COMPARISON OF TWO DIFFERENT DRYING METHODS ON PHYTOCHEMICAL CONTENT, ANTIOXIDANT PROPERTIES, AND ANTI-INFLAMMATORY ACTIVITY OF Melicope pteleifolia

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ABSTRACT

Melicope pteleifolia (Champ. ex Benth) T.G.Hartley, which is a member of the Rutaceae family and locally known as *tenggek burung*, is one of the most common medicinal herbs found in Peninsular Malaysia and other Asian countries. This herbal plant contains various nutrients that have been practiced by the local population. The different drying methods were studied because they have an important effect on plant compounds and lead to the best drying method for an herbal industry. To evaluate the effect of drying methods on *Melicope pteleifolia* quality, the content of bioactive compounds, antioxidant activity, and anti-inflammatory activity of *M. pteleifolia* dried leaves through oven drying (40°C) and vacuum drying (60°C/30 mbar, 60°C/70 mbar, 75°C/30 mbar, and 75°C/70 mbar) were compared. Vacuum drying (60°C/30 mbar, 60° C/70 mbar, 75°C/30 mbar, and 75°C/70 mbar) showed a significant difference (p < 0.05) in phytochemical content, antioxidant activity, and anti-inflammatory activity. Meanwhile, the increase in temperature of vacuum drying potent in increment of the phytochemical compound of *M. pteleifolia* with significant difference (p < 0.05) was evidenced with 166.61 mg GAE/g, 35.52 mg QE/g, 168.46 mg TAE/g, and 95.67%, respectively to TPC, TFC, TTC, and TTeC, although lower in TSC (81.73%). However, the lower temperature resulted in high antioxidant with significant difference in DPPH (IC₅₀ 18.39), ABTS (IC₅₀ 38.42), FRAP (192.99 mg TEAC/g), and anti-inflammatory activity at 250 µg/ml (96.73%). This study provides evidence on how drying processes affect the content and activity of *M. pteleifolia* bioactive compounds. The selection of a specific drying method for 'tenggek burung' leaves can now be chosen based on the intended application such as the preparation of functional foods as well as nutraceutical and pharmaceutical products enriched in bioactive compounds, antioxidant capacity, and anti-inflammatory activity.



ABSTRAK

Melicope pteleifolia (Champ. ex Benth) T.G.Hartley yang merupakan famili Rutaceae dan dikenali sebagai tenggek burung adalah salah satu herba perubatan yang paling biasa ditemui di Semenanjung Malaysia dan negara-negara Asia yang lain. Tumbuhan herba ini mengandungi pelbagai nutrien yang telah diamalkan oleh penduduk tempatan. Kaedah pengeringan yang berbeza telah dikaji kerana ia mempunyai kesan penting terhadap sebatian tumbuhan dan membawa kepada kaedah pengeringan terbaik untuk industri herba. Untuk menilai kesan kaedah pengeringan terhadap kualiti Melicope pteleifolia, kandungan sebatian bioaktif, aktiviti antioksidan dan aktiviti anti-radang daun kering *M. pteleifolia* melalui pengeringan ketuhar (40°C) dan pengeringan vakum (60°C/30 mbar, 60°C/70 mbar, 75°C/30 mbar dan 75°C/70 mbar) dibandingkan. Pengeringan vakum (60°C/30 mbar, 60°C/70 mbar, 75°C/30 mbar dan 75° C/70 mbar) menunjukkan perbezaan ketara (p < 0.05) dalam kandungan fitokimia, aktiviti antioksidan, dan aktiviti anti-radang. Dalam pada itu, peningkatan suhu pengeringan vakum yang kuat dalam peningkatan sebatian fitokimia M. Pteleifolia menunjukkan perbezaan ketara (p < 0.05) dibuktikan dengan 166.61 mg GAE/g, 35.52 mg QE/g, 168.46 mg TAE/g dan 95.67%, masing-masing kepada TPC, TFC, TTC, dan TTeC, walaupun lebih rendah dalam TSC (81.73%). Walau bagaimanapun, suhu yang lebih rendah menghasilkan antioksidan yang tinggi dengan perbezaan ketara dalam DPPH (IC50 18.39), ABTS (IC50 38.42), FRAP (192.99 mg TEAC/g) dan aktiviti anti-radang pada 250 µg/ml (96.73%). Kajian ini memberikan bukti bagaimana proses pengeringan mempengaruhi kandungan dan aktiviti sebatian bioaktif M. pteleifolia. Pemilihan kaedah pengeringan khusus untuk daun 'tenggek burung' kini boleh dipilih berdasarkan aplikasi yang ditetapkan seperti penyediaan makanan berfungsi serta produk nutraseutikal dan farmaseutikal yang diperkaya dengan sebatian bioaktif, kapasiti antioksidan dan aktiviti anti-radang.



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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
°C	-	Degree Celsius
c	-	Concentration
IC50	-	Concentration at 50% inhibition
g	-	Grams
m	-	Mass
μL	-	Microlitre
min	-	Minute
mL	-	Millilitre
mg	-	Milligram
mM	-	Millimolar
min	-	Minute
mbar	-	Millibar
Μ	-	Molarity
nm	-	Nanometre
rpm	RP	Revolutions per minute
Na ₂ CO ₃	-	Sodium carbonate
V	-	Volume
v/v	-	Volume/volume
w/v	-	Weight/volume
ABTS	-	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	-	Analysis of variance
DMSO	-	Dimethyl sulfoxide
FRAP	-	Ferric reducing antioxidant power
GAE	-	Gallic Acid Equivalent
HPODE	-	Hydroperoxyoctadecadienoic acid
LOX	-	Lipoxygenase



NDGA	-	Nordihydroguaiaretic acid
QE	-	Quercetin Equivalent
SD	-	Standard Deviation
SEM	-	Standard Error of Mean
TAE	-	Tannic Acid Equivalent
TFC	-	Total Flavonoid Content
TPC	-	Total Phenolic Content
TSC	-	Total Saponin Content
TTC	-	Total Tannin Content
TTeC	-	Total Terpenoid Content
UV-Vis	-	Ultraviolet-visible

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CHAPTER 1

INTRODUCTION

1.1 Research background

Melicope pteleifolia, also known as 'tenggek burung' is one of the most widely distributed medicinal herbs and a popular traditional fresh vegetable that belongs to the Rutaceae family in Malaysia especially in Peninsular Malaysia, as well as several other Asian countries (Mahadi et al., 2016). Young leaves and twigs are eaten fresh with rice and are said to be useful for itching and wound treatment. The roots and bark are used as an appetizer, digestive, and emmenagogue (Abas et al., 2010). Furthermore, some research found that the fresh leaves have a slightly crunchy texture and a mildly pungent lemon-lime aroma, which makes them popular in vegetable salads. Melicope pteleifolia has traditionally been used to treat a wide range of conditions, including stomach aches, wounds, fever, and itches (Kabir et al., 2017). Pharmacologically, many studies have proven that the leaf extracts of this medicinal herb have bioactive properties. In fact, leaf extracts have been shown to have antiinflammatory, antipyretic, analgesic, antioxidant, and antimicrobial properties (Mahadi et al., 2016). For instance, Zareen et al., (2013) reported that the leaves possess the antibacterial, fungicidal, antioxidant, and inhibitory activities. As such, the benefits from the bioactive compounds of *M. pteleifolia* can be obtained through the leaf extraction method as has been carried out by other researchers.

Sample drying is important before proceeding with sample extraction because it affects the amount of yield and chemical compound extractions needed. Dried samples will produce more phenolic compounds than fresh samples. As evidenced by Fatariah, Zulkhairuazha, & Rosli (2014), *Benincasa hispida* drying and heating extract



recorded the highest gallic acid concentration of 0.272 mg/100 g, followed by low heating extract (0.050 mg/100 g) and fresh extract (0.036 mg/100 g). At the cellular level, the phenolic compounds are located in the vacuoles and are separated from oxidative enzymes in an intact fruit (Sulaiman et al., 2011). This structure collapses during the drying process, releasing more phenolic constituents as well as oxidative enzymes that may destroy the phenolic compounds (Fatariah et al., 2014). Furthermore, the drying process is essential in ensuring high phytochemical recovery during extraction. Drying provides opportunities for extending shelf-life, quality preservation, product enhancement, simplifying handling, storage, and transport, besides acting as a pre-treatment for subsequent processes (Kyriakopoulou et al., 2013). Many high-tech drying techniques have been developed in recent years, offering significant benefits such as reduced drying time, energy saving, low cost, and high-quality end products. Vacuum drying, for instance, is a drying technique 0-used to dry a variety of products while retaining colour and vitamin content. According to Alibas (2007), low processing temperature, less energy consumption and increased energy conservation, enhanced drying times, and, in some cases, less product shrinkage are all important characteristics of vacuum drying. Since no research has used the drying method for *M. pteleifolia* in particular, this study was conducted to see the effect of drying conditions by vacuum oven on the bioactivity compound of the plant.



The first stage in separating preferred natural products from raw materials is extraction, which refers to acquiring pure extracts. Generations of indigenous practitioners have used the plant's crude material or pure compounds to treat a variety of ailments thus, the study of medicinal plants often begins with extraction procedures, which are critical to the extraction outcomes and subsequent assays (Stéphane *et al.*, 2021). According to the extraction principle, extraction methods include solvent extraction, distillation, pressing, and sublimation. The most common method is solvent extract antioxidant compounds from a variety of plants and plant-based foods, including plum, strawberry, pomegranate, broccoli, sage, rosemary, rice bran, sumac, wheat grain and bran, mango seed kernel, citrus peel, and a variety of other fruit peels (Sultana, Anwar, & Ashraf, 2009). Similarly, *M. pteleifolia* was mostly found to be great in extraction yield by using methanol as solvent extraction (Mahadi *et al.*, 2019).

Phytochemicals are important nutrients found in medicinal plants that are not necessary for normal human body function but are active and have beneficial effects on health or disease treatment. Süntar & Yakıncı (2020) reported that plants contain phytochemicals, and their consumption has been shown to improve health. Thus, preclinical, clinical, and epidemiological research suggests that phytochemicals, due to their antioxidant and anti-inflammatory properties, may be useful in the treatment of a variety of diseases. Likewise, these bioactive compounds are found in M. pteleifolia. Apart from being consumed raw by consumers, it can also be used as the main ingredient in food supplements. However, the processing in making a product based on *M. pteleifolia* can cause negative changes in the product's physical and chemical properties. Drying can harm the inherent nutrients and bioactive compounds, depending on the drying method and treatment conditions. Therefore, selecting a drying method and optimizing the drying process are critical for bioactive compound retention (Ozay-Arancioglu et al., 2022). Hence, the purpose of this study is to AMINA determine the effects of different drying methods on the phytochemical analysis, antioxidant, and anti-inflammatory properties of *M. pteleifolia* leaf extracts.

1.2 Problem statement



Post-harvesting of medicinal plants is critical in the manufacturing process because it directly affects the quality and quantity of active ingredients in the products sold. Moisture determines the physical and chemical properties of aromatic and medicinal plants. The first step in many postharvest operations is water removal also known as drying, which is defined as the reduction of plant moisture content with the goal of preventing enzymatic and microbial activity, thus preserving the products to extend shelf life (Rocha, Melo, & Radünz, 2011). Drying is also the most common method of storing medicinal and aromatic plants and preserving their biochemical compounds (Rahimi & Farroki, 2019). Different methods can be used to dehydrate herbs. Natural drying methods, such as drying in the shade and hot air drying, remain popular due to their low cost. However, it has many drawbacks due to the inability to handle large quantities while maintaining consistent quality standards (Sellami *et al.*, 2011).

Bhardwaj *et al.*, (2021) reported that people accept drying in an open environment because it is inexpensive and relaxed and despite a small venture, it

results in a significant reduction in product quality. Drying is not done scientifically in this case because the environmental conditions change dramatically from day to night. Furthermore, this process is observed by untrained farmers (Bhardwaj et al., 2021). As harvest is repeatedly exposed to solar radiation, essential ingredients could be depleted. Direct contact with solar radiation has an effect on desirable properties such as texture, colour, and aroma (Seerangurayar et al., 2019; Tomar, Tiwari, & Norton, 2017). Besides, when traditional procedures are followed, dehydration cannot be effectively controlled, resulting in a fairly low-quality product (Afriyie et al., 2009; Singh, Vyas, & Yadav, 2019). Shade drying also takes longer, which is already considered an excessively long-time process and may result in significant loss of functional properties in some herbs such as the total antioxidant activity of peppermint and lemon balm, as well as loss of ascorbic acid and carotenoids in dried samples (Thamkaew, Sjöholm, & Galindo, 2021). Based on the literature by Babu et al., (2018), to prevent mould growth, the drying process to sensitive humidity should be completed as soon as possible. In fact, colour, nutritional composition, and organizational structure all suffer from lengthy drying time to final moisture (Babu *et al.*, 2018).



Moreover, convective drying is the most common method of drying; nonetheless, increasing the temperature usually results in a decrease in the quality of the dried herbs. A low-pressure method of removing water from moist materials is vacuum drying, the vacuum causes the air and water vapor in food to expand, creating a frothy structure. Compared to traditional drying methods, this method offers benefits such as faster drying rates, lower drying temperatures, and high-quality products (Hee & Chong, 2015). Moreover, many studies on *Melicope pteleifolia* had used air drying method as the common procedure in the post-harvest process (Hairiah et al., 2016; Kabir et al., 2017; Li et al., 2019; Shuib et al., 2011) but studies on the different drying methods using vacuum oven drying are scare. Therefore, this study was conducted using vacuum oven drying to improve the drying treatments and conditions as well as retain the bioactivity compounds of *Melicope pteleifolia* in producing nutraceutical and pharmaceutical products. Melicope pteleifolia was chosen in this study because it has a tremendous potential value in therapeutic properties for healing itchiness and wound as well as possesses important use for antioxidant, anti-inflammatory, antimicrobial and antinociceptive properties (Mohd Din et al., 2019).

1.3 **Research objectives**

This study aims to examine the effects of different drying methods on the phytochemical analysis, antioxidant and anti-inflammatory activities of Melicope pteleifolia leaf extracts. Meanwhile, the specific objectives are as follows:

- i. To analyse the effects of different drying methods of Melicope pteleifolia methanolic leaf extracts on the qualitative and quantitative phytochemical content.
- To evaluate the effects of different drying methods of Melicope pteleifolia ii. methanolic leaf extracts on antioxidant activities by DPPH, ABTS, and FRAP assays.
- iii. To establish an anti-inflammatory activity on the different drying methods of *Melicope pteleifolia* methanolic leaf extracts using lipoxygenase assay. UN AMINAH

Scopes of the study 1.4

To achieve the objectives, the scopes of this study are outlined as follows:

- Fresh plant samples were collected from the local market. i.
- Oven drying and vacuum oven drying were used for the drying methods ii. of the samples, whereas different temperatures and pressures were applied to the vacuum oven.
- iii. Methanol was used as a solvent for extraction of the leaf sample.
- iv. Phytochemical analysis was evaluated based on the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Tannin Content (TTC), Total Saponin Content (TSC), and Total Terpenoid Content (TTeC).
- Methods for antioxidant activities were determined with 2,2-diphenyl-1v. picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric-reducing antioxidant power (FRAP).
- vi. The anti-inflammatory activity of Melicope pteleifolia leaf extracts was measured using a lipoxygenase assay method.

1.5 Significance of the study

The investigation of different drying methods is important to see how they affect the phytochemical, antioxidant, and anti-inflammatory activities of *M. pteleifolia*. If suitable drying methods are used, *M pteleifolia* extracts of the highest quality can be obtained. In addition, it is also possible to improve *M. pteleifolia* bioactive properties by determining the most suitable parameter, and the findings will consequently aid in increasing their phytochemical content, antioxidant, and anti-inflammatory properties. As a result, the medicinal industry, particularly in the production of ancillary medicines, as well as consumers, may stand to gain significantly.

This study will shed new light on the medicinal properties of 'tenggek burung' (*Melicope pteleifolia*) by focusing on its antioxidant and anti-inflammatory properties. Accordingly, the community will benefit from this study by promoting the use of medicinal plants, particularly 'tenggek burung', as a preventive measure against various diseases. Furthermore, the analysis presented in this study will provide useful information for future research regarding the various medicinal benefits of 'tenggek burung' and other medicinal plants. Moreover, this study could also help the national economy by producing nutraceutical and pharmaceutical products.



CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter covers a review of the literature relevant to the research title. In this chapter, information regarding plant species, drying methods, phytochemical, antioxidant, and anti-inflammatory properties of *M. pteleifolia* methanolic extracts from the previous studies were discussed, including the effects of different drying methods with respect to these properties. The correlation effects and statistical analysis are also briefly explained in this chapter.

2.2 *Melicope pteleifolia* (Cham. ex Benth.) T.G.Hartley)

Melicope contains approximately 233 species that range from Madagascar and the Indo-Himalaya region east to the Hawaiian and Marquesas islands, and south to New Zealand (Shuib *et al.*, 2011). *Melicope pteleifolia* (Champ Ex. Benth) T.G.Hartley, a member of the Rutaceae family and locally known as 'tenggek burung', is one of the most common medicinal herbs found in Peninsular Malaysia and other Asian countries (Sulaiman *et al.*, 2010). Besides, it is also known as 'pauh-pauh', 'medang beberas', 'cabang tiga', and 'tapak itik'. Javanese people in Indonesia called it as 'sampang', while the Siamese called it 'Uam, Sam Ngam' (Mahadi *et al.*, 2016). Table 2.1 shows the taxonomic hierarchy of *M. pteleifolia*.

According to Yao *et al.*, (2020) *M. pteleifolia* can be found in a variety of habitats, including mountains, hills, and plains. *M. pteleifolia* usually entails a shrub or tree with a height of 1-14 m, and it is rarely scandent, dioecious, or monoclinous. In the third internode, the juvenile branchlet is 2.5-4 mm broad. The leaves are

normally 3-foliolate, although they can also be 1-foliolate, with a petiole that is 1.5-14 cm long and glabrous to hairy.

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magniolopsida
Order	Sapindales
Family	Rutaceae
Genus	Melicope
Species	Melicope pteleifolia
Order Family Genus Species	Sapindales Rutaceae Melicope Melicope pteleifolia

Table 2.1: The taxonomic hierarchy of *Melicope pteleifolia*.

Young leaves are eaten fresh as a traditional salad or "ulam", a favorite Malay appetizer (Zareen et al., 2013; Shuib et al., 2011). The extract prepared from M. pteleifolia, known locally as "Ba chac", is empirically used in the treatment of high fever, colds, and other inflammatory conditions in Vietnamese traditional medicine (Nguyen et al., 2016). In China traditional uses, the leaves in decoction were used in Guangxi and Guangdong area for treating early encephalitis, exogenous disease, dermatitis, eczema, snake bite or insect sting also fresh M. pteleifolia leaves mashed solution for trauma haemostasis (Yao et al., 2020). In traditional medicine, the entire plant is used to heal itching and sores, aid digestion, and act as an emmenagogue. Moreover, according to a number of studies, the leaf extracts have antioxidant, antibacterial, fungicidal, nitric oxide inhibitory, and antinociceptive activities. Hence, M. pteleifolia has many medicinal potentials in terms of curing itching and sores (Mohd Din et al., 2019). Mahadi et al., (2016) also proved that this plant provides a wide range of health benefits and the leaves have grown in popularity as a traditional fresh vegetable among Malaysians over the years. Anti-inflammatory, antipyretic, analgesic, antioxidant and antibacterial effects have been shown in their leaf extracts. Figure 2.1 shows the whole plant of *M. pteleifolia*.





Figure 2.1: Melicope pteleifolia (Lee et al., 2021)

According to Shaari *et al.*, (2011), phytochemical research on *Melicope pteleifolia* leaves from Sri Lanka found the presence of furoquinoline alkaloids and acetophenones, whereas leaves from Vietnam revealed the presence of benzopyrans as a prominent ingredient. The presence of p-O-geranylcoumaric acid is a prominent ingredient, and various polyprenylated acetophenones and benzylisoquinoline alkaloids were discovered in the leaves of a Malaysian plant by previous studies (Shaari *et al.*, 2011). The leaves also yielded two stereoisomeric substances called melicolone A (\pm) and melicolone B (\pm), while their stems were used to isolate four novel chroman derivatives (pteleifolones A-D) and nine additional chemicals, including acronyculatin B, dictamnine, acronylin, marmesin, 5-methoxymarmesin, evolitrine, (+)-peucedanol, N-methylatanine, and atanine (Xu *et al.*, 2016).



2.3 Drying methods

Drying is an ancient and unrivalled physical method of food preservation that is utilized in the food business for both direct preparation and later processing of food products. Drying has traditionally been a vital and frequent conservation practice, enabling the year-round availability of food and medical goods. The process of drying used to be natural and simple because it was powered by solar energy, but it has since become more advanced and optimized at every level. In terms of chemical and biological changes in the products during the dehydration process, the development of innovative approaches has been thoroughly researched. Medicinal and aromatic plants can be supplied fresh or dried, depending on their intended use, while fresh plants cannot be delivered profitably to every area in the country. Thus, according to Sellami *et al.*, (2011), drying medicinal plants has the primary goal of extending product shelf life, reducing packaging requirements, and lowering shipping weights.

Consumer desire for processed products that retain most of the unique features of fresh plants has risen in recent years. As a result, drying must be done with care in order to preserve as much of the flavour, aroma, colour, appearance, and nutritional content of the plants as possible (Jin *et al.*, 2018). This is the process of eliminating moisture from a product via evaporation up to a specified threshold value. Since the activities of bacteria, enzymes, and ferments in the material are repressed, the product can be preserved for a long time (Alibas, 2007). In addition, within a fresh food item, there are two forms of moisture: bound moisture, which is defined as liquid retained in the microstructure of the solid portion, and unbound moisture, which is defined as a liquid solution retained in the solid matrix. As a result, numerous activities coexist during the heat drying of fresh food products (Calin-Sanchez *et al.*, 2020). Energy is first transmitted from the hot drying agent to the new product. Next, unbounded moisture (free water) evaporates, and water particles bound inside the cellular structure, prone to diffusion and hence migration, are eventually moved to the product's surface, where the water evaporates (Setty & Murthy, 2003).



Many different drying methods, such as freeze drying, hot air drying, vacuum drying, and microwave drying were used to remove moisture from fresh materials, and drying conditions such as time, temperature, microwave power level, and air velocity, have been linked to various energy consumption and have a significant effect on phytochemicals and antioxidant properties of the samples (Saifullah *et al.*, 2019). The most common drying method is hot air drying or oven drying; however, this can cause thermal damage and drastically alter the volatile composition of herbs as well as their colour (Rubinskienė *et al.*, 2015). Moreover, this drying method results in dried items that are shrunken and tough, with obvious browning, limited rehydration potential, and low nutritional content. In order to improve quality and or efficiency, various studies have compared the quality, drying kinetics, and energy consumption of generating dried items by hot air drying to other drying processes (Pallas & García, 2011). Some researchers found that thermolabile chemicals and or oxidizable substrates such as carotenoids, tocochromarols, and lipids are frequently degraded by oven drying, which

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