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Effects of different drying methods on essential oil yield and component profile of *Polygonum minus* root extract

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Abstract

Polygonum minus is a plant rich with bioactive components that contribute to food, pharmaceutical, and perfume industries. However, high moisture content in fresh plants will allow microbial activity that leads to the degradation of plant quality. This can be prevented by drying the fresh plants to preserve the characteristics of their bioactive components. The present work was conducted to determine the effect of different drying methods such as air-drying, oven-drying (40 and 60°C), and freeze-drying on essential oil (EO) yield and chemical compounds of *P. minus* roots. For comparison purposes, all samples were extracted by maceration with *n*-hexane at room temperature. Then, the samples were analysed and identified by using gas chromatography-mass spectrometry (GC-MS). The highest EO yield extract was obtained from freeze-drying (4.15 ± 0.5), followed by air-drying (3.79 ± 0.19). EO yield from oven-drying at 40 and 60°C was 3.4 ± 0.14 and 0.86 ± 0.04, respectively. Results showed that by increasing the drying temperature, the EO yield would decrease and cause a loss of major chemical compounds in the *P. minus* root. Air-drying was found to be the best method in preserving the presence of important chemical compound in *P. minus* roots such as β-caryophyllene (1.43%), pentadecane (4.34%), hexadecanoic acid (3.91%) and oleic acid (3.97%).

Introduction

Polygonum minus (synonym: *Persicaria minor* (Huds.) Opiz) is an aromatic plant that belongs to the polygonaceace family, which is also known as *kesum* or *laksa* leaves in Malaysia (Christapher *et al.,* 2015). It is widely used in traditional medicine to treat headache, digestive disorder, and reduce dandruff (Zakaria and Mohd, 2010). Several studies have also proven that *P. minus* extract had the capability to be a therapeutic drug for gastric ulcer (Qader *et al.,* 2012) due to its high antioxidant activity (Qader *et al.,* 2011). A similar study was reported by Ahmad *et al.* (2014) which showed that the *P. minus* extract had high antioxidant activity. In a recent study by Ahmad *et al.* (2018), *P. minus* extract showed promising results as an HIV-1 protease inhibitor.

There are several active components present in *P. minus* that have been reported to contribute to medicinal effects, such as terpenes, aliphatic compounds, and organic acids. Previous studies by Baharum *et al.* (2010) and Yaakob (1987) noted the presence of two dominant aldehydes (dodecanal and decanal) and sesquiterpenes (β-caryophyllene) in *P. minus* that contribute to the flavour and taste of this plant which have great potential to be used in food additives and perfume industries. The bioactivity of these compounds is shown in Table 1.

Lately, the demand for processed products whereby the quality remains the same as fresh ones has been increasing. Fresh plants usually have a high level of moisture content which allows for microbial activity that leads to the plant quality degradation (Mbadiko *et al.,* 2019). This can be prevented by drying the fresh plants to preserve the characteristics of their bioactive components, eliminate microbial activity (Chakraborty and Dey, 2016) and extend the shelf life of herbal products (Fiegel and Michalska, 2017). However, certain bioactive components in the plants are sensitive to temperature and will reduce product quality during drying (Tambunan *et al.,* 2001). Therefore, suitable drying techniques need to be identified to achieve high quality products.

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Table 1. Reported bioactivity on chemical compounds.

Compound	Chemical Formula	Reported Bioactivity	Reference
Aliphatic compounds			
Tetradecana	$C_{14}H_{30}$	Flavouring agent	Mohd Azhar *et al.* (2018)
Pentadecane	$C_{15}H_{32}$	Flavouring agent	Mohd Azhar *et al.* (2018)
Eicosane	$C_{20}H_{42}$	Flavouring agent	Mohd Azhar *et al.* (2018)
Heneicosane	$C_{21}H_{44}$	Flavouring agent	Mohd Azhar *et al.* (2018)
Tetracosane	$C_{24}H_{50}$	Flavouring agent	Mohd Azhar *et al.* (2018)
Organic acids			
Tetradecanoic acid	$C_{14}H_{28}O_2$	Antifungal, antibacterial, larvicidal and repellent activity	Sivakumar *et al.* (2011) Abubakar *et al.* (2015)
Hexadecanoic acid	$C_{16}H_{32}O_2$	Antioxidant, pesticide, flavouring agent	Sermakkani and Thangapandian (2012)
Oleic acid	$C_{18}H_{34}O_2$	Antifungal activity	Carolina *et al.* (2011)

Many processing techniques have been applied for drying medicinal herbs, such as air-drying, oven-drying, and freeze-drying. The air-drying method can preserve products for a longer time, but the quality of products is greatly reduced as compared to fresh samples. Another method of air-drying is done at ambient temperature which is the most economical although it requires longer drying time. Alternatives for the drying method such as oven-drying and freeze-drying have gained popularity for a variety of food including herbs. Freeze-drying, also known as lyophilisation, is a gentle dehydration technique which can maintain the quality of products, especially colour, shape, aroma, and nutritional values (Karam *et al.,* 2016). However, as reported by Diaz-Maroto *et al.* (2002), air- or oven-drying at 45°C preserved some characteristics of a bay leaf better than freeze-drying.

To the best of the authors' knowledge, there is no literature available on the effect of drying methods on the essential oil components of *P. minus* roots. The present work was therefore carried out to investigate the effect of drying methods such as air-drying, oven-drying at temperatures of 40 and 60°C, and freeze-drying on the essential oil yield and chemical composition of *P. minus* roots, in the search for a suitable drying technique to preserve the quality of product prior to extraction.

Materials and methods

Sample preparation

Fresh roots of *P. minus* were collected from Cameron Highlands, Pahang, Malaysia. The identity of the sample was confirmed by Institute of Biological Science, Universiti Kebangsaan Malaysia. The fresh samples were cleaned and washed with distilled water. The samples were stored in a refrigerator at 4°C prior to the drying experiments. The *P. minus* roots was divided into four portions of about 0.5 g each for the comparison of drying methods. One portion, labelled as fresh sample (FS), served as control, while the remaining batches were dried using one of the following methods: air-drying at ambient temperature (AD), freeze-drying (FD), and oven-drying (OD).

Drying and moisture content determination

Air-drying (AD) was carried out under natural air flow at ambient temperature (mean = 29°C). The samples were placed into trays and left in the laboratory for 7 days. Oven-drying (OD) was conducted in a ventilated oven at 40 and 60°C for 3 d and 2 d, respectively, where the samples were placed in a small Petri dish. Freeze-drying (FD) was carried out in a freeze-dryer. The frozen material was freeze-dried under vacuum and condenser temperature of -58°C for 16 h. The moisture content of the dried samples was determined using a moisture analyser.

Essential oil extraction

The dried sample (0.5 g) was extracted in a 50-mL centrifuge and soaked in *n*-hexane (22.5 mL) for 4 h at room temperature. The collected extract solutions were filtered using a filter paper and evaporated using a rotary evaporator to yield viscous mass. The extraction yield (% g/g sample) was determined using the equation.

GC/MS analysis

The GC technique with some modifications was conducted using the Agilent Technologies Model 5975C gas chromatograph with non-polar column DB-5MS (30 m long and 0.25 mm diameter) and a film thickness of 0.25 µm. Helium, with a flow rate of 1.3 mL/min, was used as the carrier gas. The splitless injection programme was set for holding at 50°C for 3 min, increased to 250°C at 6°C/min and was then held at 250°C for 5 min. All the peaks were evaluated using the NIST mass spectral library (version 2.0).

Data processing of GC-MS

All peaks exceeding signal to noise ratio (S/N) of 100 were detected. A library search was conducted for peak identification using The National Institute of Standard and Technology (NIST, version 2.0, Gaithersburg, MD, USA) database and all peaks were combined into single peak table and were then transferred into Microsoft Excel. The volatile information as extracted based on the compound name and the match and the reverse match value below 800 were filtered.

Results and discussion

Effect of drying methods on moisture content

The effects of three different types of drying methods (AD, FD and OD) were studied by comparing with fresh sample (FS) as control. The moisture content of the different methods of dried sample is shown in Table 2.

The results showed that the initial moisture content of fresh sample (FS) was 86.83%. AD at ambient temperature after 7 d yielded 19.64% moisture content, while FD for 16 h 16.76%, and OD at 40 and 60°C for 3 and 2 d yielded 18.21% and 17.15% moisture content, respectively. All samples had slight changes in colour (to light brown) due to moisture loss. However, OD samples at 40 and 60°C showed a darker colour than the other samples. OD has indeed been shown to change the colour of the sample (Arslan *et al.,* 2010).

Table 2. Moisture content of *Polygonum minus* root by three different drying methods.

Samples	Conditions	Moisture Content (%)
Air dried (AD)	Temperature : 29°C Time : 7 days	19.51 ± 0.15
Freeze dried (FD)	Temperature : -58°C Time: 16 hours	16.58 ± 0.14
Oven dried (OD)	Temperature : 40°C Time : 3 days	18.21 ± 0.07
Oven dried (OD)	Temperature : 60°C Time : 2 days	17.15 ± 0.05
Fresh sample (FS)	None	86.83 ± 0.58

Effect of drying methods on the essential oil yield

Hexane extraction of *P. minus* roots with different drying methods produced a clear liquid essential oil. The results in Figure 1 show that the drying methods had a significant effect on essential oil yield with the highest essential oil yield was obtained from FD at 4.15% g/g sample, followed by AD (3.79% g/g sample), and OD at 40 and 60°C were 3.4% g/g sample and 0.86% g/g sample, respectively.

It is apparent from Figure 1 that the amount of essential oil yield decreased with increasing temperature from 40 to 60°C, in the case of OD. This could be due to changes in biological structure of the plant cells which resulted in a loss of essential oil yield (Ghasemi Pirbalouti *et al.,* 2013). The effect of temperature drying on plant materials towards essential oil yield was also reported in basil leaves (Ghasemi Pirbalouti *et al.,* 2013), tarragon leaves (Arabhosseini *et al.,* 2007), and thyme leaves (Rahimmalek and Hossein-Goli, 2012). However, the effect of drying temperatures on essential oil yield can be different in different plants in which some species have a higher temperature range, and therefore less affected (Rahimmalek and Hossein-Goli, 2012).

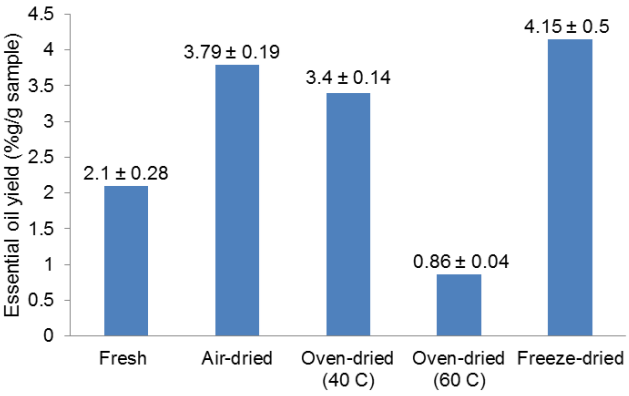

Figure 1. Comparison of oil yield from *Polygonum minus* by different drying methods.

Table 3. Compound identified in the essential oil of *Polygonum* minus root with different drying methods: (A) fresh sample, (B) oven drying at temperature 40°C, (C) oven drying at temperature 60°C, (D) air drying, and (E) freeze drying.

Compound[a]	Rt (min)	Percentage (%)[b]				
		A	B	C	D	E
Sesquiterpenes (β-caryophyllene)	12.78	1.94	1.42	1.57	1.43	0.08
Aliphatic Compound						
Nonane	6.719	0.23	-	-	-	-
Decane	8.5503	-	-	-	-	-
Undecane	10.121	0.046	-	-	-	-
Dodecane	11.163	-	-	-	-	-
Tridecane	11.924	0.34	-	-	-	-
Pentadecane	13.016	1.34	1.71	0.86	4.34	-
Hexadecane	13.454	1.14	3.45	5.00	1.76	-
Heptadecane	13.868	-	0.79	-	1.22	0.82
Octadecane	14.293	7.67	4.34	-	2.43	-
Eicosane	15.279	-	4.23	6.04	-	8.86
Organic Acid						
Hexadecanoic Acid	14.076	13.86	8.09	3.35	3.91	-
Oleic Acid	15.216	6.46	4.23	-	3.97	-

[a]Identified by GC-MS with names provided by NIST mass spectral library; [b]Percentage of component was calculated as peak area of analyte divided by peak area of total ion chromatogram times 100.

The results also showed that the duration of drying affected the essential oil yield. The essential oil yield from AD at temperature 29°C was slightly higher than OD but lower than FD. This could happen because AD had the longest drying time as compared to FD, in which AD took 7 d to dry the plant materials at a lower temperature than OD.

Effect of drying methods on essential oil chemical profile and composition

Volatile compounds present in hexane extract of *P. minus* roots were identified by GC-MS, which yielded 14 components. As shown in Table 3, for fresh *P. minus* root essential oil, 10 volatile components were identified with the most abundant being hexadecanoic acid (13.86%), followed by octadecane (7.67%), oleic acid (6.46%) and β-caryophyllene (1.93%). The highest amounts of volatiles detected in the fresh sample were organic acids (20.32%) followed by aliphatic compounds (10.76%) and sesquiterpenes (1.97%). Previous studies by Yaakob (1987), Baharum *et al.* (2010), and Ahmad *et al.* (2014) reported that the major compounds found in the *P. minus* leaves, stems, and roots were decanal, dodecanal, and β-caryophyllene. However, in the present work, no decanal or dodecanal were identified. This was probably due to less UV-B irradiation exposure towards *P. minus* roots (Steinmüller and Tevini, 1985).

To study the effect of drying methods on volatile components of *P. minus* root essential oil, the percentage of components from dried samples was compared with fresh root components. The results in Table 3 show that by increasing the temperature of OD to 60°C, many volatile components were lost as compared to the lower temperature of 40°C. It is shown that the number of volatile components decreased from eight to five, which were components lost from aliphatic compounds (heptadecane and octadecane) and organic acid (oleic acid).

The present work also demonstrated that the most abundant compounds found for OD at 40°C were hexadecanoic acid, oleic acid, pentadecane, and eicosane, which acted as the plant flavouring agent. However, by increasing the temperature to 60°C, the percentage of major compounds decreased from 8.09% to 3.35% for hexadecanoic acid while the oleic acid compound was totally lost. This could due to the biological structure of medicinal and aromatic plants which will be affected at high temperature and resulted in the collapse of the epithelial cell in the dried samples of some sensitive plants (Rahimmalek and Hossein-Goli, 2012; Ghasemi Pirbalouti *et al.,* 2013). The decrease in volatile components from medicinal plants at high temperature has been reported for tarragon leaves (Arabhosseini *et al.,* 2007) sage (Hamrouni-Sellami *et al.,* 2012), and basil leaves (Ghasemi Pirbalouti *et al.,* 2013).

In AD and FD, there were eight and four volatile components found in the essential oil, respectively. The major components obtained from AD were β-caryophyllene and pentadecane. The results also showed that essential oil by FD failed to preserve volatile components as compared to other drying methods. This could be due to the slight expansion of cell structure during FD which could release volatile components into the atmosphere (Ghasemi Pirbalouti *et al.,* 2013). A similar observation was found by Diaz-Maroto *et al.* (2002), whereby FD of spearmint resulted in the loss of most volatile compounds, especially oxygenated monoterpenes and sesquiterpenes.

Our results demonstrated that different drying methods could give significant effects on β-caryophyllene. This could be due to the fact that temperature plays a role in the production of terpenes in plants by enhancing the enzyme synthesis activity, raising terpene vapour pressure and decreasing the resistance of the emission pathway (Peñuelas and Llusià, 2001).

Additionally, a small amount of UV-B will give large biological effects which activate the plant's defence system by producing secondary metabolites (Gil *et al.,* 2013). A recent study from Zhang *et al.* (2017) showed that UV-B radiation led to a significant increase in the synthesis of bioactive compound. Our study showed that by increasing the temperature of OD from 40 to 60°C, the percentage of β-caryophyllene also increased. Therefore, the results of the essential oil components could vary with different plants or species.

In Figure 2, most chemical compounds (sesquiterpenes, aliphatic compounds, and organic acids) were present in the essential oil content from all drying methods. However, AD showed a great potential to preserve more volatile components of *P. minus* root hexane extract. Besides, there were a few similarities of compounds found in a previous study by Ahmad *et al.* (2014) which were β-caryophyllene (1.43%), pentadecane (4.34%), hexadecanoic acid (3.91%), and oleic acid (3.97%). However, the percentage of chemical compounds in the present work was lower than previously reported. This was probably due to different geographical and extraction methods, which their extraction was by the hydrodistillation method.

The reported activities of major active compound in *P. minus* roots are shown in Table 1. Aliphatic compounds (pentadecane) were found in essential oil content of *P. minus* roots and were identified as a flavouring agent with a great potential as food additives and in perfume industries. The results

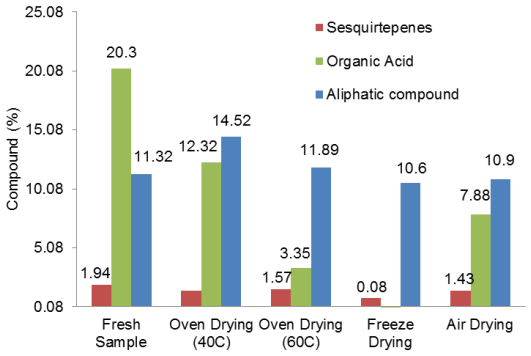

Figure 2. Chemical class compound (%) with different drying methods for Polygonum minus root.

showed that the organic acid compounds (hexadecanoic acid and oleic acid) had an important role in the application of *P. minus* as a traditional medicine. This is due to it being identified as antibacterial, antioxidant, and antifungal. β-caryophyllene is mainly found in *P. minus* roots (Ismail *et al.,* 2011; Ashraf *et al.,* 2015) which has great antifungal (Fernandes *et al.,* 2007), insecticidal (Sabulal *et al.,* 2006) and anticancer (Amiel *et al.,* 2012; Fidyt *et al.,* 2016) properties.

Conclusion

As a conclusion, both drying method and temperature had significant effects towards essential oil yield and chemical components of *P. minus* roots. Higher temperature drying (oven-drying at 40 and 60°C) decreased essential oil yield and volatile components loss. Both freeze- and air-drying gave higher essential oil yield. However, air-drying at ambient temperature is more recommended for drying *P. minus* roots because it showed the best result in preserving the *P. minus* roots' characteristics with all the majority chemical compounds being preserved. Besides, the operational cost for air-drying is cheaper than freeze- and oven-drying. In the future, different types of extraction methods should be carried out to determine the suitable method to get the highest yield of *P. minus* roots extract.

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