Effect of Drying Temperature on Rosmarinic Acid and Sinensetin Concentration in *Orthosiphon stamineus* Herbal Leaves

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**Abstract.** The objective of this work was to investigate the effects of drying temperature on the concentration of *Orthosiphon stamineus* biomarker compounds which were rosmarinic acid (RA) and sinensetin (SEN). The thin layer drying approach was used to dry *O. stamineus* leaves at various temperatures of 30, 40 and 50°C using a laboratory scale hot air dryer. The dried leaves were then extracted using 60% aqueous methanol prior to quantification. The RA and SEN concentrations in the dried leaves extracts were quantified by the high performance liquid chromatography. The concentration of RA for the dried leaves at 30 and 40°C were higher as compared to that of the fresh leaves. This may due to the response of the plant cells to abiotic stress. The concentration of RA also showed a significant reduction when the temperature was increased to 50°C. In contrast, the SEN concentration in *O. stamineus* dried leaf extract was lower than that of the fresh samples. The concentrations of SEN depicted insignificant effects by drying at 30 and 50°C, and the highest value was obtained in the samples dried at 40°C. Results showed that the drying process was found to affect the concentration of both compounds; therefore suitable drying conditions should be adopted to enhance the medicinal values of the plant species.

1. Introduction

*Orthosiphon stamineus* (*O. stamineus*) also known as misai kucing (cat whispers) or Java tea, is a popular medicinal herb in the Southeast Asia region. This herb which belongs to *Lamiaceae* family is used as a traditional medicine to cure many human illnesses and disorders especially those related to urinary tract infections and kidney stones, eruptive fever, hypertension, epilepsy, hepatitis, rheumatism, syphilis, and gonorrhea [1, 2]. A number of bioactive compounds such as rosmarinic acid (RA), sinensetin (SEN), 3'-hydroxy-5', 6,7 4'-tetramethoxyflavone, eupatorin, orthosiphol A and orthosiphol B have been identified...
and isolated from *O. stamineus*. These compounds define the medicinal properties of the herbal species and are classified as herbal marker compounds of the *O. stamineus* plant species [3-9].

*O. stamineus* is always dried for extending its shelf life during long storage period prior to downstream processing. The physical properties such as color and aroma are considered as a major criterion by the consumers in determining the optimum quality of the dried herbal plant. However, the concentration of the bioactive compounds is the determinant factor that confirms the medicinal properties of the dried herbal plants. For instance, the RA that is found in abundance in *O. stamineus* and in certain plant species is a potent antioxidant due to the presence of phenolic hydroxyl group in its structure, while SEN which is commonly found in *Orthosiphon* species was reported to exhibit potential anti-cancer characteristic [10]. Many studies have examined the effects of drying on the bioactive constituents as well as the physical properties of dried medicinal and aromatic plants. For instance, the degree of change in the color and texture of the dried mint (*Mithna cordifolia*) leaves depends on several drying parameters such as drying time, drying temperature, and oxygen level [11]. Another research work reported that the microwave drying of rosemary (*Rosmarinus officinalis*) leaves resulted in the optimum retention of minerals content and color quality as compared to the oven dried leaves [12]. A similar finding was revealed in the dried thyme (*Thymus daenensis*) leaves [13]. In addition, optimum preservation of RA content in lemon balm (*Melissa officinalis*) leaves was found under drying at a low temperature [14]. To the best of our knowledge, there is no research works that have been published regarding the drying effects on *O. stamineus* marker compounds content excluded our previous work [15]. Thus, the present research emphasizes on the effect of one of the important drying parameters that is the air temperature on the RA and SEN concentrations.

2. Materials and methods

2.1. Materials and equipment

Fresh *O. stamineus* leaves aged eight to ten weeks were harvested from the School of Bioprocess Engineering, University Malaysia Perlis, Malaysia production plot. The uniformly sized fresh leaves were packed into an airtight plastic bag and capped in a plastic container to avoid dehydration. The leaves were immediately stored in the cold room at about 5 °C prior to the experiment. Methanol (analytical grade), methanol and tetrahydrofuran (liquid chromatography grade) were obtained from Merck (Darmstadt, Germany). The standard marker compounds of RA and SEN were purchased from Indofine Chemical Co. (Hillborough, NJ USA). A customized laboratory scale hot air dryer was used for the drying experiments.

2.2. Thin layer drying of *O. stamineus* leaves

A sample size of 20 g of the fresh leaves was used for each thin layer drying experiment, which was performed twice separately. The drying experiments were performed using the hot air dryer at various air temperatures of 30, 40 and 50 °C with fix air velocity of 0.8 m/s. In addition, prior to the experiments, initial moisture content of the fresh *O. stamineus* leaves was measured by oven method (105 °C, 24 hr) using a laboratory drying oven (Binder). The samples were distributed evenly with a thickness of three leaves on the drying tray. The weights of the samples were taken manually during the drying process at particular time intervals, depending on the air temperature, until no weight change was recorded. In addition, the time taken to measure the weight at every interval did not exceed 30 s to avoid significant change in the surface temperature.

2.3. Extraction of bioactive compounds

The fresh and dried *O. stamineus* leaves were solvent extracted prior to the marker compounds quantification. The leaves were ground using a laboratory grinder. Then 1 g of the ground leaf was mixed with 100 ml of 60% aqueous methanol solution in a 250 ml conical flask. The conical flasks containing the
samples were agitated at 150 rpm for 5 h using an orbital shaker (Model BS-1, Sartorius Certomat) at 40°C. The extracts were filtered using filter papers (Whatman), and the filtrates were kept in air tight plastic bottles in a refrigerator for further analysis.

2.4. Quantification of biomarker compounds
The quantification analysis was performed using the high performance liquid chromatography (HPLC), equipped with two pumps (Model 20A, Shimadzu), auto sampler (Model SIL 20A, Shimadzu), column oven (Model CTO, Shimadzu), and UV/Vis detector (Model SPD 20A, Shimadzu). About 1.5 ml of the previously prepared extract without dilution was filtered through a 0.45 μm syringe nylon membrane filter into an HPLC vial prior to qualification and quantification. The Merck Licrochart Purospher Start RP 18 HPLC column (250 mm, 4.6 mm i.d, 5 μm pore size) was used. The mobile phase was composed of a mixture of water: methanol: tetrahydrofuran (50: 45: 5 v/v). The mobile phase was adjusted to pH 3 by adding phosphoric acid. The samples were analyzed at the mobile phase at the flow rate of 1 ml/min, and the detector wavelength was set at 340 nm at 30°C for 40 min [2].

The standard external calibration curves were used to quantify the concentrations of the standard biomarker compounds. The biomarker compounds were prepared by dissolving the chemicals in 60% aqueous methanol solution at different concentration ranges (varying from 0 to 10 mg/l for SEN and 0 to 250 mg/l for RA). For the purpose of validation, the biomarker compounds were occasionally spiked with samples for retention time and peak confirmation.

2.5. Statistical analysis
The thin layer drying temperature that affected the marker compounds of *O. stamineus* dried leaves were compared using the Analysis of Variance (ANOVA). Unless otherwise stated, the significance level was established at probability (P) < 0.05. For comparison between the means, the Student’s t-test was performed. Significance was established if the difference between the means for the treatments was more than the calculated least square difference (LSD).

3. Results and discussion
3.1. Effects of drying conditions on RA concentration
The concentrations of RA for the dried leaves at 30 and 40°C were higher as compared to that of the fresh leaves, as shown in Table 1. In general, the concentrations of this biomarker compound for both the temperatures were relatively high in comparison to other RA contained species [16]. However, the content of RA showed a significant reduction when the temperature was increased to 50°C. This result indicated that the RA in the leaves of *O. stamineus* was liable to a high-temperature range, as portrayed in the drastic reduction in concentration when the temperature was increased from 30 to 50°C. This trend is in accordance with our previous work which also revealed that when the leaf was dried with relatively higher temperature, the RA concentration easily destroyed [15]. Other previous studies also reported similar trends; the concentration of RA in rosemary and lemon balm leaves reduced with a progressive increase in the temperature [14, 16].

Besides, it is very interesting to observe that the dried *O. stamineus* leaves over 30 and 40°C contained higher RA than the fresh leaves (Table 1). Due to the narrow temperature range and lower maximum drying temperature, the leaves of *O. stamineus* tend to slowly lose their moisture content during drying, i.e., the leaf cells tend to be under stress. The concentration of RA in the dried leaf samples of *O. stamineus* might synthesize and accumulate as a defensive reaction. In addition, RA is classified as a defensive compound,
which is produced based on the biotic or abiotic stress in the plants, such as wounding, water deficit, low temperature, and pathogen attacks [17].

As shown in Table 1, the concentrations of RA in the dried leaf sample at 30 and 40 °C were significantly higher than those dried at 50 °C. This pattern may cause longer drying period consumed at 30 and 40 °C. This pattern is similar to the air-dried rosemary leaves samples with more than two weeks of drying period possessed the highest RA concentration in the air-dried samples that of vacuum and oven-dried samples [16].

Table 1 Effects of drying conditions on the concentrations of biomarker compounds for *O. stamineus* leaves

| T (°C) | RA (mg/g dw) | SEN (mg/g dw) |
|-------|--------------|---------------|
| Fresh leaf | 22.34±6.71 | 3.71±0.68 |
| 30 | 171.47±14.7a | 0.74±0.22ab |
| 40 | 98.69±25.71b | 0.88±0.12a |
| 50 | 9.10±3.58c | 0.41±0.04b |

Mean ± standard deviation with different letters in a column were significantly different (P<0.05)

3.2. Effects of drying conditions on SEN concentration

The SEN concentration in *O. stamineus* dried leaf samples was lower than that of the fresh samples, as shown in Table 1. The concentration of SEN in samples dried at 30 °C are not show a significant difference as compared with the samples dried at 40 and 50 °C. However, the SEN concentration in samples dried at an air temperature of 50 °C showed significant reduction as compared to the samples dried at 40 °C. The pattern of SEN reduction at high temperature (50 °C) may hint toward its susceptibility to heat. Apart from this, as reported in our previous work, SEN is susceptible to sunlight where it was only detected in an oven dried sample but did not appear in under shade dried and sundried samples [14]. A biomarker compound found in greater plantain (*Plantago major*), plantamojoside also reduced when the temperature increased from 30 to 50 °C [18]. Another study had revealed biomarker compounds in plantain (*Plantago lanceolata*) leaves also exhibited a similar pattern [19]. The marker compounds in plantain which are iridoidglycosides catapol and aucubin, found in the dried samples at 60 °C showed a decrease as compared to the fresh leaf samples. SEN is an important marker compound in *O. stamineus* and was reported to exert some medicinal benefits. For example, it exhibits antidiabetic property [20] and acts as a chemosensitizer in anticancer treatment [21]. Since, the drying process affects the concentration of SEN, appropriate drying conditions should be adopted to enhance the medicinal values of the plant species.
4. Conclusion

The concentration of RA significantly reduced with increasing temperature. The concentration of RA was found to be lower in the fresh samples as compared to the dried samples. This may be caused by the response of the plant cells to abiotic stress. The concentrations of SEN depicted insignificant effects by drying at 30 and 50°C, and the highest value was obtained in the samples dried at 40°C.

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