International Journal of Engineering and Advanced Technology (IJEAT)
ISSN: 2249-8958 (Online), Volume-8 Issue-3S, February 2019

Identification of Chemical Constituents of Polygonum minus Essential Oil by Gas Chromatography-Mass Spectrometry and Shelf

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Abstract: ***Polygonum minus comes from Polygonaceae family and used as fragrance and food flavoring. There were four groups of P. minus samples that were stored in cool room at 4-5 °C for shelf-life study (Day 0, Day 7, Day 21, Day 28). The essential oil of each group was extracted by using hydro-distillation apparatus and hydro-steam distillation. Nine compounds of essential oil in P. minus samples were identified by GC-MS analysis with the quality of above 80%. Decanal and dodecanal were the major compounds with the composition of 13-19% and 47-56% respectively. The concentration of decanal present in fresh sample was calculated by single point calibration. As a result the decanal concentration in P. minus essential oil was 169 519 mg/L.***

Keywords: Polygonum minus, essential oils, Total ion chromatogram (TIC), gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionisation detector (GC-FID).

I. INTRODUCTION

Polygonum minus is a native plant of Polygonaceae family at Southeast Asia. (Vikram *et al.*, 2014), (Qader *et al.*, 2012). In Malay, it is known as kesum while in English, it is called as pigmy knot weed. This plant is shrubby and lanky. The dark green leaves are narrow, lanceolate and arranged alternatively. It is also very aromatic. The stem is cylindrical, green and slightly reddish with short internodes that are easily rooted. The flowers are small, and found at the end of the shoot (Vikram *et al.*, 2014), (Narasimhulu & Mohamed, 2014). The traditional usages of *P. minus* are to treat digestive disorder and dandruff. In Malaysia, it is commonly used as food flavouring agents and served as appetizer (Qader *et al.*, 2012), (Christapher, *et al.*, 2015). In previous study, *P. minus* showed several pharmacological properties such as antimicrobial, cytotoxicity, antioxidant and anticancer (Vikram *et al.*, 2014). *P. minus* is also used as fragrance in perfume industry because it contains volatile aromatic compounds (Vikram *et al.*, 2014). Baharum *et al.* (2010) identified decanal and dodecanal as major compounds in this plant. Decanal and dodecanal are aliphatic aldehyde and act as marker compounds in the essential oil. In this research, shelf-life study and chemical constituents of *Polygonum minus* essential oil is evaluated for food safety purposes.

II. MATERIALS AND METHODS

Plant Sample

Polygonum minus was obtained freshly from Selayang Market, Selangor, Malaysia. The plant sample (leaves and stems) was cut to small pieces so that the sample could be easily inserted in the 1000 mL of round bottom flask.

Extraction by hydro-distillation method.

Plant sample (10 kg) was divided into four groups; Group 1 (Day 0), Group 2 (Day 7), Group 3 (Day 21) and Group 4 (Day 28). All groups of sample were kept in cool room at 4-5 °C. Each group was extracted by using hydro-distillation. Extraction of oil was conducted using laboratory scale hydro-distillation method in 1000 mL round bottom flask and Clevenger type distillation apparatus. Distilled water was poured into the flask containing sample until it reached the level of the sample. The flask had already been connected to a pre-setup condenser and placed on the heating mantle. Then, the sample was kept in heating process for 5 to 6 hours. From the distillation, greenish yellow oil was formed on the top layer of water.

Extraction by hydro-steam distillation method

The extraction of oil from *Polygonus minus* was also conducted by using a large scale hydro-steam distillation method in a 20 kg container. Distilled water was poured into the container until it reached a marked level after which a lid with holes was used to cover it. The sample (12 kg) was placed into the container and it was closed with cover. The closed container was connected to a pre-setup condenser.

Manuscript published on 28 February 2019.
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Throughout the process, the container was ensured to have no leakage to prevent the loss of essential oils. Then, the sample was kept in a heating process for 6 hours. The greenish yellow oil was collected from the flask.

Isolation of essential oils

The essential oils were separated from water and recovered with dichloromethane (DCM). The dichloromethane was removed from the extracted oil by using rotary evaporator. Then, anhydrous sodium sulfate was added into essential oils to absorb the water and stored in vials at 4 °C for further investigation.

Chemical constituents' analysis by Gas Chromatography - Mass Spectrometry (GC-MS)

The chromatographic analysis was carried out by using a Shimadzu QP2010-GC-MS with automatic injector. A fused silica capillary column HP5-MS (30 m x 0.25 mm x film thickness 0.25 µm) was used. Helium was used as a carrier gas and the split mode of injection was used with the ratio of 1:50. The oven temperature was maintained at 60 °C for 8 minutes. The programme of temperature was started at 60 °C and increased gradually at a rate of 3 °C min^{-1} to 300 °C and it was maintained at 300 °C for 2 minutes. The injection port temperature was at 250 °C. The sample was diluted 5 times with dichloromethane (DCM) and 1 µl was injected into the column.

Decanal quantification by Gas Chromatography - Flame Ionisation Detector (GC-FID)

Single point calibration was used to quantify amount of decanal in essential oil of *Polygonum minus* for the laboratory scale and large scale. Decanal (97%) was used as a standard solution. 10 µL of decanal standard was added to 0.5 mL hexane into 1.5 mL glass vial. The vial was shaken gently. Next, 100 µL sample of essential oil was prepared in 1.5 mL vial. A 400 µL of n-hexane was added into the vial. The standard solution and samples were analysed in triplicate by using GC-FID. The average peak area of the sample was compared to average peak area of standard solution and then the closest average peak area of the standard solution was used to determine the amount of decanal in essential oils by using single point calibration or direct calculation methods.

Gas Chromatography equipped with Flame Ionisation Detector (GC-FID) was used in this analysis. The extracted *P. minus* oil was analysed by using a Shimadzu Model GC-2010 GC equipped with flame ionization detector (FID) and an auto sampler injector AOC-2OI (Shimadzu). Separation was achieved using a middle polar capillary column RTx-5MS with 30 m length, 0.25 mm in diameter and film thickness of 0.25 µm. The injector and detector temperatures were conditioned at 250°C and 280°C respectively. The oven temperature program started from 60°C to 300°C at a rate of 3°C min^{-1} with isothermal temperature constant at 300°C for 2 minutes. Hydrogen gas was used as the carrier gas with flow rate of 30 mL min^{-1}. The mode of injection used was split mode with the ratio of 1:50.

III. RESULT AND DISCUSSION

Yield of Essential oil

The percentage yield of the essential oil from *P. minus* was extracted by using hydro-distillation extraction was shown in Table 1. The greenish yellow oil was collected from this distillation. The water content of each sample were 86.67% (Day 0), 76.67% (Day 7), 63.33% (Day 21) and 56.67% (Day 28). This showed that the water content in the samples reduced when samples were stored in cool room at longer time.

Table 1 The yield of oil extracted from each group of samples

Group of sample	Mass of sample (kg)	Volume of oil extracted |(mL)	Percent yield (%)
1 (Day 0)	2.10	0.6	0.21
2 (Day 7)	1.91	0.7	0.16
3 (Day 21)	1.73	0.5	0.079
4 (Day 28)	1.52	0.4	0.061

In this study, the percentage yield of extracted oil from the four set samples decreased at longer time of the storage. The oils might be evaporated together with water during storing process (Table 1).

Chemical constituents of essential oils

Chemical constituents of *P. minus* essential oil were identified by using GC-MS based on the quality of compound. Only compounds that had quality above 80 % were determined. There were only eight similar compounds identified by GC-MS. In each group, the compounds were decanal, dodecanal, (E)-caryophylene, α-cis-bergamotene, α-humulene, (E)-nerolidol, caryophyllene oxide and drimenol. Table 2 shows the composition of same compounds present in the oil samples. Based on table 2, there were two major compounds present in this plant which were decanal and dodecanal with around 13-19 % and 47-56 % respectively. Both of the compounds were aliphatic aldehyde that plays important roles in this plant including odor production.

Table 2 Composition (corrected area percent) of compound present in essential oils from *P. minus.*

Compounds	Group 1	Group 2	Group 3	Group 4
Decanal	18.94	17.30	15.07	13.98
Dodecanal	54.46	47.84	56.02	55.09

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(E)-caryophyllene	6.78	15.49	8.50	9.27
α-cis-bergamotene	1.34	1.26	1.36	1.42
α-humulene	5.84	4.73	5.09	5.29
(E)-nerolidol	1.03	1.60	2.28	2.46
Caryophyllene oxide	2.10	2.00	2.29	2.31
Drimenol	9.50	9.77	9.37	10.19

Figure 1 shows there was no significant different of chemical constituent composition in samples. However, the composition of decanal slightly reduces according to longer time sample stored in cool room.

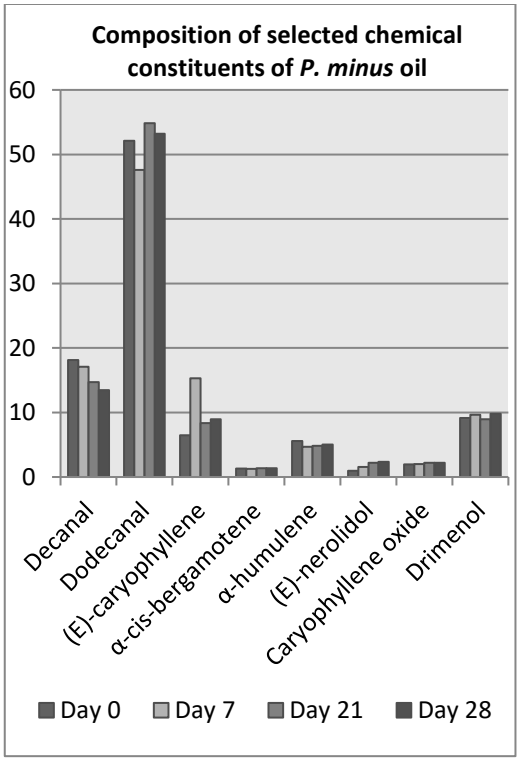

Figure 1 Composition of selected chemical constituents of *P. minus* essential oils in different days of extraction.

Quantitative analysis of decanal as a marker compound.

In this study, decanal had been chosen as one of the major constituents in the essential oil of *P. minus* to be quantified. Decanal was commonly found in natural products and an aliphatic aldehyde that had citrus-like odour (Rusdi *et al.*, 2016). In this study, decanal of *P. minus* essential oil was eluted at retention around 27.5 to 27.9 minutes from GC-FID chromatogram.

The standard solutions were analysed triplicate for each concentration by using GC-FID. Then, the average peak area of the standard solution of decanal was calculated. The amount of decanal in essential oil of *P. minus* was quantified by using single point calibration method. The average peak area of the sample was compared to single point of the standard solution that had average peak area that closest with the average peak area of sample. The average peaks of standard solution and sample solution were 2 787 080 and 5 868 389 respectively. The concentration of decanal standard solution that used was 16 102 mg/L. The concentration of decanal in the original oil sample was calculated to be 169 519 mg/L.

Comparison of chemical constituents in the Polygonus minus oil between hydro-distillation and hydro-steam distillation extraction.

Two methods of extraction were used in this study, hydro-distillation and hydro-steam distillation. The composition of the chemical constituents in the *P. minus* oils by two extraction methods was shown in Figure 2. Nine compounds were detected including two major compounds namely decanal and dodecanal from the analysis. Figure 3 shows the total ion chromatogram (TIC) of essential oils GC-MS analysis by hydro-distillation method.

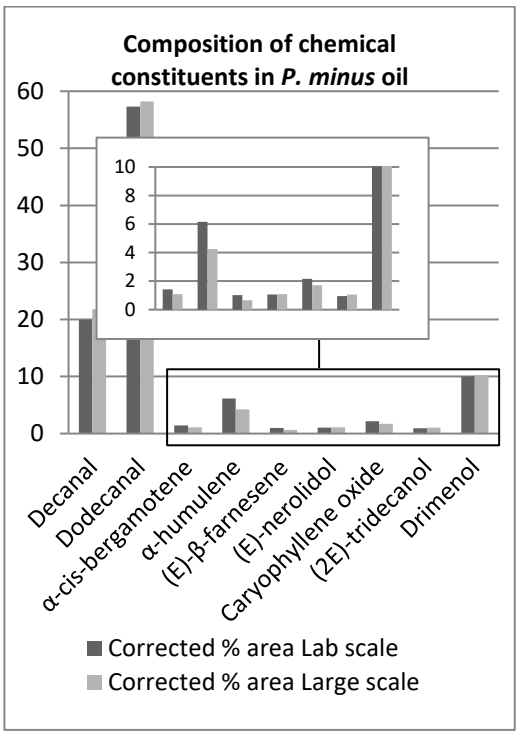

Figure 2 The composition of chemical constituents based on hydro-distillation and hydro-steam distillation extraction methods.

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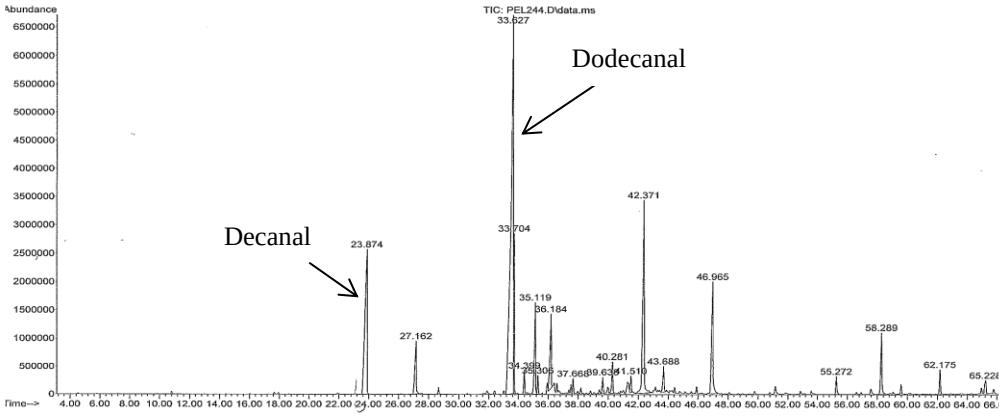

Figure 3 TIC of essential oil by hydro-distillation extraction.

IV. CONCLUSION

Essential oils of *P. minus* were extracted by using hydro-distillation and steam distillation extraction methods. In this study, the percentage yield of essential oil extracted from each group of sample reduced as longer time of storage. There were only eight similar compounds present in each group of *P. minus* samples identified by using TIC GC-MS. Both extraction methods showed that the chemical constituents found in the oils are similar. The major compounds present in essential oil of *P. minus* were decanal and dodecanal with composition around 13-19 % and 47-56 % respectively with the concentration of decanal was 169 519 mg/L.

V. ACKNOWLEDGEMENTS

Many thanks to Universiti Teknologi MARA (UiTM) for the financial support under GIP Grant Scheme, 600-IRMI/GIP 5/3 (0056/2016). The authors are also thankful to Mrs Mailina Jamil and Mr Faridz Zollfatah from FRIM for their technical assistance.

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