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Effect of Drying Methods on Metabolites Composition of Misai Kucing (Orthosiphon stamineus) Leaves

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Abstract

In this research the effects of different drying methods on the capacity of antioxidant, total phenolic and biomarker compounds concentrations of Orthosiphon stamineus leaves were studied. The biomarker compounds of the leaves studied were sinensetin (SEN) and rosmarinic acid (RA). Three different drying methods adopted were an oven at 40°C, under the shade, and under the direct sunlight. Initial moisture content of the leaves was determined as 76.6% (w.b). The total antioxidant activity of the dried leaves extract was not significantly affected by the drying methods (P>0.05). However, the total phenolic content (TPC), sinensetin and rosmarinic acid content in the dried leaves extracts were significantly affected by the drying methods (P<0.05). The sinensetin was detected in samples dried in an oven (0.197mg/g) and was not detected in leaves dried under the shade or open sunlight. In contrast, the rosmarinic acid was only detected in samples dried under the shade (0.303mg/g).

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1. Introduction

Orthosiphon stamineus Benth or popularly known in Malaysia as misai kucing is an herbal species indigenous to the South-East Asian regions. This herbal plant belongs to a genus in the family of Lamiaceae. The plant can grow to about 1.2m in height and the leaves can be harvested in 2-3 months after plantings.

Although it was first introduced to the European consumers as an herbal tea in the 20th century, this
species was only became popular, particularly in Indonesia and Malaysia in the last few decades [1]. It is believed that the bioactive compounds contained in the leaves of O. stamineus exert the effects as an anti-allergic, anti-hypertensive, anti-inflammatory, antioxidant [2, 3], and diuretic properties [4]. It was reported that the phenolic such as caffeic acid derivatives, lipophilic flavones, flavonol glycosidase and polymethoxylated flavones most likely exhibited some medicinal benefits[2]. It now accepted that sinensetin (SEN), rosmarinic acid (RA), eupatorin (EUP), and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) contained in O. stamineus leaves extract are of pharmaceutical importance. Currently, O. stamineus based products are being commercialized as food supplements available in markets in the forms of tea sachets, drinks, raw herbs, tablets and capsules.

The quality of herbal based nutraceutical and food supplement products is closely associated with the quality of raw herbal materials used. There are many factors contributed to the quality of herbs and drying is one of them. Drying of herbal plant is done by either natural or artificial methods. Natural drying is the standard practice currently adopted by most of the Malaysian herbal producers. However, artificial method provides higher drying rate and more hygienic as compared to natural method since it uses heat and operated in a closed chamber. The study of the effects of different drying methods on the quality of herbs such as dill and parsley [5], coriander [6], mint [7], rosemary [8] and olive [9] have been reported by several researchers. However, there is no such information regarding the effects of drying methods on the quality of O. stamineus herbal raw leaves. The objective of this research was to determine the relationship between drying methods and bioactive metabolites compositions of O. stamineus leaves.

2. Materials and Methods

2.1. Drying Experiment

O. stamineus plants were grown at the Malaysian University Perlis (UniMAP) Agrotechnology Research Station, Sg. Chucuh, Perlis, Malaysia. The plants were grown from cuttings and maintained according the standard practiced adopted by the majority growers in Malaysia. Fresh leaves were harvested between 10 -11 am in the morning, washed and rinsed. The leaves were then immediately removed from the twigs for subsequent drying treatments. The initial moisture content of the leaves was determined by using Sartorious Moisture Analyzer. The samples of 20 g of O. stamineus leaves were dried by using three different methods; an oven at 40°C, under the shade and directly under the sun. The samples were dried for 8 hours by oven at the temperature of 40°C. The samples in a shade and directly under the sun were dried for 4 days. The samples were replicated five times for each method and the data were recorded.

2.2. Extraction Process

Metabolites from dried O. stamineus leaves were extracted by using a Sartorious orbital shaker and water was used as extraction solvent. 5 g of dried leaves was grinded extracted in 200 ml water for five hours at 40°C and 150 rpm rotation speed. The extracts were kept in a fridge in a tight glass bottle for further analysis.

2.3. Total phenolic content (TPC) determination

The concentration of total phenolic compounds in the extracts were determined by using Follin-Ciocalteu (FC) reagent and caffeic acid was used as an external standard [10]. 0.4 ml of extract, 0.4 ml of FC reagent, 0.4 ml of methanol and 2.8 ml of 20% CaCO₃ were mixed thoroughly with a vortex mixer. The mixture was kept at room temperature in the dark for 20 minutes. The absorbance value was measured at 735nm by using a
Shimadzu UV/Vis spectrophotometer. The data was compared with the caffeic acid external calibration curve to give the concentration of total phenolic compounds as caffeic acid equivalent.

2.4. Total antioxidant activity determination

Total antioxidant activity was determined by using free radical scavenging activity (FRSA) method [11]. 2 ml of the methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was mixed with 200 μl of water extract and the mixture was added with methanol to make a final volume of 3 ml. The mixtures were left to stand for 60 minutes, then absorbance value was measured against methanol as a blank at 517nm by using the spectrophotometer. The free radical-scavenging activities (%) of the tested samples were compared with a control (2 ml DPPH solution and 1 ml methanol). The free radical-scavenging activity was measured by using this formula:

\[ \text{FRSA} = \left( \frac{A_c - A_s}{A_c} \right) \times 100 \]  

(1)

Where, \( A_c \) is absorbance value for control and \( A_s \) is absorbance value for sample.

2.5. HPLC Analysis

1.5 ml of \( O. \) stamineus leaves extract was filtered through a 0.45μm nylon membrane filter prior to HPLC analysis. A Shimadzu HPLC was utilized to perform the analysis which was equipped with autosampler, column oven and UV/VIS detector. A HPLC column used was Merck Licrochart Purospher Start RP 18 column (250mm, 4.6 mm i.d, 5μm pore size). The mobile phase used was a mixture of water: methanol: tetrahydrofuran (50: 45: 5 v/v) [3]. Each sample was analysed at the mobile phase flow rate of 1 ml/min, detector wavelength of 340nm at 30°C for 40 min. For qualification and quantification purposes, calibration curve was made with the standard marker compounds sinensetin (SEN) and rosmarinic acid (RA) purchased from Indofine Chemical Company and Sigma Aldrich respectively.

2.6. Statistical Analysis

The results were statistical analyzed using Analysis of Variance (ANOVA) in Statistical Software for Science Social (SPSS). The data were expressed as means of five replications measurements. Turkey Test was performed and the the significant value was considered if the difference of P is < 0.05.

3. Result and Discussion

The effects of drying methods on the phytochemical constituents of \( O. \) stamineus leaves dried under three different techniques are shown in Table 1.

<table>
<thead>
<tr>
<th>Drying method</th>
<th>DPPH (%)</th>
<th>TPC (mg/g dry weight)</th>
<th>Concentration (mg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven</td>
<td>80.602 (^a)</td>
<td>1.572 (^a)</td>
<td>RA nd, SEN 0.197 (_a)</td>
</tr>
</tbody>
</table>
Drying of *O. stamineus* leaves in oven had significantly reduced the content of phenolic compounds to 1.572 mg/g as compared to 4.350 mg/g when leaves were dried under the shade. However, the antioxidant capacities of the leaves dried under the three different techniques were relatively high. The inhibition rates ranged between 78% to 84% obtained were not differ significantly between the three drying treatments adopted. This may indicate that *O. stamineus* plant is a rich source of compounds which exhibit an antioxidant characteristic and at the same time can tolerate the various post-harvest drying techniques. However, on the other hand certain compounds of the phenolic group extracted from the leaves may be liable to higher heat treatment as revealed in the concentration reduction of the oven dried samples. Further works probably justifiable to characterize and quantify phenolic chemical compounds with antioxidant characteristic since *O. stamineus* leaves and other parts of this species is quite widely used as food supplements and health curative purposes.

The concentrations of the main medicinal bioactive compounds, namely rosmarinic acid and sinensetin of *O. stamineus* leaves subjected to different drying techniques were also quantified. Rosmarinic acid found in abundance in certain plant species is a potent antioxidant due to the presence of phenolic hydroxyl group in its structure, while sinensetin which is commonly found in *Orthosiphon* species was reported to exhibit potential anti-cancer characteristic[12]. The content of rosmarinic acid in the extract was only detected in the samples dried under the shade (0.303 mg/g). In contrast, sinensetin was only detected at relatively low concentration (0.197 mg/g) in the leaves dried in the oven and this compound was not detected in samples dried in open sunlight or shade. It is possible rosmarinic acid is sensitive to high temperature and direct sunlight during leaves drying. However in contrast, sinensetin may be susceptible to exposure to sunlight during leaves drying.

### 4. Conclusion

Our result revealed that the technique used for fresh leaves drying is very critical in determining the constituent of *O. stamineus* bioactive constituents. Drying leaves under shade seem to retain higher content of total phenolic, but drastically affecting the sinensetin concentration. The antioxidant capacities of the extracts were consistently high in all treatment which indicates that other compounds may also involve in the reactions. The concentration of rosmarinic acid was maintained when leaves were dried under shade but not detected in oven and open sunlight dried samples. As such an appropriate and balanced method of drying needs to be elucidated in order to retain important bioactive compounds of this species after drying.

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References


