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Article

Microwave-assisted extraction and content determination of astilbin in *Lysiphyllum strychnifolium* stems

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Lysiphyllum strychnifolium (Craib) A. Schmitz. (in Thai name, Ya nang daeng) has been traditionally used to treat fever, alcohol intoxication, cancer, allergies, and blood toxins. It can be used as a health-promoting herbal tea and contains hydroalcoholic extracts. The purpose of the present study was to develop a microwave-assisted extraction method for astilbin in *L. strychnifolium* stems. HPLC was used to determine astilbin content. Three extraction conditions were optimized: types of solvent, microwave power levels, and the number of extraction cycles. Water:methanol (40:60) was the best solvent for astilbin extraction from *L. strychnifolium* stems using 450 watts and six microwave-assisted extraction cycles. This technique offers important advantages over conventional methods, such as shorter extraction times, substantial energy savings, and a reduced environmental burden.

Keywords: Astilbin. *Lysiphyllum strychnifolium* stems. Microwave-assisted extraction.

INTRODUCTION

Microwave-assisted extraction is increasingly used as an extraction technique to obtain useful compounds from plant biomass, providing rapid and efficient extraction as microwaves generate heat by dipole rotations of solvent molecules. Microwaves directly penetrate the biomass substrates, and the irradiated materials are quickly heated from within, thus enhancing the extraction efficiency from the plant body components (Tsubaki *et al.*, 2017). Microwave-assisted extraction is a green extraction technique that offers many advantages such as the reduction of extraction time (usually from seconds to several minutes), low solvent consumption, the potential to extract multiple samples simultaneously (substantially improving sample throughput), improvement in extraction yield, and suitability of thermolabile constituents to extraction (Llompart *et al.*, 2019; Destandau, Michel, Elfakir, 2013; Delazar *et al.*, 2012). However, high microwave intensity may increase the extraction temperature, leading to oxidation or decomposition of the compound and decreased compound quality (Yang, Lambert, Sang, 2009; Cheng *et al.*, 2014; Zhang *et al.*, 2013). Therefore, the optimum extraction conditions should avoid this problem to save energy, improve efficiency, and increase the extraction yield (Chan, Yusoff, Ngoh, 2014; Li, Jiang, 2010).

The climbing herb *Lysiphyllum strychnifolium* (Craib) A. Schmitz (also *Bauhinia strychnifolia* Craib) is known in Thailand as Khayan, Khurea khayan (northern Thailand), Sayan (in Tak and Lampang province, Thailand), and Ya nang daeng (northeastern Thailand). It is an endemic plant distributed in the north, central, and eastern portions of the country (Tangnak, Hongtrakul, Keeratinijakal, 2018). A decoction of dried roots and stems has been traditionally used to treat fever, alcoholic intoxication, cancer, allergy, and blood toxins. Moreover, dried stems and leaves of

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L. strychnifolium can be prepared as an herbal tea used for detoxification and health promotion. Thai traditional doctors suggest that the brewing of the stems and leaves of *L. strychnifolium* can be used in health-promoting herbal tea and hydroalcoholic extracts. The species has been determined pharmacologically to contain the following bioactive properties: antioxidant (Maitree *et al.*, 2018; Kaewpiboon *et al.*, 2012), anti-hyperuricemic (Sutiyaporn *et al.*, 2018; Sato *et al.*, 2019), anti-inflammatory (Sato, *et al.*, 2019), and anticancer (Kaewpiboon *et al.*, 2012, Yuenyongsawad *et al.*, 2013). Previous publications report the phytochemical constituents isolated from stems of *L. strychnifolium* include flavonoids (quercetin, 3,5,6,3',5'-pentahydroxy-flavanonol-3-*O*-α-*L*-rhamnopyranoside, 3,5,7-trihydroxy-chromone-3-*O*-α-*L*-rhamnopyranoside), a triterpenoid (*β*-sitosterol), and a phytosterol (stigmasterol) (Yuenyongsawad *et al.*, 2013).

Astilbin (Figure 1), a flavonoid glycoside, is one compound recently isolated from *L. strychnifolium* (Sampaopan *et al.*, 2021), specifically found in extracts (Bi *et al.*, 2019; Lu *et al.*, 2015). The astilbin is separated from the extracts by column chromatography (Merck silica gel 60, 70-230 mesh) eluted with ethyl acetate and methanol (95: 5, %v/v), and reversed-phase column chromatography (Merck LiChroprep RP- 18, 40-63 μm) eluted with methanol and water (50: 50, % v/v). Further purification is by isocratic column chromatography using Sephadex LH-20 with methanol and preparative HPLC (Hypersil C-18 column) with methanol and water (25: 75, v/v). Astilbin is obtained as a brownish-white amorphous powder. The following were the ^{1}H-NMR spectra (600 MHz, methanol-d_4) results: δ (ppm) = 6.97 (d, *J* = 1.9 Hz, 1H, Ar-*H*), 6.86 (dd, *J* = 8.1, 2.0 Hz, 1H, Ar−*H*), 6.83 (d, *J* = 8.1 Hz, 1H, Ar−*H*), 5.94 (d, *J* = 2.1 Hz, 1H, Ar−*H*), 5.92 (d, *J* = 2.1 Hz, 1H, Ar-*H*), 5.09 (d, *J* = 10.7 Hz, 1H, Glycoside-*H*), 4.59 (d, *J* = 10.7 Hz, 1H, Glycoside-*H*), 4.29–4.24 (m, 1H, Glycoside-*H*), 4.07 (d, *J* = 1.3 Hz, 1H, Glycoside-*H*), 3.68 (dd, *J* = 9.6, 3.3 Hz, 1H, Glycoside-*H*), 3.56 (dd, *J* = 3.2, 1.7 Hz, 1H, Glycoside-*H*), 1.20 (d, *J* = 6.2 Hz, 2H, Glycoside-CH_3). Mass spectral data revealed an $[M-H]^-$ peak at *m/z* = 449 in negative mode, confirming astilbin structure as previously reported (Jusoh, Zakaria, Din, 2013; Sampaopan *et al.*, 2021). It can be used as a chemical marker to provide a basis for the quality control of raw materials, extracts, and phytopharmaceutical products. Previous research indicated that astilbin could be potentially utilized in health food and medicine because of its multiple bioactivities, such as improving immunological liver injury (Wang *et al.*, 2004), antioxidant (Zhou *et al.*, 2013; Yu *et al.*, 2014), anti-inflammatory (Yu *et al.*, 2014), and anti-arthritic (Cai, Chen, Xu, 2003) properties. In this study, astilbin was selected as a chemical marker, and its content was determined in *L. strychnifolium* stem extracts.

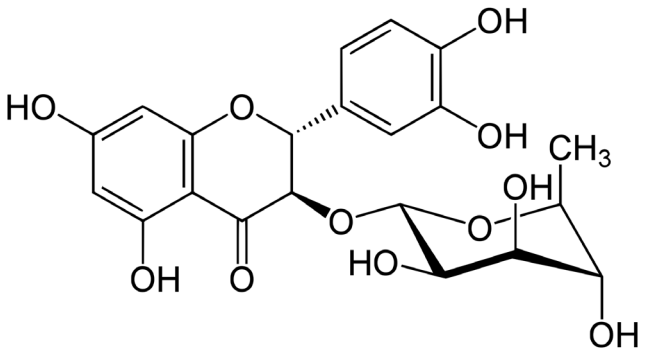

FIGURE 1 - Chemical structure of astilbin.

The objectives of this study were to assess the microwave-assisted extraction method for *L. strychnifolium* stems and determine the astilbin content in *L. strychnifolium* stem extracts. The extraction conditions optimized to maximize yield were types of solvent, microwave power levels, and the number of extraction cycles. Our results will assist future studies in analyzing *L. strychnifolium* stem extracts for anti-inflammatory properties and usage in pharmaceutical formulations.

MATERIAL AND METHODS

Materials

Acetonitrile, HPLC grade, was purchased from J.T. Baker, USA. Water, HPLC grade, was from RCI Labscan, Thailand. Glacial acetic acid, analytical-reagent grade, was purchased from Merck, Germany. Standard astilbin was purchased from Sigma, USA. All reagents were analytical grade unless stated otherwise.

Preparation of plant materials and stock astilbin

Samples of *L. strychnifolium* stem were supplied by Charoensuk Pharma Supply Co., Ltd., Nakhon Pathom, Thailand, and originated from a farm in Ratchaburi province (13°31'37.6" N 99°48'45.4" E). Niran Vipunngern identified stem samples of *L. strychnifolium*, and the voucher specimen (JS-LS001-1-11-2019) was deposited at the Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University. The dried sample was pulverized, ground into a powder, and passed through a 40-mesh sieve (mesh size is the most common measurement unit used for the sieves and screens widely used in the pharmaceutical manufacturing as well as in the quality control to determine the particle size of the sample). The *L. strychnifolium* stem powder that passed through the 40-mesh sieve had a particle size as 420 μm. The powder was stored in an air-tight container at room temperature in the dark.

Microwave-assisted extraction of L. strychnifolium stem

Five grams of *L. strychnifolium* stem powder were extracted with 200 mL of solvent in a beaker. The extraction procedure was then conducted using a microwave oven (MS23F300EEK/ST model, triple distribution system, Samsung Electronics Co., Ltd., Malaysia) placed in a fume hood for ventilation of the evaporated solvent. Intermittent microwave radiation was applied for 30 seconds ("on"), followed by 30 seconds of non-heating ("off") to avoid overheating of the extraction solvent. Thus, the total extraction time was 60 seconds per cycle. Three types of solvent (ethanol, water:methanol [40:60], and ethyl acetate), three power levels of microwave-assisted extraction (300, 450, and 600 watts [W]), and the number of microwave-assisted extraction cycles were used. All of the extraction trials were carried out in triplicate. After the process, the liquid phase was separated and filtered through a 0.45 μm Whatman No. 1 filter paper and concentrated with a rotary evaporator at 40–60ºC under vacuum.

HPLC apparatus and conditions

The HPLC apparatus (Thermo Scientific, CA, USA) equipped with a Spectra System pump P4000, a Spectra System auto-sampler AS3000 and a diode array detector Spectra System detector UV6000LP. The separation was done on a VDSpher PUR 100 C18-E column (4.6 mm × 250 mm diameter, 5 μm particle size, Chromatographie Technik GmbH, Berlin, Germany). A gradient delivery system with a flow rate of 1.0 mL/min at room temperature was used for elution. The mobile phase consisted of 2% v/v acetic acid in distilled water (solvent A) and acetonitrile (solvent B). Total running time was 50 min, and the linear gradient program was as follows: 0% to 15% B in 5 min, 15% B for 20 min, 15% to 18% B in 15 min, 18% B for 5 min, and 18% to 100% B in 5 min. The column was washed with acetonitrile for in distilled water 10 min after each analysis and equilibrated with 2% v/v acetic acid for 10 min before each injection. The detection wavelength was 290 nm. The astilbin in extraction solutions was analyzed using the same HPLC conditions as the astilbin standard. The validated HPLC method parameters for astilbin analysis were linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Analyses were conducted according to the International Conference on Harmonization guideline (ICH) (ICH, 1996).

Statistical analyses

Mean values with standard deviation for each experiment were determined using Microsoft Excel. All results were statistically analyzed by one-way analysis of variance, and the selected significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Five grams of *L. strychnifolium* stem powder were extracted in various solvent; ethanol, water:methanol (40:60), and ethyl acetate with power level of microwave-assisted extraction at 300 W and 6 cycles of microwave-assisted extraction. The *L. strychnifolium* stem extraction solutions are shown in Figure 2. The solutions varied from transparent to translucent with light brown to amber-brown color. The extraction solution using water:methanol

(40:60) as a solvent had a high viscosity, greater than that when using ethanol and ethyl acetate. This result was likely due to the water's high viscosity; the other solvents were low viscosity.

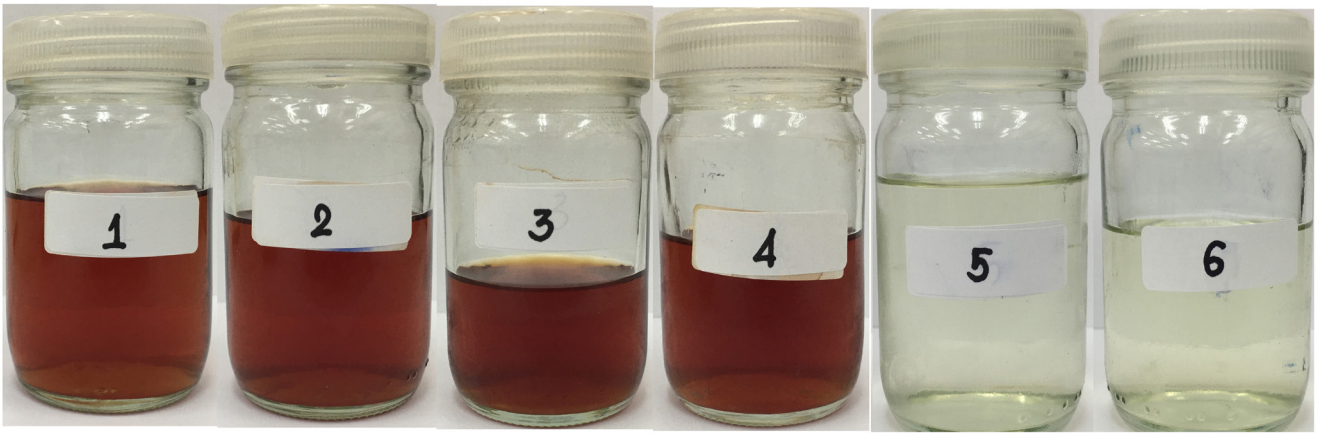

FIGURE 2 - The extraction solution using different solvents; (1) ethanol 1, (2) ethanol 2, (3) water:methanol (40:60) 1, (4) water:methanol (40:60) 2, (5) ethyl acetate 1, and (6) ethyl acetate 2.

A standard 500 µg/mL stock solution of astilbin in methanol was prepared. Further dilution was carried out using water:methanol (40:60, v/v) as the diluting solvent to achieve the desired concentration. The astilbin standard was analyzed by HPLC method of gradient elution system using 2% v/v acetic acid in distilled water as a solvent A and acetonitrile as a solvent B over the period of 50 min. The gradient program was following: 0% to 15% B in 5 min, 15% B for 20 min, 15% to 18% B in 15 min, 18% B for 5 min, and 18% to 100% B in 5 min. with a flow rate of 1.0 mL/min at room temperature. The astilbin standard was eluted at 34.06 min, which represents its retention time. The HPLC chromatogram of astilbin has been presented previously (Sampaopan *et al.*, 2021). Linearity was accessed across the concentration range of 5.55–19.40 µg/mL. The plot of the peak area versus the concentration represents the regression equation of Y = 322322 X - 211451 and a correlation coefficient (r^2) of 0.9994. The LOD and LOQ were 0.23 and 0.69 µg/mL, respectively, indicating the method's high sensitivity. The intra-day and inter-day precision as indicated by %RSD were 1.88 and 1.99, respectively. The recoveries at three astilbin concentrations were 100.52, 100.77, and 100.92%, with an average of 100.74%. The HPLC chromatogram of astilbin in different extraction solutions: ethanol, water:methanol (40:60), and ethyl acetate are shown in Figure 3. In addition, the specificity of astilbin analysis was tested by spiking a low amount of astilbin standard into each extraction solution. The result of sample spiking revealed that the analyte peak was pure, confirming the analytical method's specificity and indicating that there was no interference from the solvent in the extraction solutions.

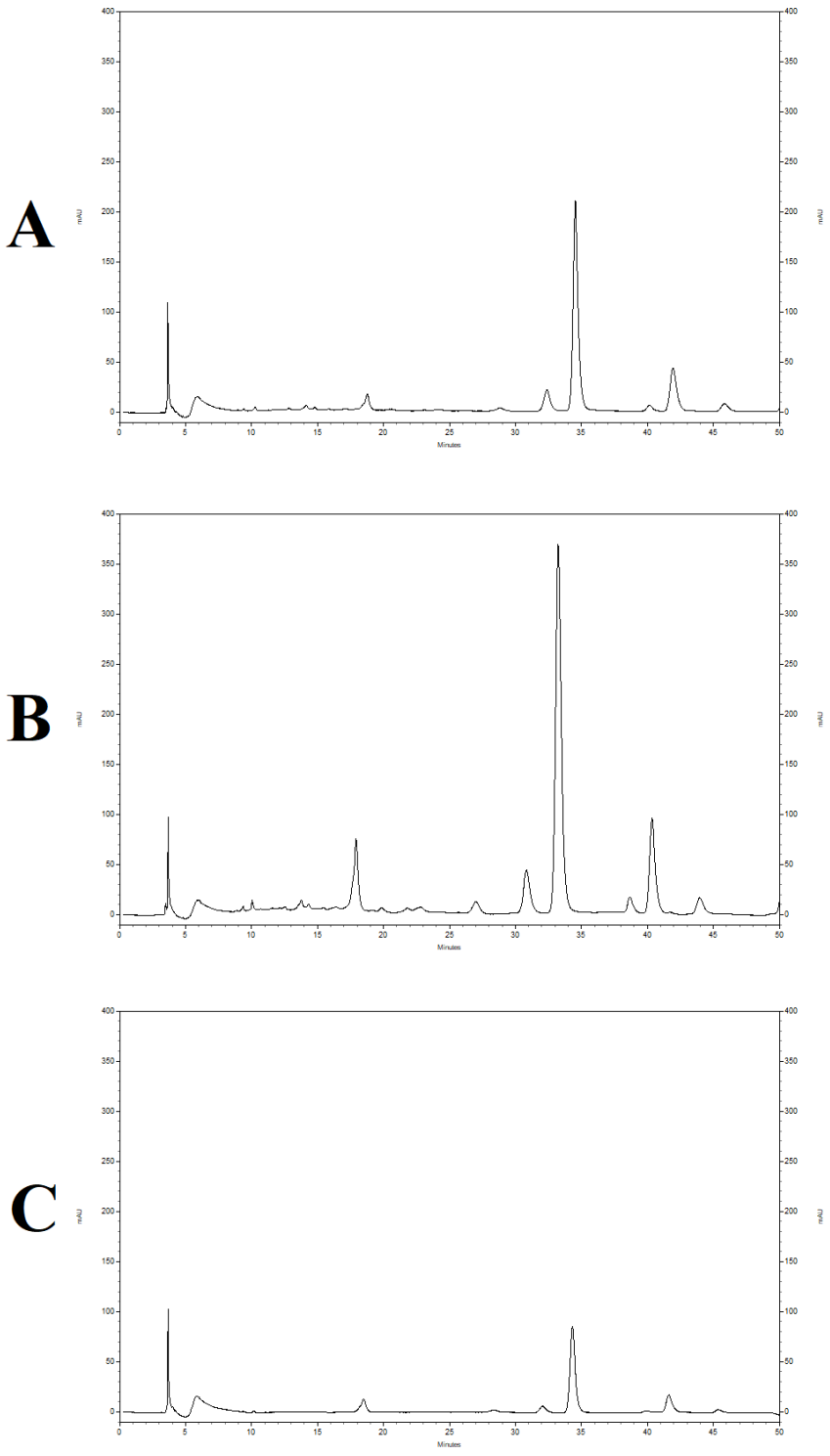

FIGURE 3 - HPLC chromatogram of astilbin using 300 W of microwave power and six cycles of microwave-assisted extraction in different solvents: (A) ethanol, (B) water:methanol (40:60), and (C) ethyl acetate.

The highest astilbin content in extraction solution was associated with water:methanol (40:60) as a solvent (675.31 ± 27.13 µg/mL), followed by ethanol (391.30 ± 5.07 µg/mL) and ethyl acetate (174.10 ± 20.08 µg/mL), respectively (Figure 4A). Solvent volume, the power level of microwave-assisted extraction, and the number of microwave-assisted extraction cycles were fixed at 200 mL, 300 W, and six cycles, respectively. Preliminary experiments show that the microwave did not destroy the structure of astilbin, confirming by previously reported (Jusoh, Zakaria, Din, 2013; Sampaopan *et al.*, 2021). The temperature of the extraction solution was measured each minute using a glass laboratory thermometer. The extraction temperatures were 75 ± 2ºC, 72 ± 2ºC, and 77 ± 2ºC with ethanol, water:methanol (40:60), and ethyl acetate, respectively, for solvent extraction. The extraction yield of astilbin content in the extraction solutions was calculated and compared with the dry powder of *L. strychnifolium* stem. The results ranged from 3.48–13.51% (Figure 4B). The water:methanol (40:60) solvent was determined to be the best of those tested for astilbin extraction from *L. strychnifolium* stem at six cycles and 300 W of microwave power. Subsequently, we varied the power level and number of cycles of microwave-assisted extraction.

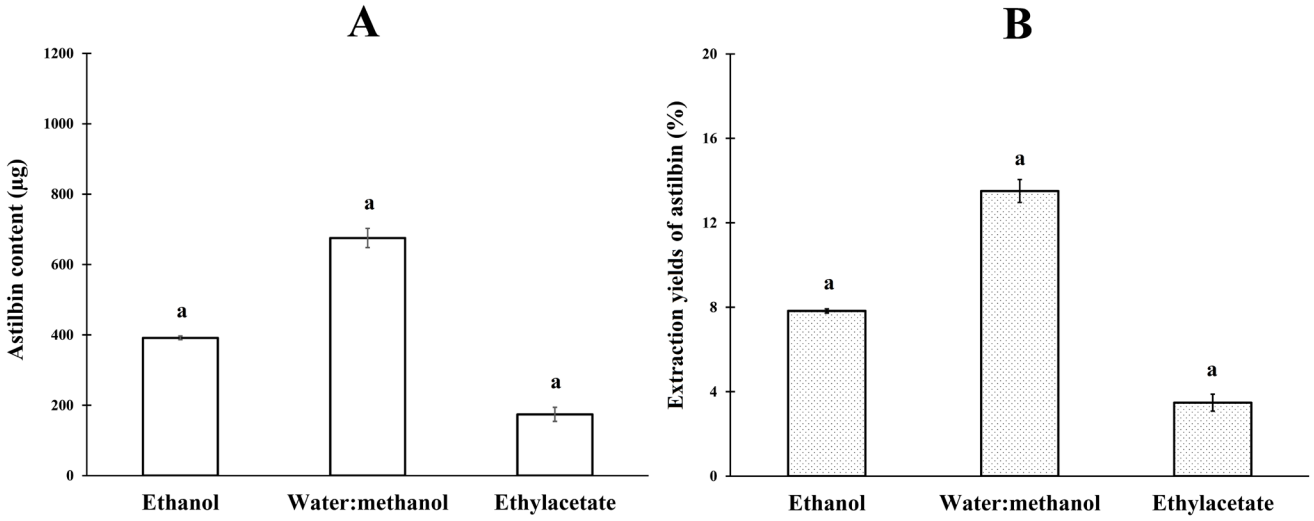

FIGURE 4 - (A) Astilbin content and (B) extraction yields of astilbin using 300 W of microwave power level and six cycles of microwave-assisted extraction in different solvents: ethanol, water:methanol (40:60), and ethyl acetate. The extraction yields of astilbin were calculated as dry powder of *L. strychnifolium* stems. [a]significantly different ($p < 0.05$).

When 200 mL of water:methanol (40:60) was used as a solvent, the number of cycles of microwave-assisted extraction was fixed at six and the power levels increased from 300 to 600 W, more astilbin was extracted from *L. strychnifolium*. The results showed that the highest astilbin content in extraction solutions was obtained when the microwave power level was 600 W (780.72 ± 26.01 µg/mL), followed by 450 W (757.54 ± 35.76 µg/mL), and 300 W (675.31 ± 27.13 µg/mL), respectively (Figure 5A). The extraction yields of astilbin content per dry powder of *L. strychnifolium* stem were 13.51 ± 0.54, 15.15 ± 0.72, and 15.61 ± 0.52% when the microwave power level was 300, 450, and 600 W, respectively (Figure 5B). However, the differences in astilbin detected between 450 and 600 W were not significantly different ($p > 0.05$). In addition, extraction using 600 W proceeded at a high extraction temperature (93 ± 2ºC), which would lead to oxidation or decomposition reaction of the compound and affect the quality of the compound (Yang, Lambert, Sang, 2009; Cheng *et al.*, 2014, Zhang *et al.*, 2013). Therefore, to save energy and improve efficiency, 450 W of microwave power was concluded to be appropriate (Chan, Yusoff, Ngoh, 2014; Li, Jiang, 2010).

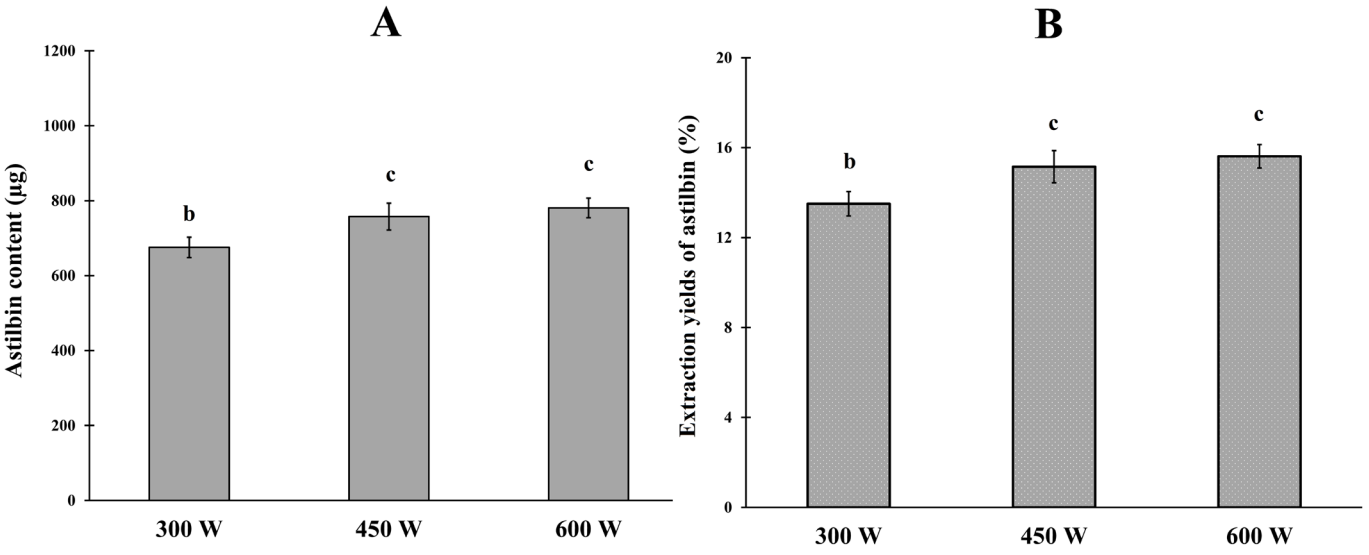

FIGURE 5 - (A) Astilbin content and (B) extraction yields of astilbin at different microwave power levels (300, 450, and 600 W) and six cycles of microwave-assisted extraction in water:methanol (40:60) solvent. The extraction yields of astilbin were calculated as dry powder of *L. strychnifolium* stem. [b]significantly different ($p < 0.05$). [c]significantly different ($p > 0.05$).

We next investigated the influence of the number of cycles of microwave-assisted extraction on astilbin content. Extraction was carried out at 450 W with water:methanol (40:60) solvent (200 mL), and the number of cycles was varied from 5–8. The results are shown in Figure 6. The astilbin content increased significantly between five and six cycles, but the astilbin content increased little when using more than six cycles ($p > 0.05$). Therefore, six microwave extraction cycles were concluded to be appropriate.

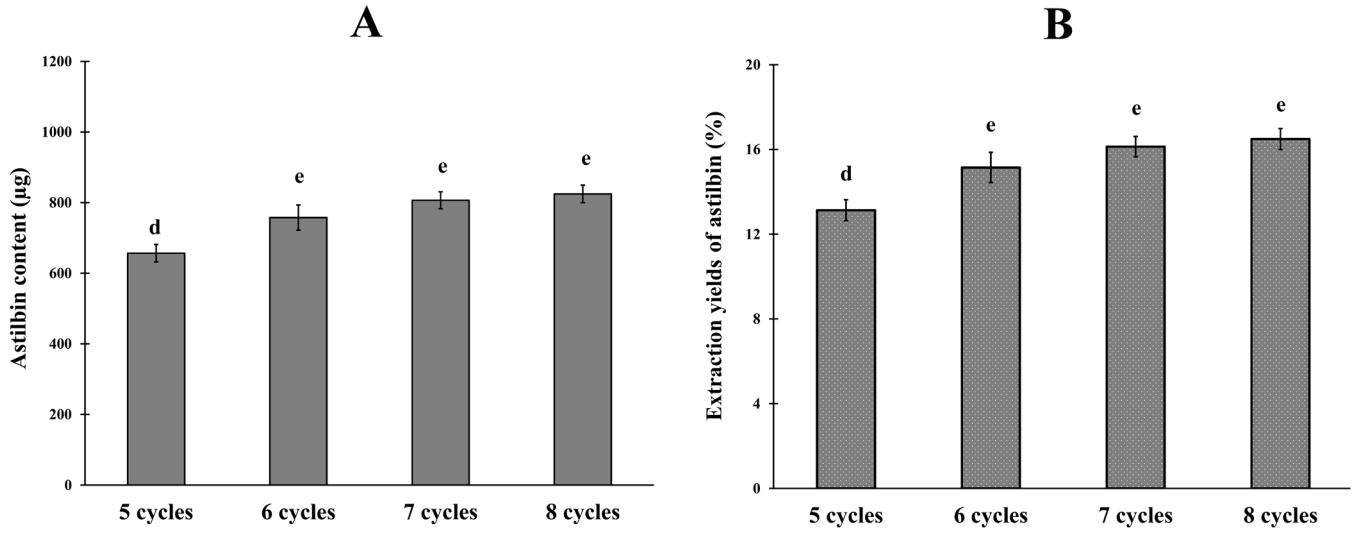

FIGURE 6 - (A) Astilbin content and (B) extraction yields of astilbin using 450 W of microwave power in water:methanol (40:60) solvent using a different number of microwave-assisted extraction cycles. The extraction yields of astilbin were calculated as dry powder of *L. strychnifolium* stems. [d]significantly different ($p < 0.05$). [e]significantly different ($p > 0.05$).

The solvent plays the most important role in the extraction of astilbin from *L. strychnifolium* stems by microwave-assisted extraction, and the highly polar water:methanol (40:60) was superior in this regard, followed by ethanol and ethyl acetate ($p < 0.05$). Six microwave extraction cycles at 450 W provided the highest astilbin extraction while minimizing energy usage and potential workload.

The dried and powdered *L. strychnifolium* stem is usually prepared by maceration with methanol for three 72-hour cycles with occasional shaking. The crude methanolic extract of *L. strychnifolium* stem is then pooled, filtered, and the solvent removed by a rotary evaporator under vacuum (Sampaopan *et al.*, 2021). While this conventional extraction method is very simple and commonly used, it nevertheless presents various disadvantages such as long extraction time, high solvent usage, and low extraction efficiency (Zhao *et al.*, 2018; Ćujić *et al.*, 2016; Heleno *et al.*, 2016; Zhang, Lin, Ye, 2018). Therefore, several new extraction methods such as ultrasound-assisted extraction (Chemat *et al.*, 2017; Xu *et al.*, 2017), supercritical fluid extraction (Pourmortazavi, Hajimirsadeghi, 2007), pressurized liquid extraction (Garcia-Mendoza *et al.*, 2017), and microwave-assisted extraction (Zhao *et al.*, 2018; Li *et al.*, 2017) can effectively improve extraction efficiency. Microwave-assisted extraction provides the specific advantages of shorter extraction times, substantial energy savings, a reduced environmental burden, low solvent usage, and high extraction efficiency compared to other methods.

In summary, the present study revealed that microwave-assisted extraction is especially suitable for astilbin extraction from *L. strychnifolium* stems. Among the three extraction variables tested, the type of solvent played the largest role in astilbin extraction. The water:methanol (40:60) was the best solvent for astilbin extraction. The order of importance on the extraction yield was the solvent's polarity, microwave power levels, and the number of extraction cycles. The optimal performance of astilbin extraction from *L. strychnifolium* stems was achieved at 450 W and six extraction cycles using water:methanol (40:60) as the solvent. Microwave extraction is an appropriate method for astilbin extraction from *L. strychnifolium* stems compared to published works (Lu *et al.*, 2015; Yuenyongsawad *et al.*, 2013).

CONCLUSION

The present study reveals that microwave-assisted extraction is especially suitable for astilbin extraction from *L. strychnifolium* stems. The solvent's polarity, microwave power levels, and the number of cycles of microwave-assisted extraction affected the extraction yield of astilbin. The best method for astilbin extraction included water:methanol (40:60) solvent and six extraction cycles at 450 W of microwave power. Microwave-assisted extraction of astilbin from *L. strychnifolium* stems has advantages of a shorter extraction time, substantial savings of energy, a reduced environmental burden, low solvent usage, and high extraction efficiency.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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