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Effect of Drying Temperatures on Chemical compounds and Antioxidant properties of *Vitex negundo l*eaves

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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, a major compound in *V. negundo* using 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 as compared to at 40°C. Whereas, antioxidant results showed that *V. negundo* leaves have significant level of phenolic content and the effect of drying at higher temperature has significantly reduce the amount of phenolics in *V. negundo* leaves. Total phenolic content of *V. negundo* leaves was highest at 50 °C drying temperature. Based on the findings of this work, the best convection oven drying condition for *V. negundo* leaves was at 50°C with the highest agnuside concentration of 502.224 mg/L and phenolic content of 286.7 ± 11.0 mg GAE/100g.
Key Words :*Vitex negundo*, agnuside, drying, phytochemical.

Introduction

The genus *Vitex* (Verbenaceae) consists of trees and shrubs, found in tropical and subtropical regions. There are about 30 species available 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,

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antiinflammatory, anti-arthritic, antimicrobial, antihistaminic, central nervous system (CNS)depressant and anti-filarial activities. These actions may be due to the various phytoconstituents present in the plant, which include iridoids, flavonoids, polyphenolic compounds, alkaloids, terpenoids etc[1].Owing to these various phytochemicals, this plant has a crucial role in phytomedicine.

Drying is an important process in material preparation because it reduces the moisture content of fresh herbs 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 antioxidant properties. *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.

On the other hand, antioxidants are well accepted as dietary supplements as it is proven to improve defense mechanism in human body[2]. During dehydration, antioxidant compounds and its activity often depleted[3].A study reported before[4] has suggested that drying is the main mechanism factor to cause the reduction in polyphenol content.Polyphenols are abundant in plants and regards as the main contributor of antioxidant capability of most plants. Many previous studies have found that antioxidant properties of plants were proportional to their phenolicscontent[5,6]. The redox properties of phenolic compounds were attributed by their hydroxyl group, allowing them to act as antioxidant[7]. Among the most common polyphenols found in the plants are caffeic acid, gallic acid and ferrulic acid[8].

In the present work, the effects of drying temperatures were studied to produce high quality dried material of *V. negundo* leaves. The quality will be evaluated based on agnuside content quantified by high performance liquid chromatography (HPLC) analysis and antioxidant activity from DPPH and total phenolic content.DPPH is a radical which is commonly used for determination of antioxidant properties of plant extracts. It is crucial to perform drying at its optimum condition to prevent any damages to the dried materials. To date, there were no previous studies reported on drying for this*V. negundo* leaves.

Material and 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, anapproximate 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[9]:

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

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

Curves Fitting

Experimental data obtained were fitted to the three well-known thin layer drying models given in Table 3.5. These models require the calculation of dimensionless moisture ratio, which is given by equation 3.3[10]:

$$\textit{Moisture ratio} = \frac{M - M_e}{M_o - M_e}$$

Where M, M_e and $M_o$are moisture content at any time, equilibrium moisture content and initial moisture content, respectively.

Table 1. Mathematical models applied to the drying curves

Model no	Model name	Model	Reference
1	Page	$MR = \exp(-kt^n)$	[11]
2	Midili	$MR = a\exp(-kt^n) + bt$	[12]
3	Logarithmic	$MR = a\exp(-kt^n) + b$	[13]

The coefficient of determination (R^2) was the primary criterion for selecting the best model to describe the drying curves[14]. In addition to R^2, root mean square error (RMSE) analysis was used to determine the goodness of the fit. The higher the value of R^2 and the lowest values of RMSE, the better the goodness of the fit[12]. They were calculated using equation 3.5 and 3.6[13]:

$$R^2 = 1 - \left[\frac{\sum_{i=1}^{N}\left(MR_{\exp,i} - MR_{pre,i}\right)^2}{\sum_{i=1}^{N}\left(MR_{\exp,i} - \overline{MR_{\exp}}\right)^2}\right]$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{\exp,i} - MR_{pre,i}\right)^2\right]^{1/2}$$

Where $MR_{exp,\,i}$ is the ith experimentally observed moisture ratio, $MR_{pre,\,i}$ is the ith predicted moisture ratio, N the number of observations.

Phytochemical Qualitative and Quantitative Analysis

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). TheChromatograph 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 at 285 nm.

Determination of Antioxidant activity of in Dried *V. negundo*

DPPH Free Radical Scavenging Activity of in Dried *V. negundo*:

Antioxidant reducing activity on DPPH radical is estimated according to the method of Blois (1958) with modification into a high-throughput microplate system. Extracts of dried *V. negundo* (50µl of 1.0 mg/ml) is added to 50µl of DPPH (FG: 394.32) (1mM in ethanolic solution) and 150µl of ethanol (absolute, AR Grade) in a 96 well microtiter plate, in triplicates. The plate is shaken (15s, 500rpm) and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution is measured spetrophotometrically at 520 nm.

Determination of Total Phenolic Content of in Dried *V. negundo*:

Determination of TPC on GTE was performed using Folin-Ciocalteu reagent according to the method of Singleton and Rossi, 1965, with modifications into high-throughput microplate system. Distilled water 0.1 ml and 0.1 ml diluted Folin–Ciocalteu reagents (0.1 ml/0.9 ml) were added to 50 µl of dried *V. negundo* extracts. The sample (*V. negundo* with Folin–Ciocalteu reagent) were set aside for 5 min before 0.1 ml 7.5% sodium carbonate (w/v) was added. After 2 hours, the absorbance was measured at 765 nm wavelength using a spectrophotometer. The calibration curve of gallic acid (GA) was used for the estimation of sample activity capacity.

Results and Discussion

Drying characteristics of *Vitex negundo* leaves

Figure 1 illustrates the change of free moisture over time for *V.negundo* leaves dried using convection oven drying.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.

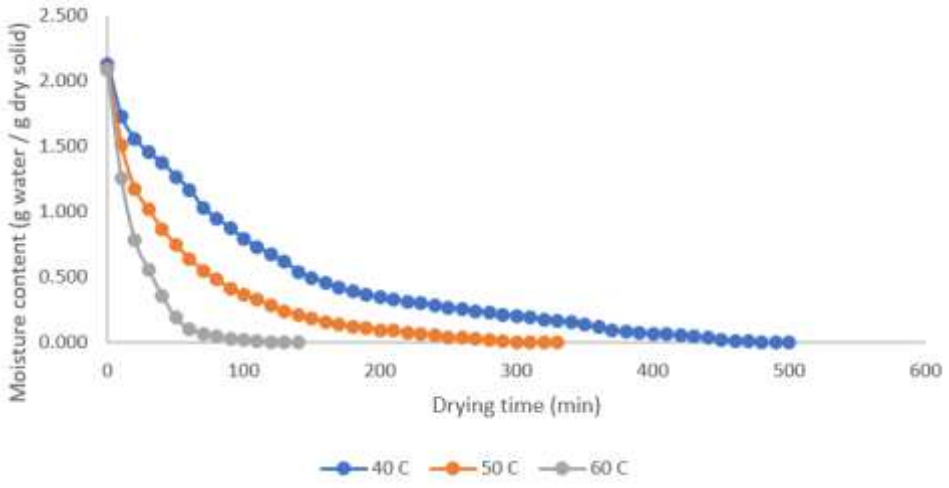

Figure 1. Drying curves of V. negundo leaves dried at 40, 50 and 60°C using convection oven

Figure 2 represents drying rate curves of *V. negundo* leaves dried using convection oven at 40, 50 and 60°C. The entire drying process took place at the falling rate period. The drying rate of the leaves dropped rapidly at the first stage of falling rate period. A slight constant rate period was observed at 40°C drying condition. stage, the rate of drying depends greatly on the mechanism by which the moisture from inside the leaves was transferred to the surface. The second falling rate period take much longer than the constant rate period because the drying is slow in order to reach its equilibrium moisture content.

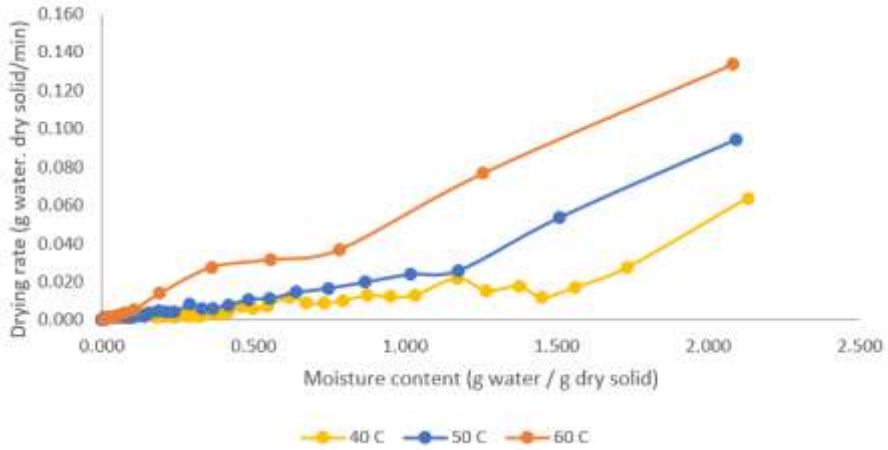

Figure 2.Drying rate curves of *V. negundo* leaves dried at 40, 50 and 60°C

Curve fitting of Drying Process by Convection Oven of *V. negundo* Leaves

By using SOLVER in Microsoft Excel 2016, the parameters of all the thin-layer models were solved for R^2 close to unity with the lowest values of RMSE. The values of the evaluation criteria for all the experiments were given in Table 2. All models showed high R^2 (R^2> 0.9) and low RMSE values (RMSE < 0.05) which proved that selected models can actually described the convection oven drying processof *V. negundo* leaves. Midili model was the most suitable model to fit the drying kinetics of convection oven drying of *V. negundo* leaveswithin studied parameters. This is because its R^2 values were closest to unity. The plot of experimental data and the best-fitted model was shown in Figure 3.

Table 2.The values of R^2, RMSE and drying constants for thin-layer drying models from convection oven drying of *V.negundo* leaves

Drying Temperature (˚C)	Drying model	Constant				Coefficients	
		k	b	a	n	R^2	RMSE
40	Midili	0.02372	-0.00005	0.98480	0.80310	**0.99915**	**0.01343**
50		0.06385	-0.00007	0.99863	0.71293	**0.99989**	**0.00427**
60		0.05804	-0.00006	0.99752	0.93977	**0.99966**	**0.00738**
40	Page	0.02151			0.83326	0.99884	0.01569
50		0.05581			0.75181	0.99953	0.00887
60		0.05599			0.95405	0.99957	0.00830
40	Logarithmic	0.00908	0.01438	0.90627		0.99825	0.01931
50		0.01899	0.02015	0.89142		0.99603	0.02571
60		0.04736	-0.00174	0.99104		0.99946	0.00935

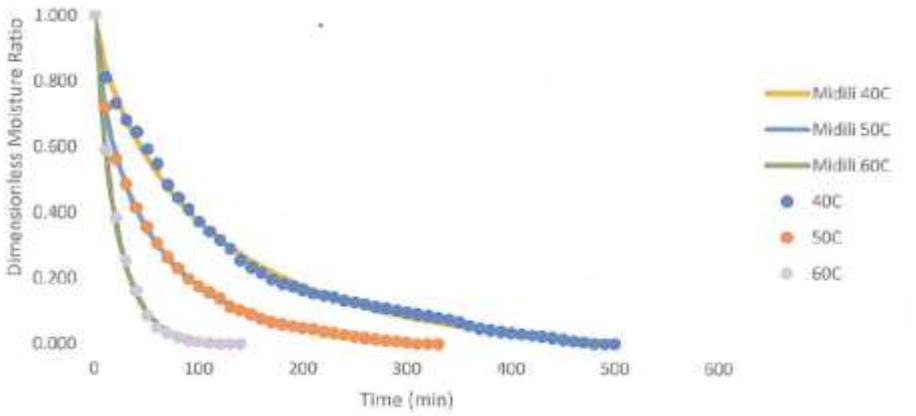

Figure 3. Comparison between the experimental data and the best fitted model for drying of *V. negundo* leaves at 40, 50 and 60°C

Phytochemical content of *V. negundo* leaves from convection oven drying

Concentration of agnuside in convection oven dried leaves of *V. negundo* and HPLC chromatograms are illustrated in Figure 4 and 5 respectively. The figure shows that the concentration of agnuside was decreasing with increasing temperatures. *Vitex negundo* leaves dried at 40, 50 and 60°C contained 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.

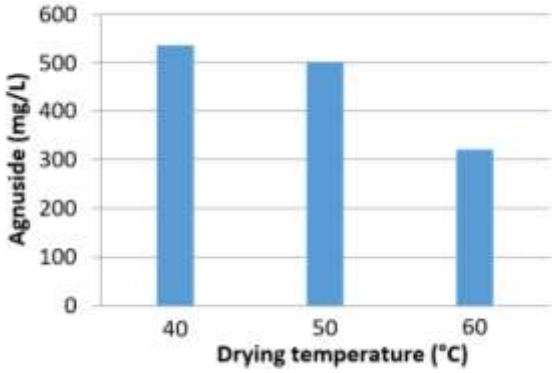

Figure 4. Concentration of *V. negundo* in dried leaves subjected to different drying temperatures

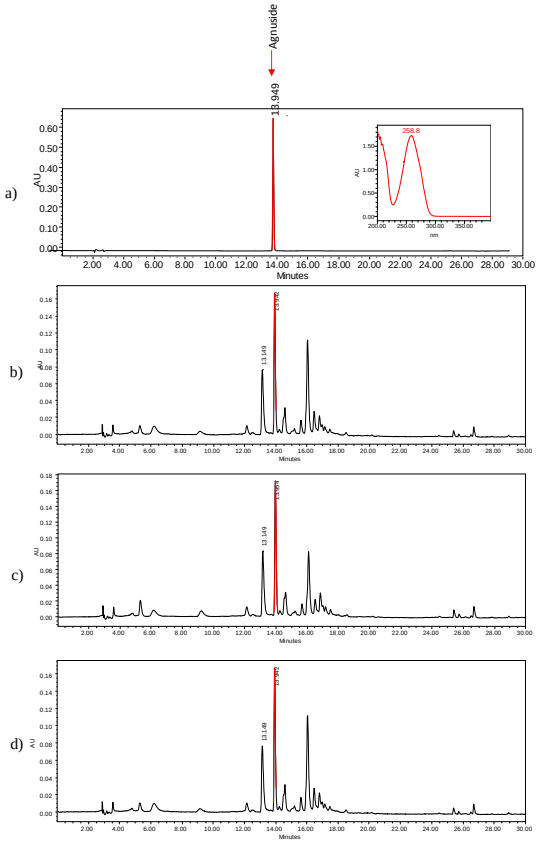

Figure 5. HPLC chromatograms of (a) standard; (b) leaves dried at 40°C; (c) leaves dried at 50°C; (d) leaves dried at 60°C

Antioxidant Activities of *V. negundo* leaves

In this study, total phenolic content of dried leaves of*V. negundo* were observed by analysing their gallic acid equivalent (GAE). The result showed that *V. negundo*leaves have significant level of phenolic content and the effect of drying at higher temperature has significantly reduce the amount of phenolics in *V. negundo* leaves. Total phenolics content of *V. negundo* leaves dried at 40, 50, 60 and 70°C were 270.8 ± 34.7, 286.7 ± 11.0, 250.8 ± 11.2 and 231.2 ± 10.9 mg GAE/100g of samples, respectively (Table 1). Green tea, which was used as positive control exhibited highest total phenolics content with 920.2 ± 71.2 mg GAE/100g of sample. These results indicated that total phenolic content of *V. negundo* leaves was highest at 50 °C drying temperature and it was inversely proportional to the drying temperature after 50 °C.

In the present study, DPPH radical scavenging activity of *V. negundo* leaves dried at different temperatures was also determined. The results indicated that the DPPH scavenging effects of *V. negundo* leaves dried at 40, 50, 60 and 70 °C were 81.53 ± 2.64, 81.46 ± 2.79, 74.35 ± 2.63 and 78.06 ± 2.43%, respectively (Table 1). These data suggest that the DPPH radical scavenging of *V. negundo* leaves dried at different temperature did not significantly altered except for 60°C drying temperature.

High drying temperature was found to give negative effect on antioxidant properties of *V. negundo* leaves as well as their phenolics content. These results were in agreement with previous study performed by previous study[15] which indicated that antioxidant capacity of plants were significantly affected by high drying temperature[15]. Similar finding[16]was found which dictate that phenolic acids are susceptible to destruction at increased temperatures. Study on the effect of different drying temperature (40, 50, 70 & 100 °C) of *V. negundo* leaves on their antioxidant properties has also been performed before[17]and their findings also produced similar results which indicated that drying temperatures of 70 and 100 °C have significantly reduced their phenolics content and antioxidant properties.

Table 3. Total phenolic content and DPPH scavenging activity of methanol extract of V. negundo leaves dried at 40 °C, 50 °C, 60 °C and 70 °C. Values with different letter are significantly different (p<0.05).

Methanol extracts of *V. negundo* leaves dried at different temperatures (°C)	Phenolic content (mg GAE/100 g samples)	DPPH Scavenging (%)
40	$270.8 \pm 34.7^{a,b}$	81.53 ± 2.64^{a}
50	286.7 ± 11.0^{a}	81.46 ± 2.79^{a}
60	$250.8 \pm 11.2^{a,b}$	$74.35 \pm 2.63^{b,c}$
70	231.2 ± 10.9^{b}	$78.06 \pm 2.43^{a,b}$

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 and 286.7 mg GAE/100 g samples of phenolic content.

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