eISBN 978-967-16145-1-8

Konvensyen Kebangsaan Kejuruteraan Pertanian Dan Makanan 2019
21 Mac 2019, Wisma Tani, Kementerian Pertanian Malaysia, Putrajaya

MSAE2019-PFE01

The Effect of Drying Temperatures on the Active Compound of *Vitex Negundo* Leaves

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Abstract
The effect of drying temperature on the leaves of *Vitex negundo* was determined. Three levels of temperatures (40, 50 and 60°C) were used in the presented study. The initial moisture content of the leaves was 69.98%. Continuous drying at the above mentioned temperature levels was conducted to determine the drying time required to achieve equilibrium moisture content. The quality of dried leaves was evaluated based on the quantity of agnuside, major compound of *V. negundo* using High Performance Liquid Chromatography (HPLC) analysis. The fastest drying of the leaves was achieved at 60°C, followed by at 50°C, but HPLC results showed that dried *V. negundo* suffered at 40% reduction in agnuside content when drying at 60°C as compared to at 40°C. Slight reduction of agnuside was found in the sample dried at 50°C. Based on the findings of this work, the best convection oven drying condition for *V. negundo* leaves was at 50°C with 502.224 mg/L of agnuside concentration.

Keywords : *Vitex negundo*, agnuside, drying, phytochemical

Introduction

The genus *Vitex* (Verbenaceae) consists of trees and shrubs, found in tropical and subtropical regions. About 30 species occur in the Malesian region. The most important medicinal species, *V. negundo* and *V. trifolia*, are widely cultivated not only for their medicinal properties but also as ornamental and hedge plants, and have sometimes naturalized. The leaf extract of *V. negundo* has been reported to reveal a wide range of biological actions including mosquito repellent activity, anti-angiogenic, hepatoprotective, analgesic, antiinflammatory, anti-arthritic, antimicrobial, antihistaminic, CNS depressant, anti-filarial activities etc. These actions may be due to the various phytoconstituents present in the plant, which include iridoids, flavonoids, polyphenolic compounds, alkaloids, terpenoids etc. (Patil, 2018). Owing to these various phytochemicals, this plant has a crucial role in phytomedicine.

Drying is an important process of dehydrated material preparation because it reduces the moisture content of fresh materials for long storage and minimizes the costs of transportation and preservation. However, drying conditions have been shown to have significant influences on the quality and stability of bioactive compounds, and their bioactivity capacity. *V. negundo*, shows the presence of numerous iridoids like agnuside, negundoside and its responsible for the different pharmacological activities. Among these iridoids, agnuside is an important chemotaxonomic marker that can be used in quality control of *V. negundo* raw material. Importantly, there is no previous study reporting on the optimal drying conditions from convection oven drying of *V. negundo* leaves. Therefore, it is necessary to identify the optimal drying conditions for preparation of dried *V. negundo* leaves for further processing steps. In the present work, the optimum drying condition was studied to produce high quality dried material of *V. negundo* leaves. The quality of the dried materials will be evaluated based on agnuside content quantified by HPLC analysis.

Methods

Raw Material

Fresh plant of *V. negundo* was harvested from Maran Research Station, Forest Research Institute Malaysia (FRIM). The plant was sorted manually for its leaves to be used in the experiments. The leaves were cleaned from dirt using tap water, rinsed and then kept in polystyrene box while being transported to the laboratory. Initial moisture content of the leaves was 69.98 ± 5.42% (wet basis) measured via calibrated Halogen Moisture Analyser (Model AND MS-70, Japan).

Drying experiments

The drying experiments were done at three selected temperatures of 40, 50 and 60°C. Lab scale convection oven dryer (UFE 500 type, Memmert, Germany) was used in this study. For the experiments, an approximate weight of 5.00 g leaves of *Vitex negundo* was distributed uniformly on aluminium tray placed in the drying chamber. Sample mass was measured by weighing the tray outside the drying chamber periodically using an electronic balance. Weights were recorded every 10 minutes until the equilibrium moisture content was reached for the calculation of moisture content during experiment. Each drying experiment was triplicated. Final moisture content was determined using Halogen moisture analyser.

The free moisture versus drying time graphs was plotted for each of the experiments. Moisture content (dry basis) of the sample was described by the percentage equivalent of the ratio of the weight of water to the total weight of the dry material. It was calculated by using equation as below (Ramaswamy and Marcotte, 2006):

$$Moisture\ content = \left({}^{M}/_{S}\right) \times 100$$

Where M is the content of water and S is the content of solid.

Qualitative and Quantitative Determination of Phytochemical in Dried V. negundo

Instrumentation:
HPLC chromatograms are generated with Waters HPLC system composing of a quaternary pump (Waters 600E), an autosampler (Waters 717) and a PDA detector (Waters 2996 PDA) scanning from 190 nm to 400 nm using a reversed phase C-18 column (4.6 i.d. x 250 nm, 5µm). The Chromatograph are processed using Empower 2 software.

Sample preparation of V. negundo leaves for HPLC analysis:
The dried and ground sample (0.5 g) is extracted in 15 ml of HPLC grade methanol by sonication in a closed vial for 15 min. the solution is filtered using 0.45 µm filter before subjected to HPLC analysis. An aliquot of 10 µL is injected for the analysis.

Preparation of standard solutions for HPLC analysis:
Stock solutions of agnuside (1 mg/mL) was prepared separately in methanol. The prepared standard solutions were sonicated and filtered through a 0.45 µm membrane filter prior to analysis

HPLC analysis:
The analysis was carried out on a RP-18 column using 0.1% formic acid (solvent A) and acetonitrile (solvent B) as mobile phase in gradient mode. The gradient elution profile used was: 0 min, 10% B; 7 min, 30% B; 15 min, 40% B; 18 min, 100% B. The column was equilibrated with 10% B for 5 min before the next injection. 10 µL of standards and sample were injected into the HPLC at a flow rate of 1 mL/min. The agnuside was analyzed 285 nm.

Results and Discussion

Drying Kinetics of Vitex negundo Leaves from Convection Oven Drying
Figure 1 illustrates the change of free moisture over time for *V. negundo* leaves with initial moisture content in range of 3.730 - 3.970 % (db). It is clear that the moisture content decreased with increasing time during drying process. It took 500, 330 and 140 minutes to dry the leaves at respective temperature of 40, 50 and 60°C. Higher drying temperature resulted in shorter drying times. Drying at 50 and 60°C could reduce about 34 and 72%, respectively of drying time as compared to drying at 40°C. This might be due to larger driving force for heat transfer at temperature 60 and 50°C as compared with at temperature 40°C.

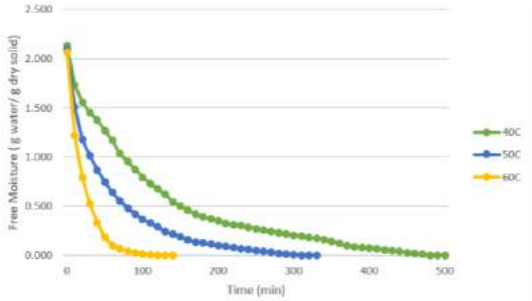

Figure 1: Drying curves of V. negundo leaves dried at 40, 50 and 60°C using convection oven

Phytochemical content of V. negundo leaves from Convection Oven Drying

Concentration of agnuside in convection oven dried leaves of *V. negundo* is illustrated in Figure 2. The figure shows that the concentration of agnuside was decreasing with increasing temperatures. *Vitex negundo* leaves dried at 40, 50 and 60°C contained remaining agnuside concentration of 535.377 mg/L, 502.224 mg/L and 320.574 mg/L, respectively. The slight reduction of agnuside was observed when the leaves were dried at 50°C as compared to at 40°C. The drop of concentration of agnuside was significant in the leaves dried at 60°C when compared to at 40 and 50°C indicates that it might be due to the destruction of agnuside during drying as a result of thermal damage.

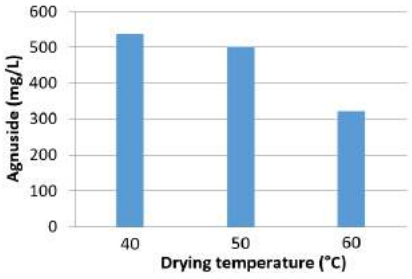

Figure 2: Concentration of V. negundo in dried leaves subjected to different drying temperatures

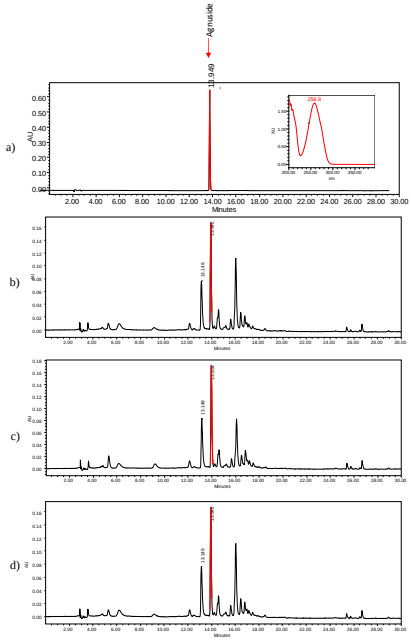

Figure 3: HPLC chromatograms of (a) standard; (b) leaves dried at 40°C; (c) leaves dried at 50°C; (d) leaves dried at 60°C

Conclusion

In term of quality with regards to drying time and phytochemical content, the best convection oven drying condition for *V. negundo* leaves was at 50°C with 502.224 mg/L of agnuside concentration.

Acknowledgements

The authors would like to thank all the staff of Natural Products Division of Forest Research Institute Malaysia (FRIM) for their support and technical assistance.

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