientific findings, it was proved to possess aphrodisiac, synergistic, antioxidant and anthelmintic effects, thus potential to become a promising agent in several critical medicinal problems (Chyang, Mustapa, & Ambia, 2018; Wan, Ahmad, & Sul'ain, 2013; Mustaffar Bakri, 2013; Dasuki, Khaizil, Emylia, Noor Izani, & Mohsin, 2012; Rahman, Fatt, & Sulaiman, 2010). However, the compositions of the plant compounds from different areas might be varied and would impact the efficacy of their functional activities.

Though the distribution areas of wild *S. myosotiflora* plants were located in the same climate regions in Malaysia, studies have showed that other factors such as local microclimates, temperature, light intensity, soil compositions and plant collection periods would 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 attributions of a plant in responses to those variations would lead to inconsistency of the bioactive compounds in the potent plant for instance the *S. myosotiflora* for the pharmacological activities. Therefore, this study was performed to evaluate the plant profile of *S. myosotiflora* from three distribution locations in Malaysia by investigating its morphology, nutritional and chemical compositions. The data is essential for theoretical foundations, knowledge and technical support in medical plant qualities determination which will contribute to the benefits 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 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 eradicate any surface pollutants and the hairs were discarded concurrently. *S. myosotiflora* tubers sample was prepared according to 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. The grinding process was performed using a power grinder machine (Golden Bull, Malaysia) to obtain the powdery sample. Prior to that, dried tubers were broken down into smaller pieces using a mortar pastel set. The ground tubers were sieved through a 200 meshes tray to obtain the fine powder. The powder samples were kept in the tightly sealed containers at 4ºC until future use.

2.2 Morphological evaluation

Scanning electron microscope (SEM) analysis was carried out to investigate the morphology of the *S. myosotiflora*. The powder was mounted on the carbon stubs and coated with gold coating in sputter coating (Leica, Germany) for 15 min. The *S. myosotiflora* coated samples were viewed at 20KV with field emission SEM (Fei, Switzerland). The energy-dispersive X-ray (EDX) spectroscopy was done for quantitative analysis of the elemental composition of the *S. myosotiflora* tubers. The analysis was performed 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 with Ng *et al*. (2020) and the official standard methods of the Association of Official Analytical Chemists procedures (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 capture the calorific values of the samples. The carbohydrate content of *S. myosotiflora* tubers were estimated by difference of crude protein, crude fat, moisture and total ash (Method of Analysis of Nutrition Labelling AOAC). The Soxhlet method with petroleum ether as the extract agent was conducted to determine the amount of crude fat in the samples (AOAC 936.15). *S. myosotiflora* moisture was determined gravimetrically after drying the sample overnight using hot air oven method at 105°C (AOAC 931.04). For crude

protein quantification, semi-micro Kjeldahl method was applied using Kjeldahl analyzer unit (FOSS, Denmark) with the nitrogen conversion factor of 6.25 (AOAC 991.20).

2.4 Total of phenolic and flavonoid contents (TPC & TFC) assays

Fine ground tubers, 10mg were stirred in 10mL distilled water (dH_2O) at room temperature for three hours before filtered through the Whatman no.1 to gain the *S. myosotiflora* filtrate. Then, the filtrate would be used to determine the total phenolic and flavonoid contents of *S. myosotiflora* quantitatively. The used of water in this study as the extracting solvent was intended to imitate the common way on how the plant was prepared and used in the folk medicine as according to Rosdi *et al*. (2022).

The TPC of the plant was determined using the 96-well microplate 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 with two hours incubation in the dark. The absorbance of samples was measured at 765nm. The *S. myosotiflora* TPC was expressed as mg GAE/g DW (mg gallic acid equivalent per gram of dry weight used) based on the calibration curve by gallic acid (12.5-400mg/L with R^2=0.99). The gallic acid dilutions (10-400mg/mL) were used as standards of the calibration.

Meanwhile, the TFC of *S. myosotiflora* was quantified using the aluminium chloride colorimetric in 96-well plate method 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 omitted. The absorbance of the

sample mixture was determined at 510nm against blank and the TFC value was expressed as mg QE/g DW (mg quercetin equivalent per gram of dry weight sample used), based on the standard calibration curve of quercetin (12.5-400mg/L with R^2=0.99). TPC and TFC assays were analyzed 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 using chloroform for overnight to form the *S.myosotiflora* chloroform extract (SMCE). The SMCE was dissolved in methanol and dichloromethane (50:50) before injected into the GC-MS system equipped with a 5975 Mass Selective Detector (Agilent, USA) and a HP-5 MS capillary column (30m length x 0.25mm internal diameter x 0.25μm film thickness). Samples were run simultaneously using the modified method based on Zubair *et al*. (2017) where the carrier gas was 99.9% helium at a constant flow rate 1.5mL/min. The temperature of the injector was begun at 300°C prior to the injection of sample, 1.0μL. The temperature program was set as followed; initial temperature 150°C and held for 1 min, then ramping at a rate of 10°C/min up to 290°C 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 the 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 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 compositions, TPC and TFC were calculated according to the duplicate or triplicate readings from three independent experiments. Data was firstly determined for their normality using Kolmogorov-Smirnov test. The determination of significant difference was calculated using one-way ANOVA or Kruskal Wallis test followed by Mann-Whitney when not normally distributed. All statistical analysis was performed using GraphPad PRISM Version 6.0 by GraphPad Software Incorporated Company, California. *P*-value< 0.05 was defined as level of significant difference between groups.

3. Results and Discussion

3.1 S. myosotiflora samples preparations

The preparation of *S. myosotiflora* samples in the study was according to Wan *et al*., (2016) method. Figure 3 showcased the origin powder of tuber samples and after they were subjected to the total ash evaluation in nutritional analysis. Figure 3(A) exhibited the *S. myosotiflora* powdery samples which finely ground and sieved using 200 meshes tray. Even though 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 more whitish. Literally, all samples were collected from their actual habitat where Kelantan's was obtained from the forest of Tahan range while Perak's from Titiwangsa range and Pahang's from Pantai Timur range (Figure 1). Therefore, this can be claimed that *S. myosotiflora* plants grew wildly and visibly away from the interference of industrial contaminations and wastage. However, the variations of the tubers granule's phenotypic

and physiology are yet attributable by the origins of the geographical harvesting place, climate change and the divergent collection periods where it able to alter the biochemical compositions of the soil and cause the yield of a plant varied (Kosanic *et al*., 2018; Wang, Tang, Fu, Huang, & Zhang, 2016). Other external factors for instances surrounding temperature, sample storage and handling technique in the laboratory also able to contribute to the variability physicochemical properties of the plant (Kaur, Singh, Ezekiel, & Sodhi, 2009).

Meanwhile, Figure 3(B) displayed the residue of the samples after an overnight incineration when determining the total ash content. As can be seen, the incinerated tuber samples derived different colour than theirs before. Residues of *S. myosotiflora* Kelantan were white ash while the whitish Perak have transposed to firebrick colour and Pahang's to cantaloupe colour. Total ash content is the inorganic residue consisted of minerals that remained after the combustion of carbon, moisture, fibers and so forth from the samples (Chanda, 2014). Hence the distinctive colour of ash in the *S. myosotiflora* as in the figure may represent the presence of various incombustible minerals such as sodium, aluminum, nickel, calcium, magnesium, silicon or iron in the plants which variably gained through the soil of their origin places. Those minerals work as the essential nutrients in sustainability of growth and yield of the plant (Veeresham, 2012). The present study established that variation of minerals exists in the same plant species from different harvesting areas.

3.2 Morphological evaluation

The SEM-EDX analysis was subjected to the tubers of *S. myosotiflora* to determine a comparative morphological and elemental compositions evaluation among the samples. According to the SEM images in the Figure 2, the granules of *S.*

myosotiflora tubers were relatively irregular in shapes and sizes. However, no distinct observation on the micromorphology of the granules in all samples except Pahang's displayed 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 range of 5-50µm and possessed roughish, irregularities, fragmented surfaces (Figure 2: Panels D, E and F). The Figure 2 also showed the elemental compositions on the surface of *S. myosotiflora* ground tubers using the EDX analysis. The compositions of carbon (C), oxygen (O) and potassium (K) were primarily presence in all samples at a comparable intensity with the existence small amount of calcium (Ca) solely in the *S. myosotiflora*'s Kelantan sample.

From these findings, the wild growing plant of honey little devil from three different locations possessed a corresponding micromorphology and chemical constituents except low percentage of Ca in Kelantan sample. Though this *Smilax* species signified rather less discrepancy between all samples, many have reported that the environmental factor for example, climate, temperature and soil composition have high correlation with the attributions of morphology and chemical compositions in the plant (Abdelsalam *et al*., 2019; Alcántara-Ayala *et al*., 2020; Backouchi, Aouida, Khemiri, & Jebara, 2015; Yusuf *et al*., 2020). Those elements would modify the mineral contents in the soils which eventually affect to the nutrient intake of the plants and alter the morphology and biochemical compositions (Kosanic *et al*., 2018). Therefore, even the variability were less occurred in the tuber, it is recommended to investigate the morphology and biochemical contents from other parts for instances the leaves, flowers or bud to further investigate any diversification potential in the plant.

3.3 Proximate analysis

Proximate analysis which consisted of the total ash, crude fat, crude fiber, protein, moisture, carbohydrate and protein were performed on the *S. myosotiflora* finely ground samples. Figure 4 outlines the nutritional compositions of the tubers from three different states in Malaysia; Kelantan, Perak and Pahang. According to the figure, the *S. myosotiflora* tubers exhibited significant differences in several nutritional compositions between the regions except the TDF and calories. It revealed that the sample of Kelantan was significant differences with Perak for 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). Whereas 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 accordingly. While the *S. myosotiflora* samples of Perak and Pahang were significantly different only in carbohydrate content (%) with (87.23 ± 1.33 vs 89.50 ± 0.65). The value of calories were comparable with each other with Perak's 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 variants of nutritional compositions also exist in wild growing plants such as *S. myosotiflora* even their growth were only influenced by the nature forces. According to the previous studies, type of soils, moisture and exposure to environmental factors could lead to the modifications of the physicochemical and phytochemical properties which contribute to the nutritional composition diversification in a same plant species (Chanda, 2014; Ogundola, Bvenura, & Afolayan, 2018;

Veeresham, 2012). Therefore, for the purpose of phytopharmaceutical development, clinical research or manufacturing, consistency on the harvesting area is critical as the divergent could affect the effectiveness of the compounds and its bioactivity. The data manifested that *S. myosotiflora* is a 'high calorie, low fat' plant which not only can be used for medicinal purposes but also potential as a supplementary diet.

3.4 TPC and TFC assays

Phenolic and flavonoid compounds are among the most functional bioactive ingredients from the plant due to their significant contributions to the health benefits and crucial roles in the antioxidant, anticancer and aphrodisiac activities (Chittasupho, Manthaisong, Okonogi, Tadtong, & Samee, 2022; Dasuki *et al*., 2012; 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 colorimetric methods accordingly. In the Table 1, *S. myosotiflora* manifested no significant different in their TPC and TFC values despite of they had been harvested from different places and time. Even so, the *S. myosotiflora* from Pahang derived the lowest amount of TPC (3.64 ± 0.26 mg GAE/g DW) and TFC (23.43 ± 1.05 mg QE/g DW) compared to the rest. Meanwhile, Perak's possessed the highest TPC and TFC with 5.15 ± 0.57mg (GAE/g DW) and 27.48 ± 0.44 mg (QE/g DW) accordingly.

Based on the data of the three examined *S. myosotiflora* samples, ironically, geographical variations and other environmental factors were imperceptible to impact the production of phenolic and flavonoid content in the tubers after the amounts were barely similar. Previously, studies have evidenced that the content of phenolic and flavonoid in a plant were mainly influenced by the geographical, climate and environmental factors such as day length, 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 variations in their TPCs and TFCs resulted from the aforementioned circumstances. Therefore, the findings from this study was in contrast with the reported studies earlier. Nevertheless, before any conclusion could be made, it is recommended to perform more research on these secondary metabolite contents in the *S. myosotiflora* to scrutinize 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 different extraction solvent to determine the TPC and TFC also explain the variability of the phytochemicals in the plant. The organic solvent with high polarity for examples methanol or ethanol may enhance the solubility and extraction of complex and high molecular weight compounds such as polyphenol. However, due to the reason of simplicity and applicability, water has been widely used in the traditional medicine as the extracting agent. Its ability to extract high content of other compounds such as carbohydrates, proteins and organic acids could interfere the quantification of phenolics and flavonoids in the plant. More research should be conducted using the organic solvent to explore more on 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 of three examined *S.*

myosotiflora samples, 15 constituents were detected and tentatively identified in the SMCEs (Table 2). Although the SMCEs compounds revealed considerably different percentage, overall, 2-methyl-7-phenylindole (RT: 19.772) was the major compound found accounted for 3.63 to 15.17% from the total compound contents. In the SMCE Kelantan, it was noticeable that there were a few compounds that detected greater than in Perak or Pahang SMCEs for instances 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 abundant most 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 Perak and Kelantan accordingly.

The chemical constituents of *S. myosotiflora* traced using a non-polar solvent, chloroform, displayed almost identical constituents which mostly were volatile compounds from the straight chains of alkanes, alkenes, fatty acids ester and methyl esters functional groups. The secondary metabolites of 2-methyl-7-phenylindole from lactone group which was discovered to be the most prominent compound in the SMCEs was reported to have strong correlation with antimicrobial and antiparasitic activities in the plants (Bloch, Vijay, Singh, Minna, & Sougata, 2021; Norouzi, Hejazy, Shafaghat, & Shafaghat, 2021; Raj, Vijayakumari, Jebarubi, & Kavitha, 2022). Meanwhile, eicosane, pentacosane, docosane and tetracosane are the compounds from alkanes, the

biggest group compound detected in the SMCEs. The group is a series of long chain saturated hydrocarbons compounds with single covalent bonds. In agricultural sector, alkanes were synthesized as part of the epicuticular leaf wax for terrestrial plants and as a plant chemotaxonomy biomarker (Bush & McInerney, 2013). Therefore, the alkanes compounds established in the SMCE could be developed as the chemotaxonomy attributions 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 of *S. myosotiflora* from Kelantan, Perak and Pahang states in Malaysia. Some significant variations were found in the primary metabolite compounds (nutritional contents) among the samples for instances in carbohydrates, crude fiber, crude fat, total ash and moisture. For the secondary metabolite, no distinct variation 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 the variability does exist in the plant. Despite of all, Titiwangsa range in Perak was the most potential place to harvest *S. myosotiflora* plant due to its highest TPC, TFC and most compounds obtained from 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 the diversification in the quality pharmaceutical development.

Since this is the first study to report on the variability of the promising honey little devil, future works are strongly suggested in order to optimize the utilizations of the plant.

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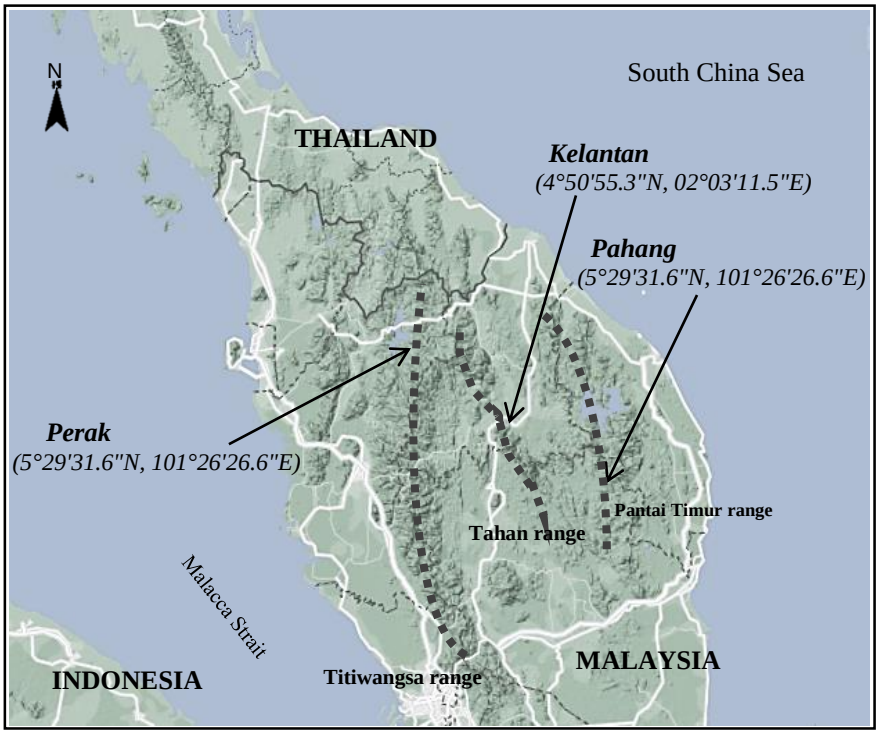
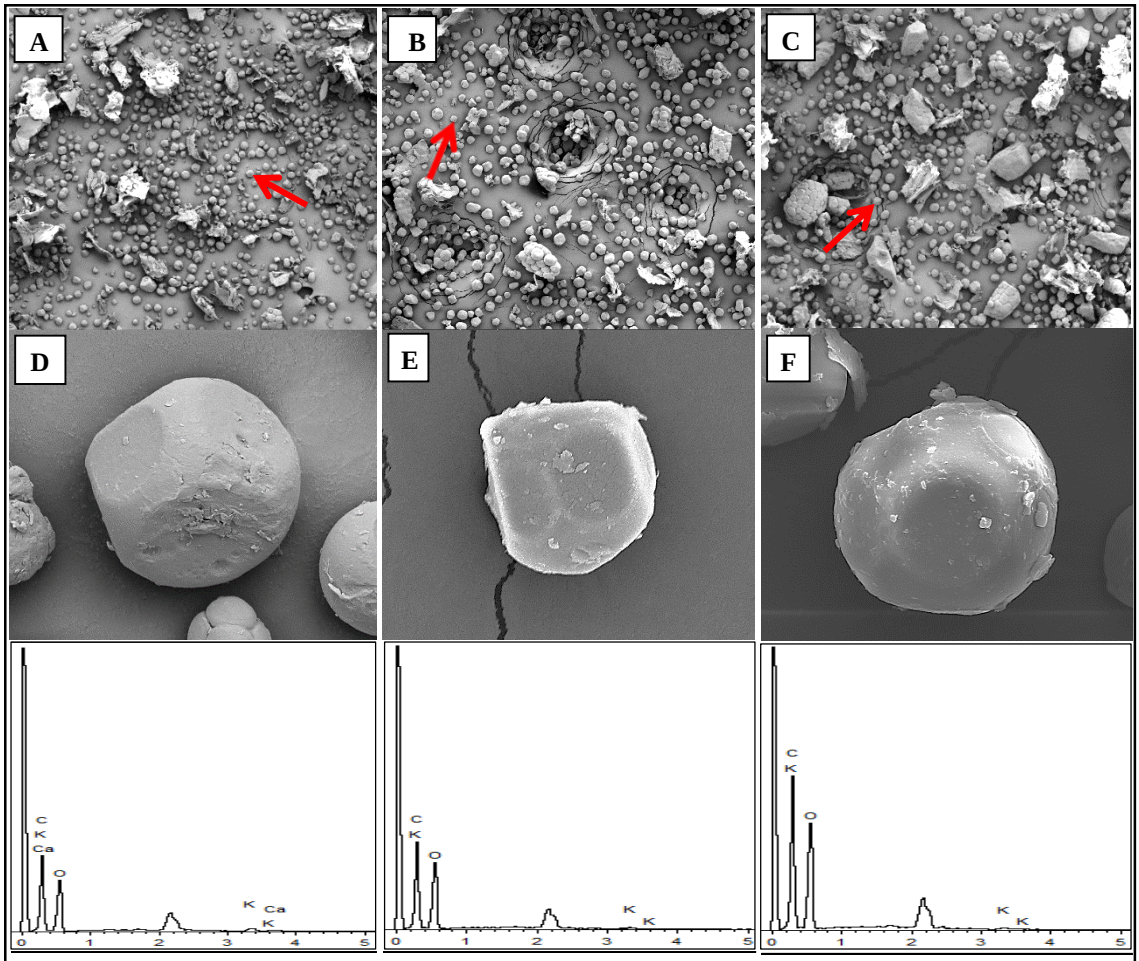


Figure 1 *S. myosotiflora* harvesting locations; Kelantan, Perak & Pahang in Malaysia



Element	Weight (%)	Atomic (%)
C K	48.9	56.59
O K	49.18	42.73
K K	1.28	0.45
Ca K	0.63	0.22
Total	100	

Element	Weight (%)	Atomic (%)
C K	48.68	55.94
O K	50.88	43.9
K K	0.44	0.16
Total	100	

Element	Weight (%)	Atomic (%)
C K	49.78	57
O K	49.89	42.89
K K	0.32	0.11
Total	100	

Figure 2 SEM-DEX analysis of *S. myosotiflora* tubers at magnifications 250x and 5000x accordingly; Kelantan (A & D), Perak (B & E) and Pahang (C & F)

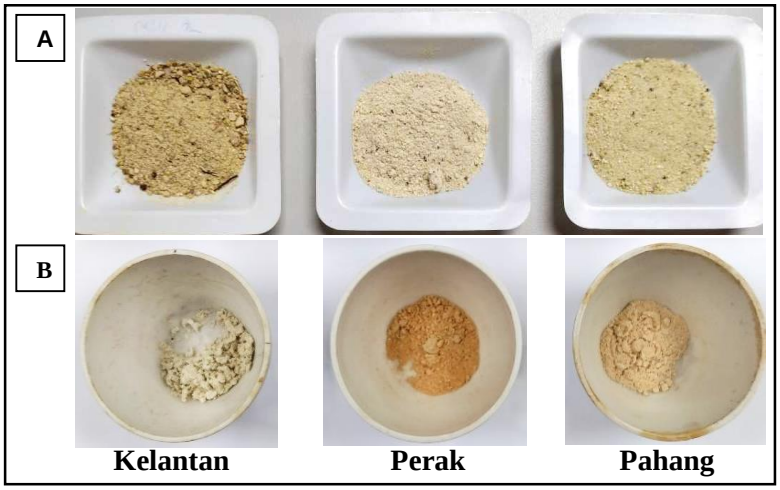


Figure 3 *S. myosotiflora* dried tubers in powder form (A) and after went through 550°C overnight ashing in total ash determination (B)

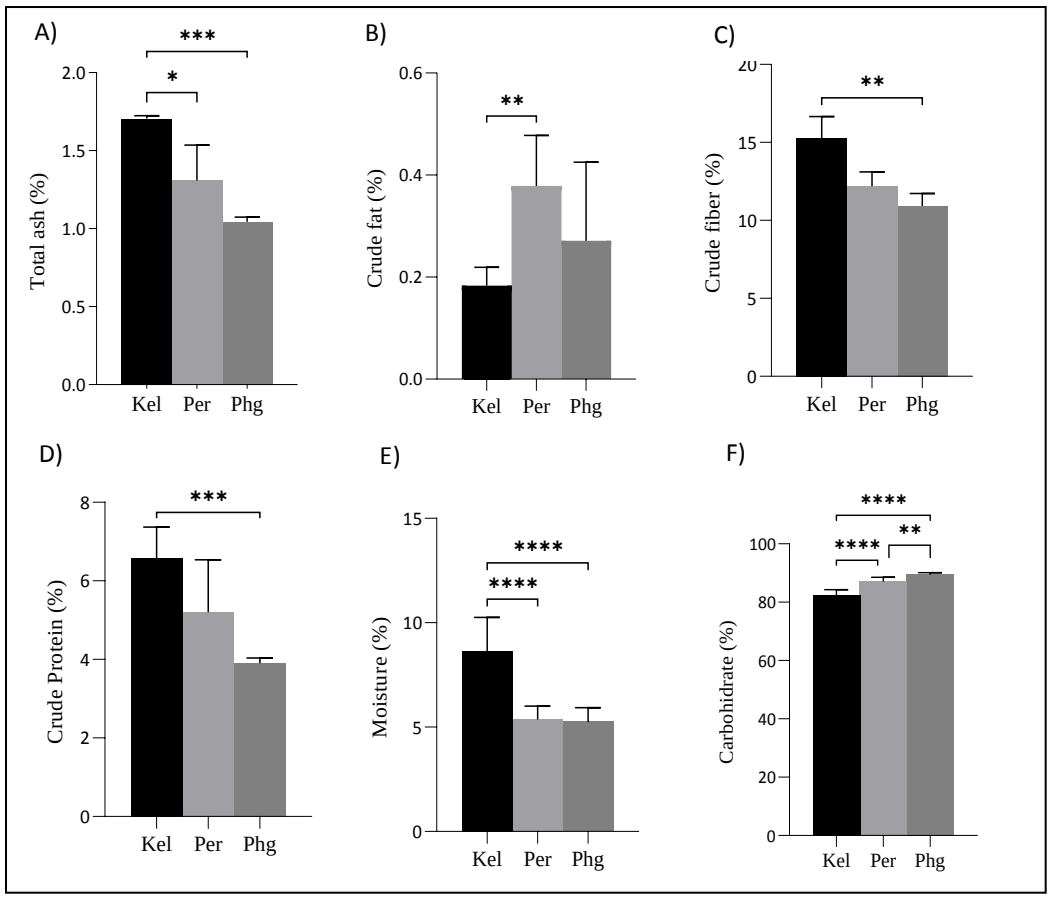


Figure 4 Percentages of the elements in proximate analyses of *S. myosotiflora* from three different places; Kelantan (Kel), Perak (Per) and Pahang (Phg). Abbreviations: * - *P*-value < 0.05, ** - *P*-value < 0.005, * - *P*-value ≤ 0.005, **** - *P*-value < 0.0001**

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 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.

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

No.	RT	Compound name	Formula molecule	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.