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Mass Spectrometry Profiles for Identification of *Lansium domesticum* Corr. Ethanolic Leaf Extracts Harvested from Two Different Locations in Malaysia

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ABSTRACT

Lancium domesticum Corr. is a woody tree species from the Meliaceae family and is mainly cultivated for its fruits in Southeast Asia. Parts of the plant have been used in traditional medicine to treat inflammation, gastrointestinal problems, fever, and intestinal worm infection in children. However, phytochemicals in a plant may vary from one location to another due to environmental conditions and soil properties. This study aimed to identify the composition of the *L. domesticum* ethanolic extracts collected from different locations. *L. domesticum* leaf ethanolic extracts were prepared from leaves collected from two different locations in Malaysia, namely Perlis and Selangor. Next, the chromatographic pattern and identification of the component from both extracts were carried out by LC/MS-QTOF analysis and accurate mass database screening. The results tentatively identified six compounds, including flavonoid glycosides, phenolics, and terpenes. Interestingly, α, γ- onoceradienedione and lansic acid, onoceranoid triterpenes known to present in the fruit peel of *L. domesticum* were detected in both leaf extracts. The ion intensity of both compounds was different in both leaf extracts, thus enabling the discrimination of the geographic locations where the *L.domesticum* leaves were collected. From the results, *L. domesticum* leaves from Perlis contains more lansic acid and are recommended to be collected for future biological activity studies.

Keywords: *LC/MS-QTOF, phytochemicals, Lansium domesticum, location*

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INTRODUCTION

Chromatographic profiling of plant extract using HPLC with detectors such as DAD and MS has been employed to give information on the phytochemical composition of the plant extract that later could be correlated to the biological property of the extract. Other than detecting and identifying compounds of interest, determination of compound concentration is also possible. Consequently, several studies have proposed detection and quantification methods for compounds of interest [1]. This is also beneficial in developing fingerprints and markers for quality purposes, especially when the discrimination of plants is impaired once they are pulverised into powder. In addition, information such as the effect of solvent extraction, method of extraction, and even the geographical information such as location or biotic influences on phytochemicals have been generated from chromatographic profiling [2,3]. The application of plant extract profiling is essential in herbal industries to standardise natural products required by government agencies.

Lansium domesticum Corr., or *Dokong* as it is locally known in Malaysia, is a woody tree species from the Meliaceae family. In the countries of Southeast Asia, the plant is mainly cultivated for its fruits. The resin, bark, and leaves of the plant have been traditionally used to treat inflammation and gastrointestinal problems such as dysentery, colic of the gastrointestinal tract, flatulence, swellings, and stomach cramp. In contrast, powdered seeds alleviate fever and intestinal worm infection in children [4]. Research on the extract of *L. domesticum* leaves from previous studies showed exciting findings. A study on the antiplasmodial activity of *L. domesticum* leaves was first initiated by reports on the use of *L. domesticum* to combat episodic fevers and malaria by indigenous peoples of Sabah, Malaysia. The results indicated that methanol extract of *L. domesticum* leaves reduced parasite populations of the drug-sensitive strain (3D7) and the chloroquine-resistant strain (T9) of *P. falciparum* by 35% and 58%, respectively [5]. Thus, it was postulated that the leaves of *L. domesticum* contain compounds that, either singly or in combination, have antimalarial properties [5].

Previous studies showed that *L. domesticum* extracts were rich in terpenes [6], whereas the presence of alkaloid [7, 8] tannins and phenols were also reported [9, 10]. Five onoceranoid types of triterpenes that were isolated from *L. domesticum* leaves extract exhibited antimutagenic activity in the Ames test. The structure−activity relationships suggested that lansiolic acid with a carboxylic acid moiety showed more potent antimutagenic effects than its analogues with an ether methyl ester moiety. Next, three more onoceranoid types of triterpenes were isolated from the methanolic extract of *L. domesticum* leaves as part of an ongoing research program to discover new antimutagenic agents [11]. Comparing the antioxidant activity of *L.domesticum* leaves extracts from Northern, and Eastern Thailand showed that methanolic extracts from the eastern exhibited free radical scavenging, lipid peroxidation inhibition, and metal chelating activity. However, the extracts of northern exhibited only free radical scavenging activity. The study also reported that the total phenolic content of *L. domesticum* leaves prepared in the cold was significantly higher than those prepared in hot methanol; nevertheless, there was no significant effect of temperature on the total flavonoids content [12]. However, the profiles of the extracts were not reported.

In this study, the *L. domesticum* leaves were collected from two locations. Perlis and Selangor are states located in Malaysia's north region and central region, respectively. This study aimed to identify the composition of the *L. domesticum* ethanolic extracts collected from different locations. The composition of

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the ethanolic extracts was identified using LC/MS-QTOF. LC/MS-QTOF detection has been a powerful tool in providing accurate mass measurements, including the elemental composition of parent and fragment ions, which is vital for identification. In addition, a library constructed to identify compounds based on the peaks and their respective spectra facilitates the screening of constituents from the plant extract using LC/MS-QTOF [13]. Results from this study will help propose a suitable location for the *L. domesticum* leaves to be collected for future biological activity studies.

EXPERIMENTAL

Plant Materials

Lansium domesticum leaves were obtained from two geographic locations. The first location was Tropical Fruits Garden, Taman Botani Negara Shah Alam. The leaves were collected from trees with codes IDG41 to IDG52. The second location was Felda Chuping, Perlis. A voucher specimen (ID numbers: PID 020117-02) was deposited at the Herbarium of Forest Research Institute Malaysia (FRIM).

Chemicals

Ethanol 95% was obtained from White Lab, Malaysia, while the methanol HPLC grade, formic acid and acetonitrile were obtained from Merck, Darmstadt, Germany.

Preparation of Samples

L. domesticum leaves from the two locations were separately washed, air-dried and pulverised to a coarse powder. Three kilograms of powdered leaves were macerated in 95 % ethanol (7.5L) for three days at room temperature (25 ± 3 °C). The extracts were filtered with Whatman's No. 1 filter paper. To produce the crude extract, the extract was concentrated in the rotatory evaporator (Butchi, Switzerland) at 40 ^{0}C. The leaves were reextracted with 95 % ethanol, and the processes were repeated four times until the leaves were exhaustively extracted. The crude extract was cleaned-up by using C18 SPE cartridges containing 1 g of sorbent (Agilent Bond Elut, USA).

The cartridge was first conditioned with 5 mL of methanol. Then, 100 mg of sample in 1 mL methanol (HPLC grade) was loaded into a cartridge, followed by 5 mL methanol and the eluate was collected. 100 µL of clean-up sample was centrifuged at 13500 *g* for 5 minutes. The supernatants were collected and dried using a vacuum concentrator. Before analysis, the samples were weighed and reconstituted to 1 mg/ml in methanol (HPLC grade).

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LC/MS-QTOF Analysis

Analyses were carried out using LC/MS-QTOF system (LC 1200 Series Agilent Technologies). The system was equipped with MS/Q-TOF mass spectrometer (G6510A), binary pump (G1312B), well-plate sampler (G1367D) and thermostated column compartment (G1316B). Chromatographic separations were performed using a ZORBAX Eclipse Plus C18 column of 2.1 × 100 mm × 1.8 microns particle size (Agilent Technologies SA, USA). The mobile phases consisted of water (A) and acetonitrile (B), containing 0.1 % formic acid. The oven column temperature was set at 40ºC with a 0.25 ml/min flow rate. The elution gradient was set up as follow: 0 min, 30 % B; 0-36 min, 95 % B; 36-41 min, 95 % B; 41-48 min, 5 % B. The sample injection volume was 2 µl. The mass spectrometer was operated in positive electrospray ionisation (ESI) mode, and spectra were recorded by scanning the mass range from *m/z* 100 to 1000 in MS mode. Nitrogen was used as drying, nebulising and collision gas. The drying gas flow rate was 10 L/min. The heated capillary temperature was set at 325 °C and nebuliser pressure at 30 psi. The source parameters capillary voltage (VCap), fragmentor, skimmer and octupole voltages were set to 3500 V, 175 V, 65 V and 750 V, respectively.

Phytochemicals Database Screening

TOF data (MS1 mode) was processed using the Molecular Feature Extraction (MFE) algorithm of the MassHunter Qualitative Analysis B.06.00 software (Agilent Technologies, Santa Clara, CA, USA). This algorithm searches all ions in the data file that represent actual compounds. Specific search criteria for *L. domesticum* extracts were set as follows: for extraction, the peak intensity threshold was 1000 counts, and ion species of H^+, Na^+, K^+ and NH_4^+ were selected as positive adducts. Assigned charge states were limited to a maximum of 2, and a compound filter was used to search only compounds with absolute height equal to and higher than 1×10^4. Accurate mass data of compounds detected by MFE were then compared to the exact masses of compounds in the database using the Database Search algorithm (5 ppm tolerance). The database contains a total of 5382 compounds comprised of compounds from the PlantCyc 12.0 (PMN) database and known compounds that were reported presented in *L. domesticum* Corr.

RESULTS AND DISCUSSION

Using the described phytochemical database screening, six compounds were identified in the extract from Perlis (Compound *a,b,c,d,e* and *f*). In contrast, four of those six compounds were identified in the *L. domesticum* leaves extract from Selangor (compounds *b,c,e,* and *f*). The QTOF-MS spectra of the compounds are presented in Figure 1, whereas the retention time and measurement of accurate mass are shown in Table 1. The molecular structures of identified compounds are shown in Figure 2.

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Table 1 : Compounds identified from *L. domesticum* ethanolic leaf extracts by LC/MS-QTOF

Cpd	Identification	Perlis			Selangor			Fragments (*m/z*)
		RT (min)	*m/z*	Error (ppm)	RT (min)	*m/z*	Error (ppm)	
a	Quercetin 3-O-rhamnoside-7-O-glucoside	5.02	611.1603	0.70	ND	ND	ND	121, 269, 303, 449, 465
b	Kaempferol-3-glucoside	5.25	449.1091	2.17	5.25	449.1080	0.87	121, 287
c	Scopoletin	5.60	193.0498	1.65	5.62	193.0499	2.15	127, 133, 137, 150, 187
d	Ferruginol	14.17	287.2375	1.81	ND	ND	ND	125, 133, 137, 147, 151, 163, 201, 229, 243, 257, 269
e	α, γ-Onocera-dienedione	34.52	439.3593	4.72	34.57	439.3590	3.84	149, 191, 205, 217, 233, 421,
f	Lansic acid	35.13	453.3361	0.92	35.18	453.3372	2.01	219, 397, 407, 435

(ND=not detected)

Electrospray ionisation (ESI) is the most widely applied ionisation technique. It is suitable for the analysis of highly polar compounds. ESI is a soft ionisation technique producing little fragmentation; Therefore, for many compounds, their molecular ions can be detected by mass spectrometry [14]. In the positive ESI mode (Figure 1), compound *a* produced molecular ion $[M+H]^+$ at *m/z* 611. The full scan electrospray positive mass spectrum for a compound *a* showed the presence of molecular ions at *m/z* of 465, 449 and 303, which corresponded to the loss of rhamnose, glucose and both, respectively, as reported in previous studies [15, 16]. The molecular ions observed at *m/z* 269 and 121 correspond with the fragment spectra reported in the PMN database. Thus, compound *a* was identified as quercetin 3-O-rhamnoside-7-O-glucoside.

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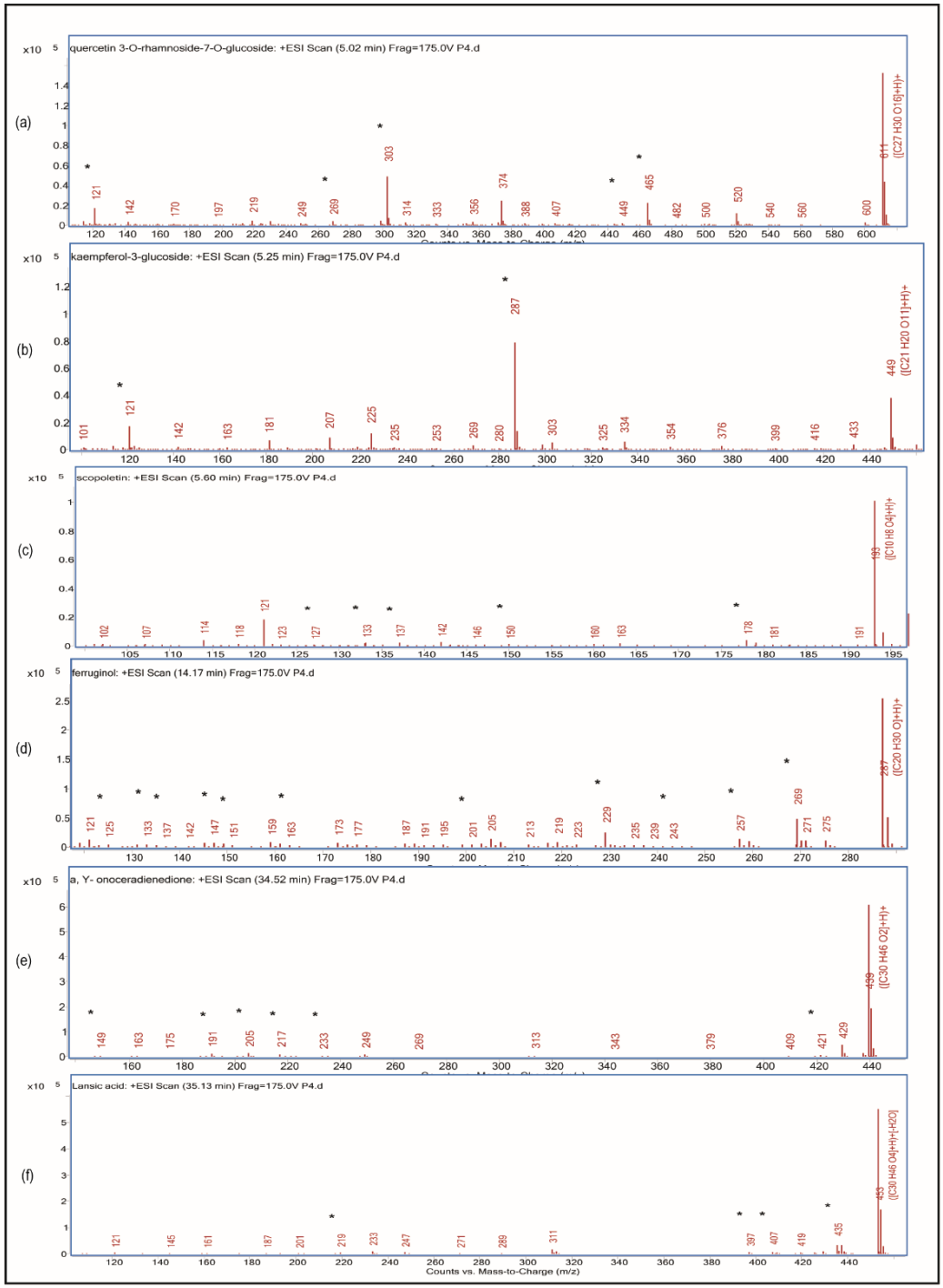

Figure 1: The QTOF-MS spectra of (a) quercetin 3-O-rhamnoside-7-O-glucoside, (b) kaempferol-3-glucoside, (c) scopoletin, (d) ferruginol, (e) α, γ-onoceradienedione and (f) lansic acid in positive ionization mode

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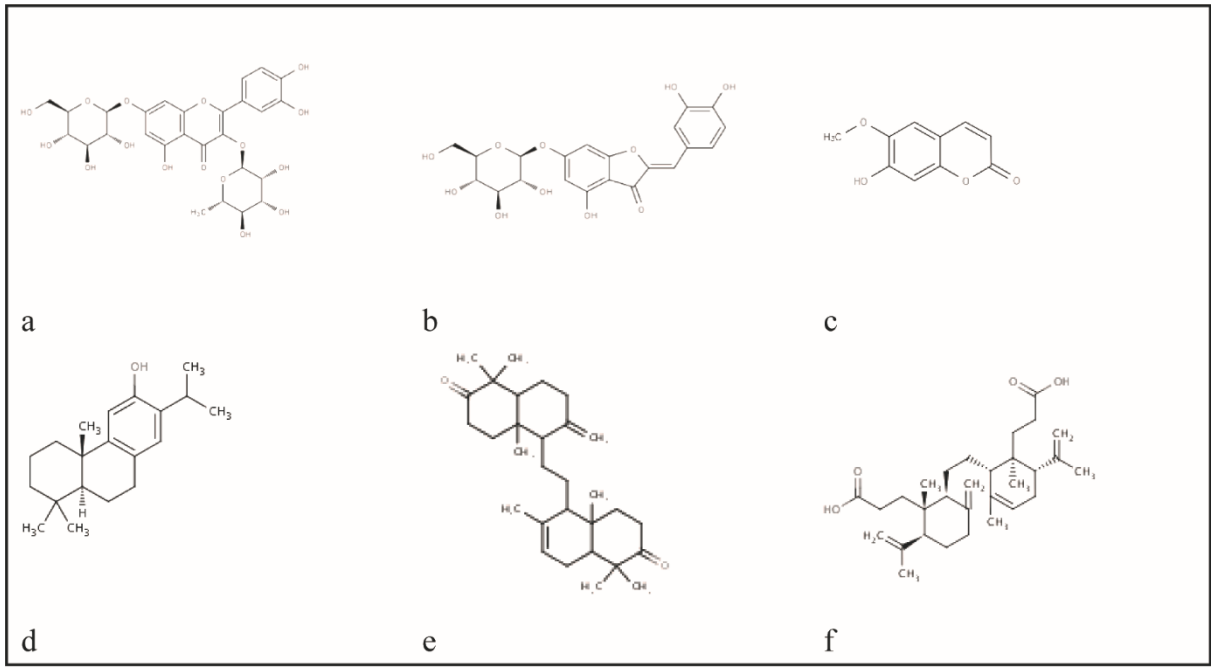

Figure 2: Molecular structures of (a) quercetin 3-O-rhamnoside-7-O-glucoside, (b) kaempferol-3-glucoside, (c) scopoletin, (d) ferruginol, (e) α, γ-onoceradienedione and (f) lansic acid

The full scan mass spectra of compound *b* showed molecular ion $[M+H]^+$ at *m/z* 449. Molecular ions observed at *m/z* 287 were due to loss of one glucose moiety, inferring the presence of aglycon kaempferol [17, 18]. Molecular ions observed at *m/z* 287 and 121 correspond with the fragment spectra reported in the PMN database. Compound *b* was identified as kaempferol-3-glucoside. A molecular ion $[M+H]^+$ was observed from the full scan mass spectra at *m/z* 193. Molecular ions observed at *m/z* 127, 133, 137, 150 and 178 were characteristic fragments of this compound reported in a previous study [19]. Compound *c* was identified as simple phenolic; scopoletin. Three other compounds (*d,e,f*) were identified as terpenes. Compound *d* was identified as ferruginol producing molecular ion $[M+H]^+$ at *m/z* 287. Its characteristic fragment ions were at *m/z* 269, 213, 187, 159 and 145 [20]. While fragments ions at *m/z* 257, 243, 163, 151, 147, 137, 133 and 125 were characteristic fragment ions of ferruginol reported in the PMN database. Compound *e* and *f* were putatively identified as α, γ- onoceradienedione and lansic acid, respectively. Compound *e* showed molecular ion $[M+H]^+$ at *m/z* 439, whereas compound *f* showed molecular ion $[M+H]\text{-}H2O]^+$ at *m/z* 453. These triterpenoids are present in the seed and peel of *L. domesticum* [21-24], but their presence in leaves ethanolic extract of this species has not yet been reported. Although the fragmentation mechanism of both α, γ- onoceradienedione and lansic acid has not been studied, their predicted characteristic fragments obtained from the PMN database were included in Table 1.

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Comparison of LCMS Profiles

Both profiles of *L. domesticum* leaves ethanolic extracts from each origin were observed. Based on both profiles (Figure 3), a similar base peak chromatogram pattern was observed in addition to the identification of α, γ-onoceradienedione and lansic acid from both extracts, which are reported as terpenoids from *L.domesticum*. These indicated that the plants collected from two locations are of the same species. The LCMS profiles obtained can also be regarded as the chromatographic fingerprints of *L. domesticum* leaves ethanolic extract prepared by the extraction procedure described in this study. Base peak chromatogram is the chromatogram obtained by plotting the signal of the ions. From the base peak chromatogram, extract from Perlis shows peaks with higher intensity than extract of Selangor. Peak X is the peak with the highest relative ion abundance for *L.domesticum* extract from Perlis, but it was not identified by the database used in this study.

Mass spectrometry (MS) offers a highly sensitive detection technique that ionises the sample components, separates the resulting ions in a vacuum based on their mass-to-charge ratios (*m/z*) and measures the intensity of each ion. The signal intensity is dependent not only on the absolute amount of analyte entering the ESI source but also on the eluent flow and concentration of the analyte [14]. Since both extracts were analysed in the same LCMS condition, the signal intensity of identified compounds was implied as to the abundance of the respective compounds in the leaves extract.

Quercetin 3-O-rhamnoside-7-O-glucoside, kaempferol-3-glucoside, and scopoletin are phenolic compounds, whereas ferruginol is an abietane diterpene. According to literature, these compounds are common and have been reported from other plants [25, 26] but are not yet reported from the leaves extract of *L. domesticum* previously. Both α, γ-onoceradienedione and lansic acid are onoceranoid type triterpenes. Both compounds were previously isolated from the fruit peel of *L.domesticum*. Consequently, both compounds were synthesised to study the versatile synthetic procedures of this class of natural products. However, their biological activity was not reported [23].

Compound *a* and *d* were not detected in the extract from Selangor. However, it was apparent that the intensity of the ions corresponded to their location (Figure 4), thus suggesting that it can be used to discriminate the locations of the *L.domesticum* leaves collected. This finding is in accordance with other studies that found plants of the same species but grown in different localities showed differences in the concentration of phytochemicals [2, 3]. Furthermore, the synthesis of phytochemicals is frequently influenced by abiotic factors such as temperature, water supply, salinity, and light and biotic factors such as bacterial infection and attack from herbivores and insects [27].

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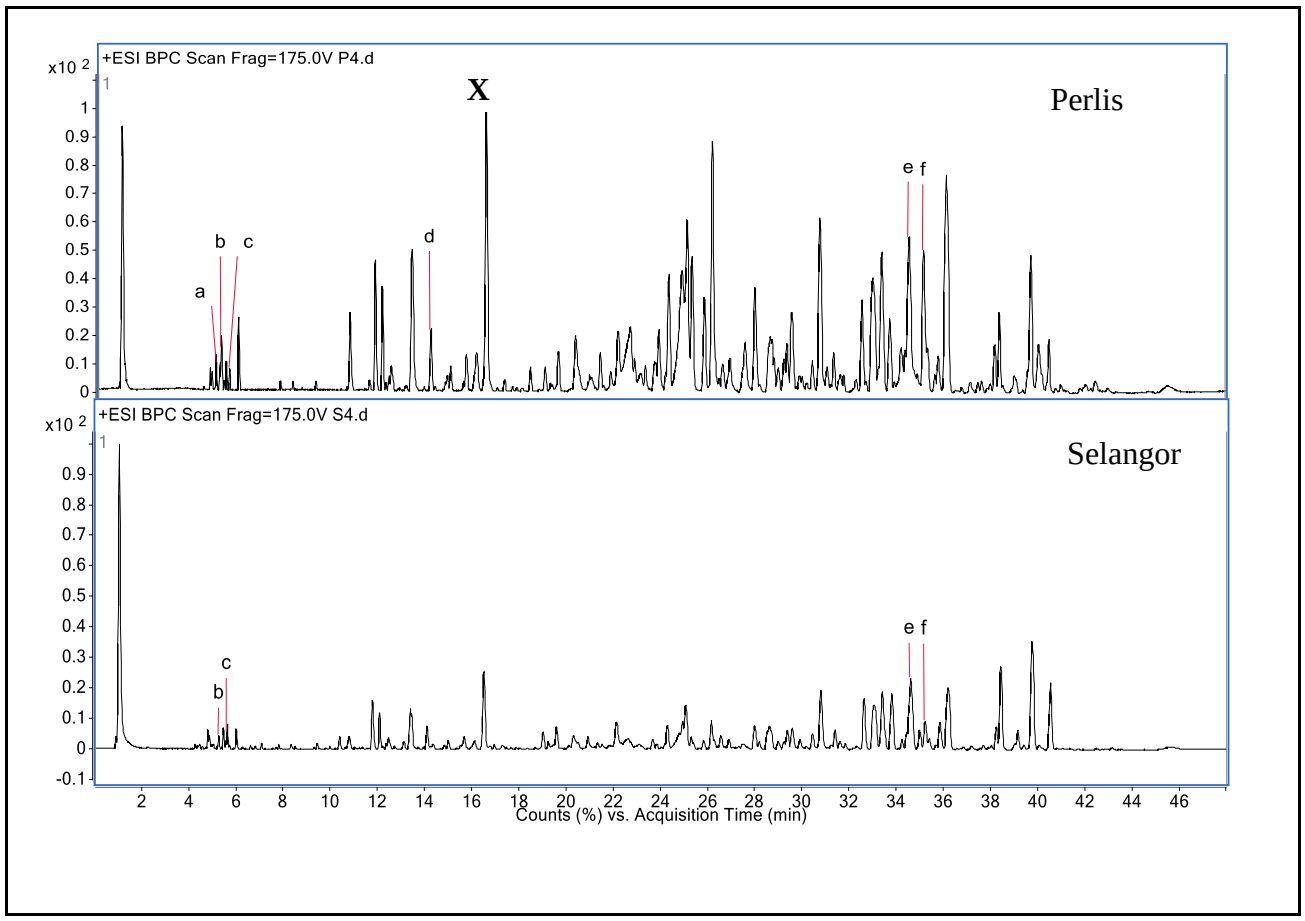

Figure 3: The LCMS base peak chromatogram (BPC) of ethanolic leaf extracts from two locations; Perlis and Selangor. Peak labelling designates the identified compounds.

There are six approaches to the selection of plant material for biological study. The approaches have been applied to programs that are aimed at drug discovery. These are the locally random selection, the taxonomic, the ethnomedical, the phytochemical, the information-based and serendipity [28]. For instance, an excellent antimicrobial activity of α, γ-onoceradienedione, towards *Pseudomonas aeruginosa* was reported [24]. Based on the abundance of α, γ-onoceradienedione and lansic acid in each extract (Figure 4), it can be postulated that the extract from Perlis could give a better antimicrobial activity than the extract from Selangor. Thus, *L. domesticum* leaves from Perlis can be suggested as plant material for antimicrobial study or could be the plant material desirable for other biological studies. However, a comparison of activity from both extracts is recommended.

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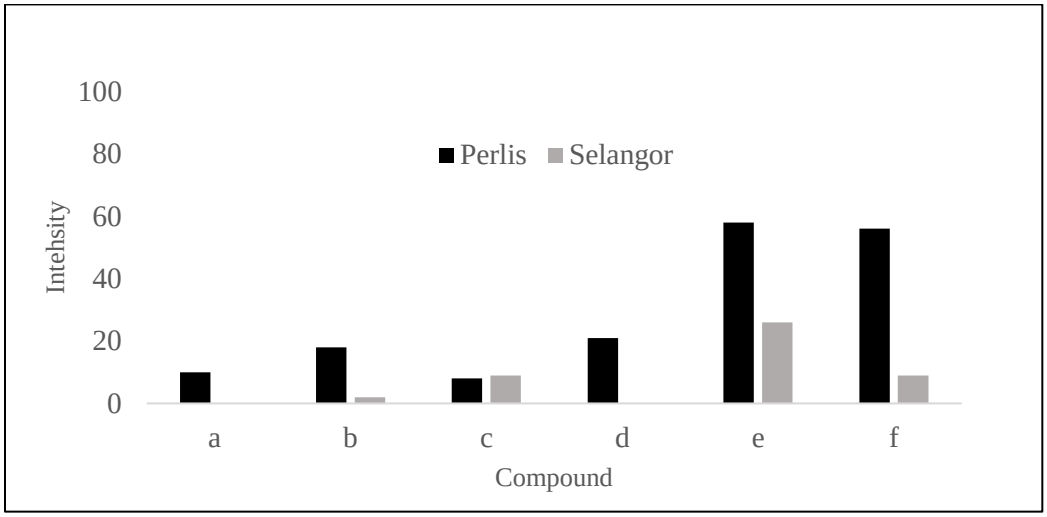

Figure 4: The abundance of compound (a) quercetin 3-O-rhamnoside-7-O-glucoside, (b) kaempferol-3-glucoside, (c) scopoletin, (d) ferruginol, (e) α, γ-onoceradienedione and (f) lansic acid in *L. domesticum* leaf ethanolic extracts from Perlis and Selangor.

CONCLUSION

The ethanolic extracts of *L. domesticum* leaves collected from Perlis and Selangor showed a similar LCMS profile in this study. However, the intensity of the base peak chromatogram from both extracts is different. The base peak chromatogram of *L. domesticum* extract from Perlis showed higher peak intensity than Selangor. Six compounds were tentatively identified in this study. Although identifying the peak of highest intensity in the extract from Perlis is limited by the fragmentation behaviours provided by the library/database, results from this study suggested that the α, γ-onoceradienedione and lansic acid can be used to discriminate the two locations where *L.domesticum* leaves were collected. Furthermore, identifying compounds from the extract helps propose suitable *L. domesticum* leaves to be collected and its potential biological activity studies. Therefore, the study could be extended to *L.domesticum* leaves collected from other states of Malaysia.

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AUTHOR'S CONTRIBUTION

Noor Fahimah Saari carried out the lab work, and Salfarina Ramli wrote the article. Richard Johari James had conceptualised the central research idea and provided the theoretical framework. Richard Johari James, Salfarina Ramli, Mohd Salleh Rofiee, Hasseri Halim, Teh Lay Kek and Mohd Zaki Salleh had designed the research, analysed data and revised the article.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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