

Application of Microwave-Assisted Drying to Shorten Granules Drying Process for the Preparation of *Thunbergia laurifolia* Lindl. Leaf Tablets

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Abstract

Thunbergia laurifolia Lindl. is a medicinal plant that belongs to the family of Acanthaceae. It possesses several biological and pharmacological activities. According to the Thai National List of Essential Medicines, *T. laurifolia* leaves are used for the treatment of fever. This work sought to optimize microwave-assisted drying of *Thunbergia laurifolia* Lindl. leaf granules to shorten the granule drying process by the 3² full factorial design. Power was varied from 300, 450 and 600 W for 5, 10 and 15 min, to minimize moisture content and maximize rosmarinic acid content. Results showed that the optimal microwave-assisted drying condition within the design space and control space was the power of 450 W for 13 min. This condition gave the moisture content of 4.77 ± 0.24 % and rosmarinic acid content of 0.0566 ± 0.0075 %. The HPLC method was validated; it showed good linearity, specificity, precision, and accuracy. Furthermore, the system suitability was achieved. The *T. laurifolia* leaf granules obtained from the optimal microwave-assisted drying condition were further used to prepare *T. laurifolia* leaf tablets by the wet granulation method. Three batches of 650 mg tablets containing 250 mg of *T. laurifolia* leaf powder were prepared using different compression forces: 500, 1,000 and 1,500 psi. The suitable compression force was 1,500 psi; the tablet formulation had suitable hardness (9.88 ± 0.52 kP), low friability (0.20 %), and a short disintegration time (23.17 ± 0.76 s). Rosmarinic acid marker was dissolved from the tablets for approximately 75 % within 4 h when 0.5 % sodium lauryl sulfate aqueous solution was used as dissolution medium. In conclusion, microwave-assisted drying could be applied in preparing *T. laurifolia* leaf tablets by shortening the granules drying time.

Keywords: Factorial design, Moisture content, Optimization, Rosmarinic acid, Wet granulation

Introduction

Thunbergia laurifolia Lindl. is a medicinal plant that belongs to the family of Acanthaceae. It possesses several biological and pharmacological activities, including, antidiabetic activity [1,2], anti-inflammatory activity [2], antimicrobial activity [1,2], antioxidant activity [2,3], antipyretic activity [4], cytotoxicity [5], detoxifying effect [1,2,4], hepatoprotective activity [1,2], etc. Its leaves are composed of several chemical constituents such as apigenin derivatives, glucopyranosides, grandifloric acid, and iridoid glucosides. Its leaves and flowers are composed of delphinidin derivatives and phenolic acids (including, chlorogenic acid, caffeic acid, gallic acid, and protocatechuic acid). Furthermore, it is composed of rosmarinic acid which is previously used as a standard marker of *T. laurifolia* [3,5]. As revealed in the 2021 Thai National List of Essential Medicines, *T. laurifolia* leaf capsules are used for the treatment of fever. The administration dose is 500 mg to 1 g 3 times daily before a meal [4].

Tablet is the dosage form that is most accustomed to the patients. About 70 % of all medicines are administered in tablet form. They have several advantages such as providing a stable and accurate dose of drugs. Tablets are capable of large-scale economic production with a high degree of tablet uniformity. The easiest method for the preparation of tablets is the direct compression method. Unfortunately, some herbal powders are unable to be compressed into tablets by the direct compression method due to poor powder

flowability. The bulky characteristic of some herbal powder also cannot be compressed due to poor compressibility properties. The simplest procedure of formulating herbal tablets of poor flowability powder is by mixing herbal powder and pharmaceutical excipients to form a uniform powder mixture as granules. The granulation process is an important step to preserve uniformity of the drug, endorsing acceptable flowability with enhanced compressibility, circumventing particle segregation, and promising both physical and chemical stability [6]. Wet granules must be dried to give good flowability dried granules before being compressed into tablets by a tableting machine. Among several drying techniques, fluidized bed drying and tray drying is the most frequently used method for drying granules [6]. However, both methods are time consumable procedures. So, finding a modern and innovative drying method with an eco-friendly and economical manner to shorten the granules' drying process is required.

Microwave-assisted drying is a modern, green, and innovative drying technique that is applied in several fields. Previously, microwave-assisted drying succeeded in applying to the granule preparation process. Aspirin, a moisture-sensitive drug, was used as a model drug to evaluate the effectiveness of microwaves for granules drying. Results showed that microwave-assisted drying preserved the drug from degradation [7,8], due to its shortening drying time of the granules, so aspirin less degrades because of short exposure to moisture compared with the other techniques [9]. The microwave-assisted drying technique is compared with fluidized bed drying for ibuprofen. It was found that microwave-assisted drying has a shorter duration time, consequently, decreasing energy consumption and production cost [10,11]. So, microwave-assisted drying could be used as an alternative method for granule drying of pharmaceutical as well as herbal products. Besides granules drying, microwave irradiation succeeded in drying wet molded tablets [12,13], as well as extraction of phytochemical compounds [14-16] before being compressed into tablets [17]. However, microwave impact chemical compounds, for example, microwave enhanced emissions of volatile compounds [18].

This work aimed to optimize microwave-assisted drying of *T. laurifolia* leaf granules to shorten the granule drying process by the 3² full factorial design, before the preparation of *T. laurifolia* leaf tablets for the treatment of fever according to Thai traditional medicines. Furthermore, the high-performance liquid chromatographic (HPLC) method was validated and system suitability was tested to confirm the reliability of the chemical analysis.

Materials and methods

Materials

Standard rosmarinic acid (purity 99.44 %) was purchased from Chengdu Biopurify Phytochemicals Ltd., Sichuan, China. Acetonitrile (99.99 %) and methanol (99.90 %) (HPLC grade) were purchased from Fisher Chemical, Leicestershire, UK. Glacial acetic acid (99.97 %) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Microcrystalline cellulose (Avicel® PH-101) was purchased from Sigma-Aldrich Co., Missouri, USA. Polyvinyl pyrrolidone (PVP K30) (Kollidon® 30) was purchased from BASF, Ludwigshafen, Germany. Sodium starch glycolate was obtained as a gift from Onimax Co., Ltd., Bangkok, Thailand. Magnesium stearate was purchased from Changzhou Kaide Imp. & Exp. Co., Ltd., Changzhou, China. Colloidal silicon dioxide was purchased from P.C. Drug Center, Bangkok, Thailand. Talcum was purchased from Nitika Pharmaceutical Specialities Pvt. Ltd., Nagpur, India. Sodium lauryl sulfate (SLS) was purchased from EMD Millipore Corporation, Massachusetts, USA.

Preparation of plant sample

T. laurifolia leaves were harvested from Sun Herb Thai Chinese Manufacturing, Rangsit University, Pathum Thani, Thailand. They were washed with tap water and dried in a hot air oven at 60 °C for 4 h. They were pulverized, passed through a 60-mesh sieve, and stored in a dry place until use. The fresh plant samples were identified by Ajarn Nirun Vipunngun, a plant taxonomist at the Department of Pharmacognosy, College of Pharmacy, Rangsit University to confirm the correct plant species. The voucher specimens were coded as CM-TL1-09-21 and deposited at the college's Drug and Herbal Product Research and Development Center.

Preparation and drying optimization of *T. laurifolia* leaf granules

The 3² full factorial design was applied for the design of the experiment of *T. laurifolia* leaf granules drying using a microwave (Table 1). The design composes of 2 factors, *i.e.*, microwave powers of 300, 450 and 600 W, and microwave times of 5, 10 and 15 min. Two responses, *i.e.*, moisture content and rosmarinic acid content were monitored.

The working formula (for 40 tablets) was composed of *T. laurifolia* leaf powder of 10 g (equivalent to 250 mg per tablet) as an active ingredient, PVP K30 of 1.04 g (equivalent to 26 mg per tablet) as a binder, sodium starch glycolate of 0.78 g (equivalent to 19.5 mg per tablet) as a disintegrant, magnesium stearate of 0.26 g (equivalent to 6.5 mg per tablet) as a lubricant/anti-adherent, colloidal silicon dioxide of 0.26 g (equivalent to 6.5 mg per tablet) as a glidant, talcum of 0.78 g (equivalent to 19.5 mg per tablet) as a glidant/anti-adherent, and microcrystalline cellulose of 12.88 g (equivalent to 322 mg per tablet) as a filler. All ingredients were passed through a 60-mesh sieve before being used to produce homogeneous size, except colloidal silicon dioxide. According to granules preparation, they were prepared by mixing *T. laurifolia* leaf powder, microcrystalline cellulose, and polyvinyl pyrrolidone in a mortar and pestle by the geometric dilution method. After the powder mixture was homogeneous, the damp mass was produced by adding 21.5 mL of water and mixed until the damp mass was obtained. The wet granules were produced by sieving the damp mass through a 14-mesh sieve. The wet granules were spread on a pan of a microwave oven (MS23F300EEK, Samsung, Bangkok, Thailand) and dried using the specific microwave power and time as shown in **Table 1**.

Table 1 The factors and responses of the 3² full factorial design.

Conditions	Factors		Responses	
	Power (W)	Time (min)	Moisture content (%)	Rosmarinic acid content (%)
1	300	5	12.59 ± 0.94	0.0510 ± 0.0022
2	300	10	5.28 ± 0.14	0.0515 ± 0.0002
3	300	15	4.56 ± 0.34	0.0434 ± 0.0002
4	450	5	4.10 ± 0.17	0.0556 ± 0.0006
5	450	10	3.88 ± 0.33	0.0648 ± 0.0002
6	450	15	3.43 ± 0.12	0.0444 ± 0.0005
7	600	5	3.49 ± 0.40	0.0570 ± 0.0005
8	600	10	3.76 ± 0.31	0.0589 ± 0.0004
9	600	15	3.29 ± 0.50	0.0573 ± 0.0009
Standard method (Hot air oven, 60 °C for 4 h)			5.45 ± 0.50	0.0464 ± 0.0002

Moisture content and rosmarinic acid content data obtained from each microwave-assisted drying condition were analyzed by license Design-Expert® (version 11.1.2.0) software. The predicted equations were reported. The contour plots of moisture content and rosmarinic acid content were created. The design space that moisture content and rosmarinic acid content of granules was equal to or greater than hot air drying at 60 °C for 4 h was constructed. Furthermore, the control space that moisture content was equal to or less than 5 and rosmarinic acid content was equal to or higher than 0.05 was also produced. The optimal microwave-assisted drying condition within the control space was selected to prepare the *T. laurifolia* leaf granules for the verification step. The experimental value obtained from the optimal condition was compared with the predicted value to verify the accuracy and reliability of the prediction by computer software. Percent error was calculated as Eq. (1).

$$\text{Error (\%)} = \left(\frac{\text{Experimental value} - \text{Predicted value}}{\text{Experimental value}} \right) \times 100 \quad (1)$$

Determination of moisture content of granules

T. laurifolia leaf granules for approximately 2.0 g were analyzed in their moisture content using moisture balance (MAC 50/NH, Radwag, Bracka, Poland) based on the loss on drying technique (n = 3). The temperature was controlled at 120 °C throughout the test. The moisture content was collected when the weight change was less than 1 mg within 2 min. The average value and standard deviation (SD) were reported.

HPLC condition for determination of rosmarinic acid content

Analysis of rosmarinic acid content was done using Agilent 1,260 Infinity (Agilent Technologies, California, USA). The stationary phase was the Luna C18 column (250×4.6 mm², internal diameter, 5 μm) (Phenomenex Inc., California, USA). The column temperature was controlled at 25 °C. The mobile phase was composed of acetonitrile (A) and 1 % acetic acid aqueous solution (B). The gradient elution system was started at 15 to 45 % A within 10 min, decreased to 15 % within 1 min, and maintained for 2 min before being injected the next sample. The flow rate of the mobile phase was 1 mL/min. The injection volume was 10 μL. The signal of rosmarinic acid was detected using a photodiode array detector at 328 nm.

Method validation

HPLC Method validation was performed based on the ICH guideline [19]. It was performed on 5 topics, *i.e.*, linearity and range, limit of detection (LOD) and limit of quantitation (LOQ), specificity, precision, and accuracy.

Linearity and range

The rosmarinic acid (5.0 mg) was dissolved in 10 mL of methanol in the 10-mL volumetric flask to obtain the concentration of 500 μg/mL stock solution. Then, it was diluted to 60 μg/mL, followed by diluted to 30, 15, 7.5, 3.75, 1.875, 0.9375 and 0.46875 μg/mL using a 2-fold serial dilution technique. They were filtered through a nylon syringe filter with a 0.45 μm pore size and injected into the HPLC instrument (n = 3). A calibration curve of rosmarinic acid was constructed. The 3 parameters, *i.e.*, linear equation, coefficient of determination (R²), and test range were reported.

LOD and LOQ

LOD and LOQ were evaluated by diluting the 0.46875 μg/mL of rosmarinic acid and analyzed by an HPLC instrument. The concentration level of rosmarinic acid that gave the signal-to-noise ratio of 10:1 was reported as LOQ. Then, it was further diluted and analyzed by the HPLC instrument, the concentration level that gave the signal-to-noise ratio of 3:1 was reported as LOD.

Specificity

The UV spectra at the upslope, apex, and downslope of the peak of rosmarinic acid in *T. laurifolia* leaf powder were collected. The specificity was accepted when the similarity of UV spectra of the 3 regions was achieved and were similar to the UV spectrum of standard rosmarinic acid. Furthermore, the specificity was confirmed by injection of a placebo tablet. The method was specific when no peak of the placebo tablet eluted at the same retention time of rosmarinic acid.

Precision

The 3 concentration levels of standard rosmarinic acid of 1.875, 7.5 and 30 μg/mL were prepared, filtered, and analyzed by HPLC instrument (n = 3). The percent relative SD (percent RSD) of the analysis on the same day was reported as intraday precision. The percent RSD of the analysis on 3 consecutive days was reported as inter-day precision.

Accuracy

The accuracy of the method was evaluated using a spiking method. Briefly, 5 mg/mL of *T. laurifolia* leaf powder in methanol was sonicated for 15 min before being filtered. The 4 mL of the filtrate was added to a volumetric flask. The standard rosmarinic acid solution was added to the volumetric flask containing *T. laurifolia* leaf filtrate. The final concentrations of standard rosmarinic acid were 24, 30 and 36 μg/mL, which corresponded to 80, 100 and 120 % of the test concentration, respectively. They were filtered through a syringe filter with a 0.45 μm pore size and analyzed by the HPLC instrument (n = 3). Percent recoveries of each concentration level were reported.

System suitability

System suitability can be regarded as the performance qualification, where characteristics related to the analytical method, the equipment, and the software are used. Generally, these will all contribute to indicating whether the system (*i.e.*, the stationary phase (column) and mobile phase) is suitable to analyze the sample. Furthermore, it could confirm the accuracy and reliability of the quantitation. The system suitability was tested according to US FDA guidance [20]. Four topics: Number of theoretical plates, capacity factor, asymmetry, and resolution were performed. Each value was obtained from the HPLC software. All data were obtained from the 6 replicated injections of rosmarinic acid 30 μg/mL, except

resolution was obtained from the 6 replicated injections of *T. laurifolia* leaf powder (5 mg/mL). Number of theoretical plates, capacity factor, asymmetry, and resolution of more than 2,000, more than 2, not more than 2, and more than 2 were acceptable values [20].

Preparation of *T. laurifolia* leaf tablets

Dried *T. laurifolia* leaf granules obtained from the optimal microwave-assisted drying were passed through a 20-mesh sieve. They were mixed for 3 min with the premix which consisted of sodium starch glycolate, colloidal silicon dioxide, magnesium stearate, and talcum. The obtained mixture was individually weighed for 650 mg and compressed into a tablet using an in-house assembled hydraulic press connected with a pressure gauge. Each tablet was compressed using the specific compression force and maintained force for 10 s. Three compression forces: 500, 1,000 and 1,500 psi were compared.

Evaluation of *T. laurifolia* leaf tablets

The physicochemical properties of *T. laurifolia* leaf tablets were evaluated in 6 topics, *i.e.*, weight, thickness, diameter, hardness, friability, disintegration time, and rosmarinic acid content.

Weight

The weight of individual tablets ($n = 20$) was measured by analytical balance (Entris224i-1S, Sartorius AG, Göttingen, Germany). The average value and SD were reported. Furthermore, the weight variation of not more than 5 % was acceptable.

Thickness and diameter

The thickness and diameter of *T. laurifolia* leaf tablets ($n = 20$) were measured using a thickness gauge. The average value and SD were reported.

Hardness

The hardness of *T. laurifolia* leaf tablets ($n = 10$) was measured by a hardness tester (TBH 220 TD, Erweka GmbH, Heusenstamm, Germany). The average value and SD were reported.

Friability

Ten *T. laurifolia* leaf tablets were dedusted and weighed (W_a) using an analytical balance. The friability was determined by a friability tester (K.S.L. Engineering Co. Ltd., Bangkok, Thailand). The drum of the friability tester was rotated for 100 rounds. Then, the tablets were removed from the drum, dedusted, and weighed again (W_b). The friability was calculated using Eq. (2).

$$\text{Friability (\%)} = \left(\frac{W_a - W_b}{W_a} \right) \times 100 \quad (2)$$

Disintegration time

The disintegration time of *T. laurifolia* leaf tablets ($n = 6$) was evaluated using a disintegration tester (BJ-2, Tianjin Guoming Medicinal Equipment Co., Ltd., Tianjin, China). The disintegration medium was water at 37 ± 0.5 °C. The average value and SD were reported.

Rosmarinic acid content

The content of rosmarinic acid in *T. laurifolia* leaf tablets was determined by grounding ten tablets before being added 650 mg powder into a 50-mL volumetric flask ($n = 3$). Methanol was used to adjust the volume before being ultrasonicated for 30 min. They were filtered and analyzed rosmarinic acid content by HPLC instrument.

Dissolution test

Three *T. laurifolia* leaf tablets were tested for rosmarinic acid marker dissolution from the tablets using a modified beaker method using a 250-mL beaker. The dissolution medium was 100 mL of 0.5 % SLS aqueous solution. The temperature of the dissolution medium was controlled at 37 ± 0.5 °C. They were shaken at 100 rpm. The medium was sampled for 3 mL at 5, 10, 15, 30, 60, 120, 180 and 240 min. The fresh medium was replenished to maintain the volume of the dissolution medium. The withdrawn medium was filtered and analyzed by HPLC. The dissolved rosmarinic acid was calculated and the dissolution profile of rosmarinic acid from the tablets was produced.

Results and discussion

Optimal microwave-assisted drying of *T. laurifolia* leaf granules

The responses, *i.e.*, moisture content and rosmarinic acid content, obtained from different microwave-assisted drying conditions are shown in **Table 1**. The predicted equations of each response are shown in Eqs. (3) - (4).

$$\text{Moisture content (\%)} = 25.56 - 0.04(\text{Power}) - 1.47(\text{Time}) + (2.6 \times 10^{-3})(\text{Power} \times \text{Time}) \quad (3)$$

$$\text{Rosmarinic acid content (\%)} = 0.05 + (3.03 \times 10^{-5})(\text{Power}) - (6.17 \times 10^{-4})(\text{Time}) \quad (4)$$

The above equations were used to construct the contour plots of moisture content and rosmarinic acid content for estimation of their values when microwave power and time were altered. Contour plots of moisture content and rosmarinic acid content are shown in **Figure 1**. Results showed that increasing microwave power and microwave time decreased moisture content. Furthermore, decreasing moisture content was observed when microwave power interacted with time (**Figure 1(a)**). Rosmarinic acid content was increased when microwave power increased while increasing microwave time decreased rosmarinic acid content (**Figure 1(b)**).

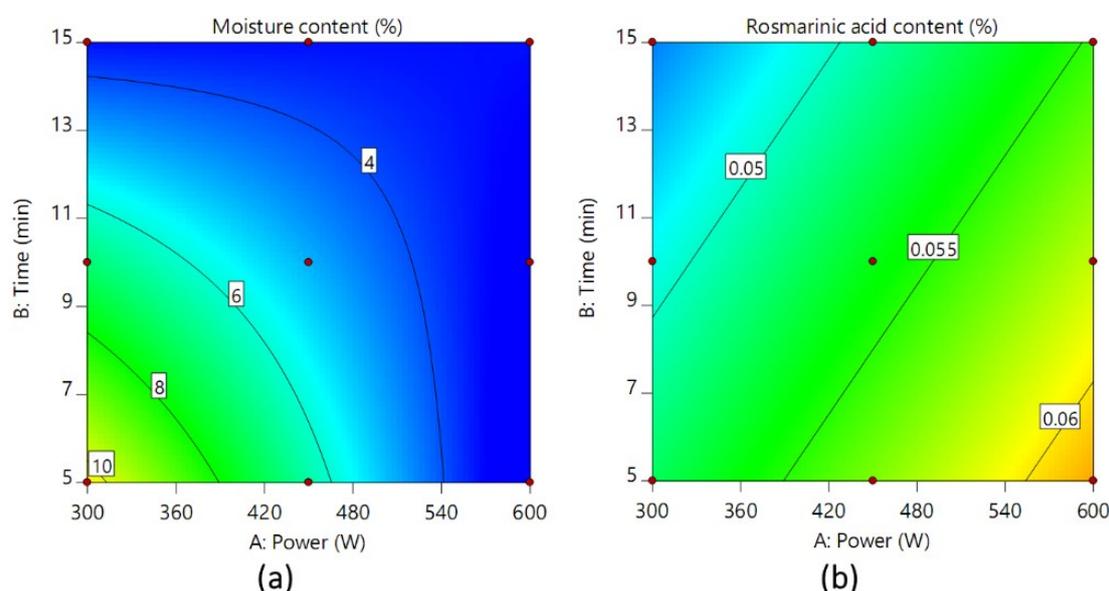


Figure 1 Contour plots of (a) moisture content and (b) rosmarinic acid content when microwave power and time were varied from 300 to 600 W and 5 to 15 min, respectively.

The *T. laurifolia* leaf granules dried in a hot air oven at 60 °C for 4 h had a moisture content of 5.45 % and rosmarinic acid content of 0.0464 %. These results were used as a standard value for the evaluation of the effectiveness of granules drying by microwave method. The design space that which moisture content was equal to or less than 5.45 % and rosmarinic acid content was equal to or higher than 0.0464 %, was constructed as shown in **Figure 2(a)**. Medium to high microwave power as well as microwave time was achieved. The control space that moisture content was equal to or less than 5 % and rosmarinic acid content was equal to or higher than 0.05 %, which was narrowed from the design space shown in **Figure 2(b)**. Based on the control space, the optimal condition selected for the verification step was 450 W for 13 min. The limitation of selecting the optimal condition was the difficulty in adjusting the microwave power, due to the machine could not adjust the fine values.

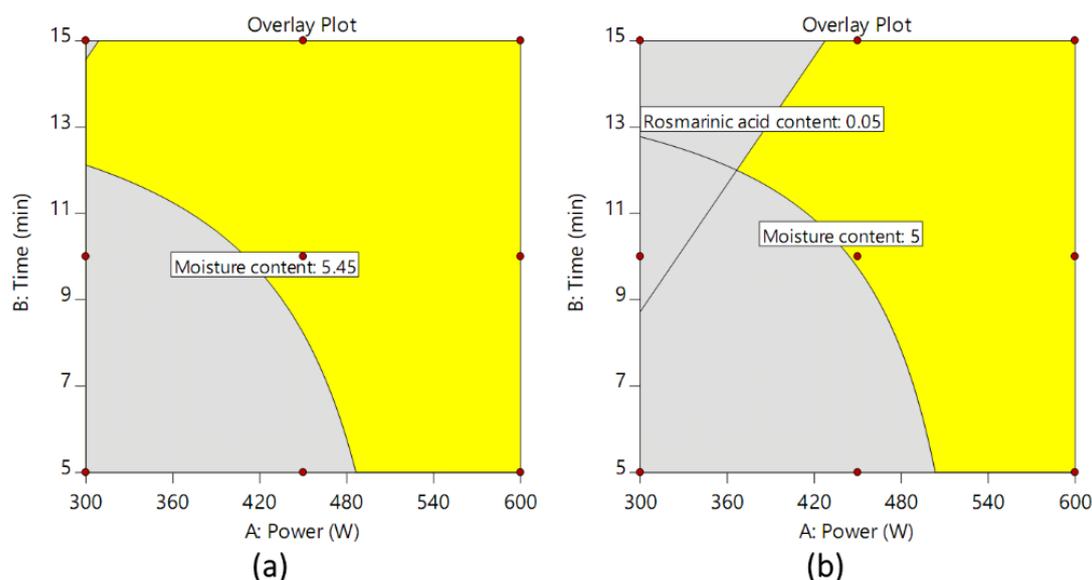


Figure 2 Overlay plots represented (a) design space where moisture content and rosmarinic acid content were equal to or better than hot air drying (moisture content was equal to or less than 5.45 % and rosmarinic acid content was equal to or higher than 0.0464 %) and (b) control space that moisture content was equal to or less than 5 % and rosmarinic acid content was equal to or higher than 0.05 %.

The optimal condition was used to dry the granules to confirm the accuracy of the prediction by the Design-Expert® software. Results showed that the error of the moisture content was relatively high; however, the experimental value was within the 95 % CI range, indicating that the variation was acceptable. In the case of rosmarinic acid content, the percent error was low and within the 95 % CI range (**Table 2**). The verification results indicated the prediction by the Design-Expert® software was accurate and reliable.

Table 2 Verification data consisted of predicted values, experimental values, error, and lower and upper values of 95 % CI.

Responses	Predicted values	Experimental values	Error (%)	95 % CI (lower)	95 % CI (upper)
Moisture content (%)	4.04	4.77 ± 0.24	15.30	2.07	6.00
Rosmarinic acid content (%)	0.0519	0.0566 ± 0.0075	8.30	0.0461	0.0578

Method validation and system suitability

The method validation was done to confirm the reliability of the chemical analysis by HPLC. The HPLC chromatograms of standard rosmarinic acid, *T. laurifolia* leaf powder, and optimal *T. laurifolia* leaf granules are shown in **Figures 3(a) - 3(c)**, respectively. Rosmarinic acid was eluted at the retention time of 10.195 min.

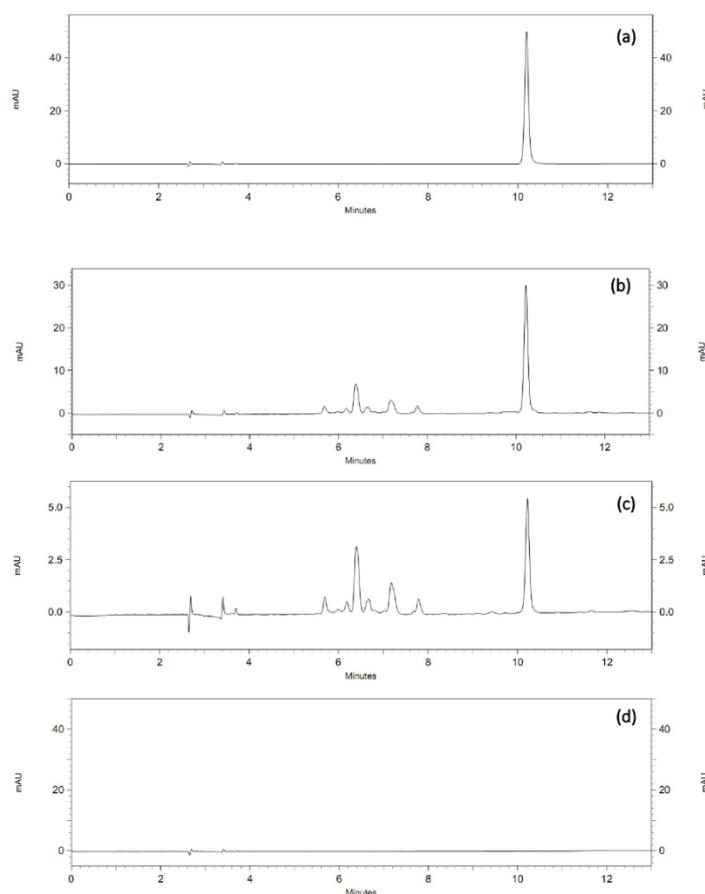


Figure 3 HPLC chromatograms of (a) standard rosmarinic acid (30 $\mu\text{g/mL}$), (b) *T. laurifolia* leaf powder (5 mg/mL), (c) optimal *T. laurifolia* leaf granules (5 mg/mL), and (d) placebo tablet (650 mg/50 mL or 1 tablet/50 mL).

The linear equation was $y = 22,590x - 3,218.4$ ($R^2 = 0.9995$) in the test range of 0.46875 - 60 $\mu\text{g/mL}$. The LOD and LOQ obtained from the signal-to-noise ratio were 6.91 and 23.03 ng/mL, respectively. According to specificity, the UV spectrum of the peak of rosmarinic acid in *T. laurifolia* leaf powder (**Figure 4(b)**) was similar to the UV spectrum of standard rosmarinic acid (**Figure 4(a)**). However, the absorbance of rosmarinic acid in *T. laurifolia* leaf powder at 330 nm was lower than those of standard rosmarinic acid, due to lower rosmarinic acid content of *T. laurifolia* leaf powder compared to standard rosmarinic acid. Furthermore, the specificity was also confirmed by analysis of placebo tablets (**Figure 3(d)**). There was no peak found at the same retention time of rosmarinic acid, which indicated that the method was specific. In the case of precision, the percent RSD of intraday precision and inter-day precision were less than 2 % for all concentration ranges, indicating that the method was precise. The accuracy represented as recovery was 92.22 - 108.22 %. This result showed that the HPLC method was accurate. So, the HPLC method for the analysis of rosmarinic acid had good linearity, specificity, precision, and accuracy.

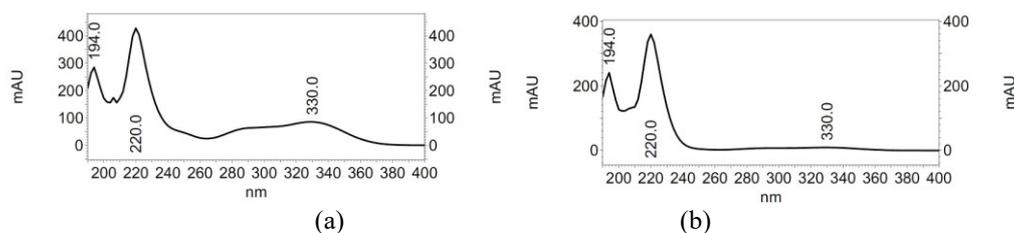


Figure 4 UV spectra of (a) standard rosmarinic acid (30 $\mu\text{g/mL}$) and (b) rosmarinic acid in *T. laurifolia* leaf powder (5 mg/mL).

Table 3 Precision and accuracy results.

Concentration ($\mu\text{g/mL}$)	Precision (RSD, %)				Spike concentration ($\mu\text{g/mL}$)	Accuracy Recovery (%)
	Intraday			Inter-day		
	Day 1	Day 2	Day 3			
1.875	0.91	1.83	1.70	1.48	24	108.22 \pm 0.22
7.5	0.73	0.28	0.34	0.91	30	92.22 \pm 1.54
30	1.20	1.05	0.26	0.81	36	93.93 \pm 0.40

The system suitability results are shown in **Table 4**. The number of theoretical plates was more than 2,000, the capacity factor was more than 2, asymmetry was not more than 2, and the resolution was more than 2. All system suitability parameters indicated that the HPLC system was suitable.

Table 4 System suitability (n = 6).

Parameters*	Values (mean \pm SD)
Retention time (min)	10.195 \pm 0.004
Peak area	661,556 \pm 5,573
Number of theoretical plates	66,813 \pm 168
Capacity factor	2.877 \pm 0.001
Asymmetry	1.088 \pm 0.014
Resolution	3.240 \pm 0.074

*All data obtained from rosmarinic acid 30 $\mu\text{g/mL}$, except resolution was obtained from *T. laurifolia* leaf powder (5 mg/mL).

Physicochemical properties of *T. laurifolia* leaf tablets

The *T. laurifolia* leaf granules dried by optimal microwave-assisted drying condition were mixed with the excipients and then prepared in tablet form. Three compression forces were applied to evaluate the effect of compression force on the physicochemical properties of the tablets. Physicochemical properties of *T. laurifolia* leaf tablets compressed with different forces are shown in **Table 5**. Among several physicochemical properties, tablet weight and rosmarinic acid content were comparable for all compression forces due to each mixture powder being individually weighed during the tablet preparation step. In addition, the tablet diameter did not change due to it being fixed by the die. Increasing compression force decreased tablet thickness and friability, while hardness and disintegration time were increased.

Table 5 Physicochemical properties of *T. laurifolia* leaf tablets compressed with different forces.

Compression force (psi)	Weight (mg)	Thickness (mm)	Diameter (mm)	Hardness (kP)	Friability (%)	Disintegration time (s)	Rosmarinic acid content (mg/tablet)
500	648.80 \pm 1.51	6.44 \pm 0.06	12.89 \pm 0.20	1.84 \pm 0.11	5.28 %	9.56 \pm 0.76	0.38 \pm 0.04
1,000	647.82 \pm 1.57	5.37 \pm 0.07	12.91 \pm 0.02	4.47 \pm 0.37	0.76 %	17.30 \pm 0.76	0.40 \pm 0.03
1,500	647.91 \pm 2.02	4.68 \pm 0.08	12.83 \pm 0.04	9.88 \pm 0.52	0.20 %	23.17 \pm 0.76	0.46 \pm 0.04

The selected tablets compressed using 1,500 psi were sampled for the dissolution test. The dissolution profile in 0.5 % SLS aqueous solution of rosmarinic acid from *T. laurifolia* leaf tablets is shown in **Figure 5**. It was found that rosmarinic acid was dissolved for 74.81 \pm 18.66 % within 240 min.

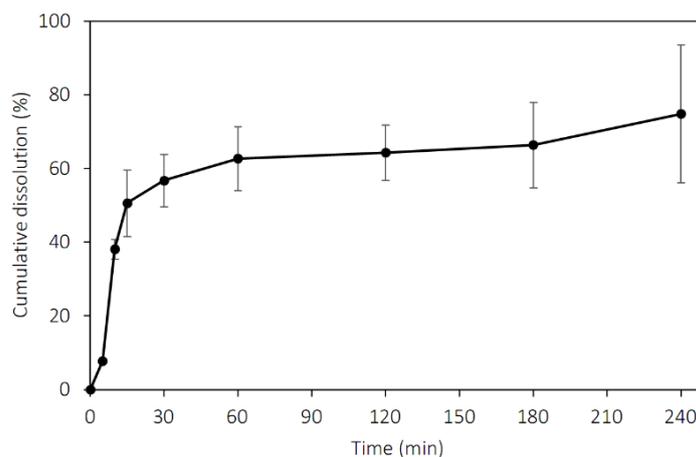


Figure 5 Dissolution profile of rosmarinic acid from *T. laurifolia* leaf tablets in 0.5 % SLS aqueous solution.

Microwave power applied to the plant materials is a key factor that affected moisture removal from the plant matrix [21]. The drying rate constant is increased by increasing microwave power. The other important factor that affected plant materials drying was microwave time. Increasing microwave time also decreased the moisture content of the materials. When single or both factors, microwave power and microwave time, were increased, the moisture content was decreased [22]. According to the thermo-stable phytochemicals, increasing microwave power as well as microwave time increased active compounds higher than degradation. For example, when microwave power of 630 W was used, increasing microwave time from 20 to 60 min reduced the water content of sliced fresh turmeric. In the case of volatile oil content and total curcuminoids content were increased because of decreased water content [23]. In some cases, microwave heating could enhance the biological activity of the plant, for example, microwave heating boosted the antioxidant activity of tomatoes (*Solanum lycopersicum*) by enhancing polyphenols and flavonoids [24]. However, microwave-assisted drying could alter the thermo-labile volatile oils [25].

According to the present work, when microwave power and microwave time were increased, higher moisture content was removed from the *T. laurifolia* leaf granules, which corresponded to the previous works. In the case of rosmarinic acid, it could be destroyed by increasing temperature. Rosmarinic acid in spearmint (*Mentha spicata*) was lost when the drying temperature was increased from -50 , 35 or 45 to 80 °C, consequently decreasing in total antioxidant capacity [26]. Similar results were also found in the other work. Rosmarinic acid contained in *Orthosiphon stamineus* leaves was decreased from 0.802 to 0.002 % of dry weight when the extraction temperature was increased from 40 to 100 °C. Decreasing rosmarinic acid could affect the free radical scavenging activity of its extract [27]. The other research team also investigated the stability of rosmarinic acid in *O. stamineus* leaves. Increasing drying temperature from 30 to 40 and 50 °C decreased rosmarinic acid content from 171.47 to 98.69 and 9.10 mg/g dry weight, respectively [28]. However, the present work found that increasing microwave power from 300 to 600 W increased the rosmarinic acid content of *T. laurifolia* leaf granules. No degradation of rosmarinic acid was observed. It can be described by the moisture removed from the granules caused the phytochemicals contained in the granules more concentrate, so rosmarinic acid content based on the weight of the dried granules was increased. However, decreasing rosmarinic acid content was observed when microwave time was increased from 5 to 15 min. It can be concluded that microwave power increased rosmarinic acid content, which was opposed to the effect of microwave time.

The results indicated that microwave-assisted drying of *T. laurifolia* leaf granules was equal to or better than the drying by a hot air oven in terms of moisture content and rosmarinic acid content. Hence, microwave-assisted drying is a motivating method for drying granules of herbal plants for the preparation of herbal medicinal products in tablet form, because microwave-assisted drying had quick heating rates and short operating time, so, it could save energy. Moreover, it gave desired product properties and does not generate secondary waste, so it is recognized as an eco-friendly method [29].

Tablets with suitable hardness with low friability became a good performance indicating that tablets had sufficient physical strength and did not lose components due to abrasion, friction, or mechanical shock [30]. Moreover, tablets with a short disintegration time provided rapid drug absorption and drug acting compared with the tablets with a longer disintegration time [31]. Using compression force of $1,000$ psi had a high friability value which was closed to the borderline of the acceptable value: 1 %. The friability of the

tablets compressed with 500 psi failed the acceptable friability because the friability value was relatively high: 5.28 %, which exceeded 1 %. So, the present work found that the compression force of 1,500 psi was the most suitable force for the preparation of *T. laurifolia* leaf tablets because it provided suitable hardness, low friability, short disintegration time, and a high percentage of rosmarinic acid marker could be dissolved. The limitation of the dissolution test of this work was a low rosmarinic acid content: 0.46 mg per tablet. In case of 900 or 500 mL of dissolution medium were used, the final concentrations of rosmarinic acid were 0.51 or 0.92 µg/mL, which could be lower than the lower range of the calibration curve. So, the pharmacopeia compendial method could not be used in this work. The authors modified the dissolution test by decreasing the volume of the dissolution medium to increase the final concentration of rosmarinic acid. Furthermore, the surfactant, 0.5 % SLS, was added to the medium to increase the water solubility of the low water-soluble compound. According to traditional use. The administration dose for the treatment of fever is 500 mg to 1 g of *T. laurifolia* leaf powder 3 times daily before a meal [4]. However, no optimum range of rosmarinic acid content for the treatment of fever is reported. So, the developed *T. laurifolia* leaf tablets must be administered 2 - 4 tablets 3 times daily.

Conclusions

The 3² full factorial design was applied to optimize microwave-assisted drying of *T. laurifolia* leaf granules. This work revealed that microwave-assisted drying of *T. laurifolia* leaf granules was equal to or better than the drying by a hot air oven, a standard method, in terms of lower moisture content and higher rosmarinic acid content. The optimal microwave-assisted drying condition providing the low moisture content with high rosmarinic acid content was the power of 450 W for 13 min; the *T. laurifolia* leaf granules dried by the optimal microwave-assisted drying condition were used to prepare *T. laurifolia* leaf tablets. The suitable compression force was 1,500 psi; it provided suitable hardness, low friability, short disintegration time, and high rosmarinic acid marker dissolved. In conclusion, this work succeeded in applying microwave to drying *T. laurifolia* leaf granules for the preparation of tablets by shortening the granules drying time.

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