ORIGINAL ARTICLE

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J. Chem. Metrol. XX:X (202X) X-XX

journal of chemical metrology

Microwave-assisted extraction for the tetermination of shanzhiside methyl ester andb in *Barleria prionitis* L.

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(Received April 12, 2025; Revised May 26, 2025; Accepted May 28, 2025)

Abstract: The study focused on the application of microwave-assisted extraction (MAE) to determine the presence of Shanzhiside Methyl Ester (SME) and Barlerin (BLR) in *Barleria prionitis* L. (*B. prionitis* L.) The impact of the extraction parameters, including solvent concentration, microwave power, and duration of extraction. The findings of the current investigation reveal that the solvent concentrations of 90% and 70% ethanol produced significant yields of SME and BLR, measuring at 4% and 0.5%, respectively. The power of microwave irradiation ranges from 100 W to 600 W. Notably, at 600 W, the extraction efficiencies of SME and BLR match those observed at 450 W. Therefore, we opted for a microwave irradiation power of 450 W for the extraction of *B. prionitis* L. The findings demonstrated that the yield of SME and BLR rose with the extension of MAE time during the initial phase of extraction. The yield may attain a peak of 4% within 5 minutes during the MAE process. Consequently, a duration of 5 minutes was selected as the ideal timeframe for MAE to achieve the maximum yield.

Keywords*: Barleria prionitis* L.; barlerin; shanzhiside methyl ester; iridoid glycoside; microwave.

1. Introduction

Barleria prionitis Linn. (family: Acanthaceae) is an annual shrub distributed across the tropical regions of India, Sri Lanka, and South Africa. *B. prionitis* L. is referred to as "Aungkabnoo" in Thai. The entire plant measures approximately 1-3 feet in length, with flowers that are broad and tubular, predominantly yellowish or whitish, and measuring around 3-4 cm in length. The fruits are ovoid and capsular, while the seeds are flattened, covered with matted hairs, measuring approximately 8 mm in length and 5 mm in width. The elliptic leaf measures approximately 3-10 cm in length and 1.5-4 cm in breadth, featuring spines that range from 5 to 20 mm in length. In traditional medicinal systems, nearly all parts of the plant are utilized for their antiviral [1-2], antioxidant [3-5], antiinflammatory [6-7], antidiabetic [8], antibacterial [9-12], and various other properties.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology Month-Month 2025 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.115.2504.3488
Available online: June 07, 2025

The isolation and identification of SME, 8-O-acetyl shanzhiside methyl ester (barlerin), 6,8-O,O-diacetyl shanzhiside methyl ester (acetylbarlerin), 6-O-trans-p-coumaryl-8-O-acetyl shanzhiside methyl ester, and its cis-isomer have been achieved through previous published research on the iridoid constituents (monoterpene lactone glucosides) of Barleria species [13-14]. SME has been observed in plants belonging to numerous genera [15]. The iridoids are typically found as glycosides. A wide variety of biological activities have been demonstrated in vitro, in vivo, and in clinical studies by iridoids or iridoid-rich plants. These activities include antiarthritic, antiinflammatory, antibacterial, antifungal, anticancer, anticoagulant, antioxidant, antiviral, antispasmodic, immunomodulatory, wound-healing, and neuroprotective effects [16-18].

Microwave-assisted extraction (MAE) is a technique that uses microwave radiation to heat solvents in contact with a sample, facilitating the partitioning of analytes from the sample matrix into the solvent. The capacity for fast heating of the sample solvent combination is intrinsic to MAE and is its primary benefit. The use of closed containers enables extraction at increased temperatures, hence expediting the mass transfer of target chemicals from the sample matrix. A standard extraction technique takes 15 to 30 minutes and utilizes minimal solvent quantities between 10 and 100 milliliters. These quantities are about tenfold lower than those used by traditional extraction methods. MAE, recognized for its environmental sustainability and economic benefits compared to conventional extraction procedures, has been used to extract biologically active chemicals from various materials [19]. Recently, numerous research studies have been conducted on the advancements of MAE methods for extraction, including the extraction of glycyrrhizic acid from licorice roots, camptothecin from Nothapodytes foetida, saikosaponins from Bupleurum falcatum root, and essential oil from cardamom. Numerous studies have shown the advantageous impacts of MAE on medicinal plants, demonstrating considerable enhancements compared to traditional extraction procedures, including reduced extraction time and increased efficiency [24-26].

According to a survey of the relevant literature, the two principal active ingredients of a great number of Barleria species are SME and BLR However, there is no information that has been revealed about the procedures of microwave extraction. To address this, the first article presents a time-saving extraction methodology that utilizes microwave radiation to increase the yield of both BLR and SME from *B. prionitis* L.

2. Experimental

2.1. General Procedures

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AVANCE 400 FT-NMR spectrometer functioning at 400 MHz for ^{1}H and 100 MHz for ^{13}C. High-resolution mass spectra were acquired with the Bruker micrOTOF-Q II mass spectrometer. Column chromatography (CC) was performed with Merck silica gel 60 (particle size less than 0.063 mm) and Pharmacia Sephadex LH-20. Merck precoated silica gel 60 F254 plates were used for TLC. Spots on TLC were identified using UV light and by using anisaldehyde-H_2SO_4 reagent, followed by heating. Reversed-Phase High-Performance Liquid Chromatography (ThermoFisher). All organic solvents and chemicals were of analytical reagent and HPLC quality (E-Merck, Mumbai, India) and were used as received.

2.2. Plant Material

The whole plant of *B. prionitis* L. were gathered from Ladkrabang, Bangkok, Thailand, in January 2019. A voucher specimen of this plant No. is NS-101 archived in the Department of Plant Sciences, Faculty of Science, Mahidol University, Bangkok.

2.3. Microwave-Assisted Extraction (MAE)

B. prionitis L. (5.0 g) was heated in 100 mL of solvent using a Samsung microwave oven (model GE711K/XST), manufactured by Electrical Appliances Manufacturing Co., Ltd., Foshan, China. The microwave irradiation power ranged from 100 W to 600 W, with exposure times between 1 s and 15 s. Following extraction, the resulting mixture was diluted to 10 mL using ethanol (technology Co., Ltd., Dongguan, China) and filtered through a nylon membrane with a pore size of 0.45 μm. The analysis of SME, BLR, and the solution was conducted using the RP-HPLC method on a ThermoFisher system. This system included a C18-4E Shodex column (4.6 × 250 mm, 5 μm), a Thermo intelligent pump (SpectraSYSTEM P1000), a precision loop injector (Rheodyne), a variable wavelength UV/Vis detector (SpectraSYSTEM UV1000), and ChromQuest software (version 5.0). The solvent system comprised ACN-methanol-water (1:1:4, v/v) and the analysis was performed over 45 minutes at 25°C, with detection at 254 nm and a flow rate of 1.5 mL min^{-1}. The injection volume for the marker compounds was 20 μL.

2.4. Isolation, Purification and Characterization of BLR and SME

The whole plant of *B. Prionitis* L. (1.0 kg) was air-dried, milled, and successively macerated with n-hexane. Filtration and evaporation of the solvents under reduced pressure yielded an n-hexane extract of 103.0 g. Subsequently, maceration with EtOAc and MeOH yielded 85.0 g of EtOAc and 209.0 g of MeOH extract. The methanol extract (9.2 g) underwent fractionation via column chromatography employing Sephadex LH-20 with 100% methanol as the solvent. The eluates were analyzed using TLC, resulting in the acquisition of three combined fractions (ME1-ME3). Fraction ME2 underwent column chromatography on silica gel with a CH_2Cl_2–MeOH (100:10) solvent system, yielding compound **1** as BLR (43.5 mg). Fraction ME3 underwent column chromatography on silica gel with a CH_2Cl_2–MeOH (90:10) solvent system, yielding compound **2** as SME (52.1 mg). The isolated and purified compounds from the methanol extract were identified using ^{1}H and ^{13}C-NMR, as well as positive ESI-MS, with their spectral data compared to previously reported findings [10–13].

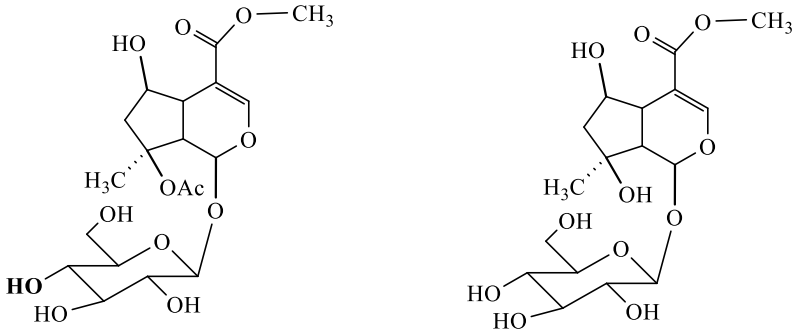


Figure 1. Chemical structure of barlerin (**1**) and shenzhiside methyl ester (**2**)

*Barlerin (**1**):* Pale yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): aglycone moiety δ 6.33 (1H, d, J = 2.1 Hz, H-1), 7.55 (1H, d, J = 1.6 Hz, H-3), 3.39 (1H, dd, J = 8.9, 1.6 Hz, H-5), 4.63 (1H, d, J = 2.4 Hz, H-6), 2.03, 2.39 (1H, dd, J = 14.5, 5.1 Hz, H-7), 3.47 (1H, dd, J = 8.9, 2.1 Hz, H-9), 1.51 (3H, s, H-10), 3.44 (3H, s, H-12), 1.72 (3H, s, OCOCH3), glucose moiety 5.22 (1H, d, J = 8.1Hz, H-1'), 3.86 (1H, m, H-2'), 4.12 (1H, t, J = 8.9 Hz, H-3'), 4.05 (1H, t, J = 9.1 Hz, H-4'), 3.89 (1H, ddd, J = 8.6, 5.9, 2.4 Hz, H-5'), 4.16, 4.42 (1H, br.d, J = 10.7 Hz, H-6'). ^{13}C-NMR ($CDCl_3$, 100 MHz): aglycone moiety 95.6 (C-1), 153.7 (C-3), 109.7 (C-4), 42.2 (C-5), 78.2 (C-6), 47.6 (C-7), 89.7 (C-8), 49.9 (C-9), 22.2 (C-10), 169.0 (C-11), 51.8 (C-12), 173.2, 22.2 (C-13), glucose moiety 100.3 (C-1′), 73.8 (C-2′), 77.9 (C-3′), 71.5 (C-4′), 75.9 (C-5′), 62.9 (C-6′). HR-TOFMS (ES^+): *m/z* 471.1462 $[M+Na]^+$, calcd. for $C_{19}H_{28}O_{12}$+Na.[13]

,*Shanzhiside methyl ester (**2**)*: *P*ale yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): aglycone moiety δ 5.50 (1H, d, J = 2.7 Hz, H-1), 7.37 (1H, d, J = 1.5 Hz, H-3), 3.01 (1H, dd, J = 10.5, 2.5 Hz, H-5), 4.03 (1H, m, H-6), 1.81 (1H, dd, J = 3.2, 4.4 Hz), 1.97 (1H, dd, J = 13.2, 4.4 Hz, H-7), 2.59 (1H, dd, J = 10.5, 3.5 Hz, H-9), 1.25 (3H, s, H-10), 3.72 (3H, s, H-12), glucose moiety 4.61 (1H, d, J = 7.5 Hz, H-1'), 3.0 (1H, dd, J = 8.7, 8.0 Hz, H-2'), 3.35 (1H, t, J = 8.7 Hz, H-3'), 3.16 (1H, t, J = 8.1 Hz, H-4'), 3.24 (1H, m, H-5'), 4.16, 3.87, 3.63 (1H, dd, J = 12.3, 4.1 Hz, H-6'). ^{13}C-NMR ($CDCl_3$, 100 MHz): aglycone moiety 95.1 (C-1), 153.1 (C-3), 111.6 (C-4), 41.5 (C-5), 77.8 (C-6), 48.7 (C-7), 79.4 (C-8), 52.2 (C-9), 25.1 (C-10), 170.1 (C-11), 50.3 (C-12), glucose moiety 100.2 (C-1′), 75.0 (C-2′), 78.4 (C-3′), 72.0 (C-4′), 78.8 (C-5′), 63.3 (C-6′). HR-TOFMS (ES^+): *m/z* 429.2682 $[M+Na]^+$, calcd. for $C_{17}H_{26}O_{11}$+Na. [13]

2.5. Preparation of Sample

The whole plant of *B. prionitis* L. was collected, shaded, dried, and ground into a fine powder. The powdered plant material (5 g) was subjected to extraction using ethanol in water at ratios of 90:10, 70:30, 50:50, 30:70, and 10:90, respectively, with a total volume of 100 mL, at room temperature for a duration of 3 days. Subsequently, filtration and evaporation of the solvents under reduced pressure yielded each extract. The powdered plant material (5 g) was extracted using microwave assistance at electric power settings of 100, 300, 450, and 600 Watts, employing ethanol in water ratios of 90:10, 70:30, 50:50, 30:70, and 10:90, respectively (100 mL). Subsequently, filtration and evaporation of the solvents under reduced pressure yielded each extract.

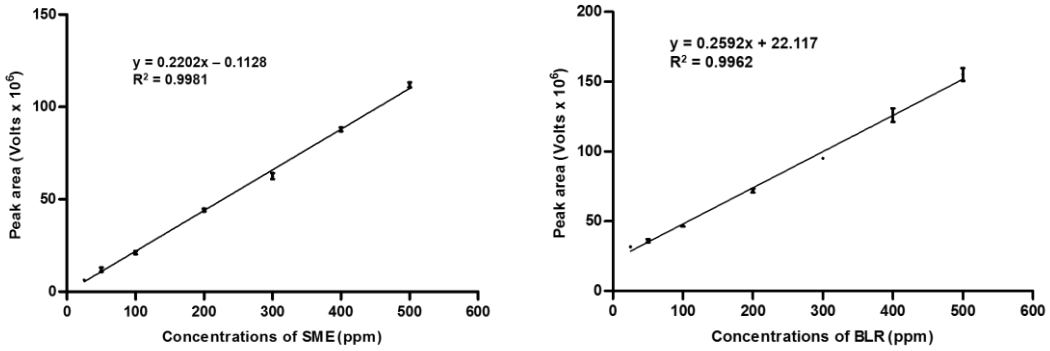
2.6. Determination of Purity of SME and BLR by RP-HPLC Method

The analytical purity of SME and BLR was assessed utilizing a reverse-phase high-performance liquid chromatography method on a ThermoFisher apparatus. This setup featured a C18-4E Shodex column (4.6 × 250 mm, 5 μm), a Thermo intelligent pump (SpectraSYSTEM P1000), a precision loop injector (Rheodyne), a variable wavelength UV/Vis detector (SpectraSYSTEM UV1000), and ChromQuest software (version 5.0). The solvent system comprised ACN-methanol-water in a ratio of 1:1:4 (v/v), with the analysis conducted over 45 minutes at a temperature of 25°C, monitoring at 254 nm and a flow rate of 1.5 mL/min. The volume of injection for these marker compounds was set at 20 μL. The purity levels of SME and BLR were determined to be 99.42% and 99.35% w/w, respectively.

2.7 Calibration Curves of SME and BLR

Stock solutions of SME and BLR at a concentration of 100 μg/mL was prepared separately in methanol. Various concentrations of stock solutions were utilized, specifically 25, 50, 100, 200, 300, 400, and 500 ppm. The peak area data plotted against the corresponding concentrations were analyzed using linear regression, as illustrated in Figure 2. The pre-analyzed sample was supplemented with an additional 50%, 100%, and 150% SME and BLR, and the resulting mixtures were reanalyzed using the proposed method in triplicate. This was conducted to assess the recovery of SME and BLR at various levels in the

extract. The results indicated that the percentage recoveries for both SME and BLR ranged from 95.64% to 99.63% and 98.40% to 99.92%, respectively, as presented in Table 1.



(**A**)

(**B**)

Figure 2. Calibration curve of (**A**) Shenzhiside methyl ester (SME), (**B**) Barlerin (BLR)

Table 1. Recovery study of SME and BLR.

Marker compound	Amount present in the extract (ppm)	Amount added (ppm)	Theoretical content (ppm)	Amount found (ppm)	Recovery (%)	Average recovery	% RSD	SE
SME	100	0	100	95.64	95.64	97.69	1.57	0.87
	100	50	150	146.44	97.63		2.01	1.70
	100	100	200	199.25	99.63		1.03	1.19
	100	150	250	244.63	97.85		1.63	2.29
BLR	100	0	100	98.40	98.40	98.92	1.30	0.74
	100	50	150	149.89	99.92		1.36	1.17
	100	100	200	195.78	97.89		0.71	0.80
	100	150	250	248.69	99.48		1.03	1.48

Mean ± standard deviation (SD, $n = 3$)

3. Results and Discussion

This study examines the impact of extraction conditions, including solvent concentration, microwave irradiation power, and extraction time. The results are presented in Tables 2-4, as well as Figures 4-6. Water and ethanol serve as effective and safe solvents for the extraction of active constituents in herbal medicine. Water is a highly polar molecule that not only absorbs microwave radiation but also readily dissolves compounds containing hydroxyl groups in plants. Ethanol exhibits characteristics akin to those of water. The concentration results of ethanol solvent ranging from 10-100% are presented in Table 2 and Figure 4. The experimental conditions included 100 mL of solvent, 5 g of *B. prionitis* L. sample, 450 W microwave power, and microwave irradiation for 15 minutes, with subsequent analysis conducted via HPLC. Ethanol concentrations of 90% and 70% resulted in high yields of SME and BLR, measuring 4% and 0.5%, respectively.

Table 2. The effect of different ethanol concentrations.

Ethanol concentration in water (%w/v)	MAE[a)]		SOAK[b)]	
	SME (%w/v)	BLR (%w/v)	SME (%w/v)	BLR (%w/v)
0	1.247 ± 0.215	0.130 ± 0.002	0.031 ± 0.012	-
10	1.591 ± 0.181	0.293 ± 0.004	0.314 ± 0.281	-
30	2.835 ± 0.157	0.312 ± 0.013	0.950 ± 0.175	0.001 ± 0.000
50	3.760 ± 0.212	0.472 ± 0.012	2.133 ± 0.181	0.005 ± 0.000
70	4.810 ± 0.293	0.570 ± 0.023	4.417 ± 0.240	0.011 ± 0.001
90	4.681 ± 0.245	0.568 ± 0.019	4.735 ± 0.175	0.018 ± 0.001
100	4.795 ± 0.385	0.590 ± 0.033	4.766 ± 0.241	0.025 ± 0.001

[a] Experimental conditions: 100 mL of solvent, 5 g of *B. prionitis* L. sample, 450 W microwave power, microwave irradiation for 15 min.

[b] Experimental conditions: 100 mL of solvent, 5 g of *B. prionitis* L. sample, soak for 3 days.

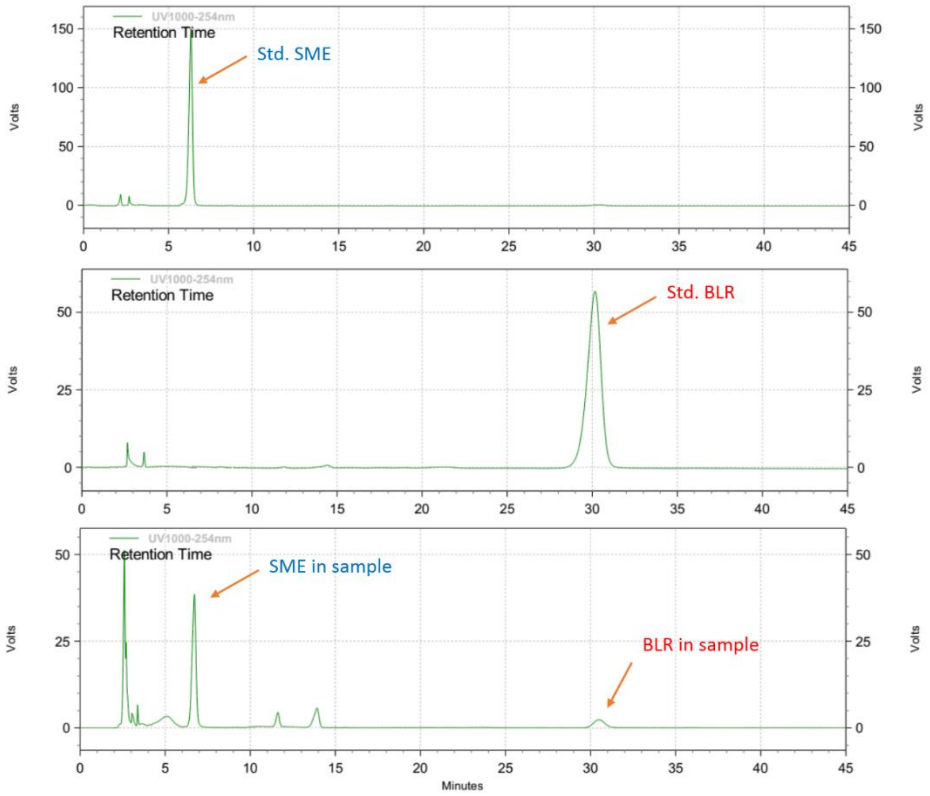


Figure 3. Chromatograms of (A) Shenzhiside methyl ester (SME), (B) Barlerin (BLR), and (C) *B. prionitis* L. extract

Table 3. The effect of different microwave power

Power (Watt)	SME (%w/v)	BLR (%w/v)
100	2.874 ± 0.221	0.191 ± 0.049

300	3.49 ± 0.099	0.236 ± 0.059
450	4.810 ± 0.293	0.570 ± 0.123
600	4.891 ± 0.282	0.563 ± 0.134

[a] Experimental conditions: 100 mL solvent, 5 g of sample, microwave irradiation for 5 min.

[b] The temperature reading on the curve of 70% ethanol at different microwave power.

[c] Holding for 60 min at the pressure and temperature.

Table 4. The effect of microwave irradiation time (min) on the yield of SME and BLR.

Time (min)	MAE		SOKE	
	SME (%)	BLR (%)	SME (%)	BLR (%)
1	1.224 ± 0.214	0.111 ± 0.007	-	-
2	1.580 ± 0.282	0.236 ± 0.019	-	-
3	2.947 ± 0.319	0.314 ± 0.021	-	-
4	3.921 ± 0.191	0.485 ± 0.027	-	-
5	4.810 ± 0.293	0.570 ± 0.023	-	-
6	4.794 ± 0.171	0.563 ± 0.016	-	-
7	4.809 ± 0.190	0.579 ± 0.024	-	-
8	4.812 ± 0.320	0.571 ± 0.017	-	-
9	4.788 ± 0.325	0.574 ± 0.015	-	-
10	4.809 ± 0.166	0.580 ± 0.025	-	-
11	4.684 ± 0.288	0.575 ± 0.009		
12	4.817 ± 0.131	0.582 ± 0.012		
13	4.881 ± 0.290	0.572 ± 0.022		
14	4.758 ± 0.112	0.573 ± 0.018		
15	4.810 ± 0.121	0.581 ± 0.031		
1day	-	-	2.370 ± 0.118	0.004 ± 0.001
2day	-	-	3.452 ± 0.259	0.009 ± 0.003
3day	-	-	4.417 ± 0.249	0.011 ± 0.003

[a] Experimental condition: 100 mL solvent, 5 g of sample, 450 microwave power

[b] The temperature reading on the curve of 70% ethanol at different microwave power.

[c] Holding for 60 min at the pressure and temperature.

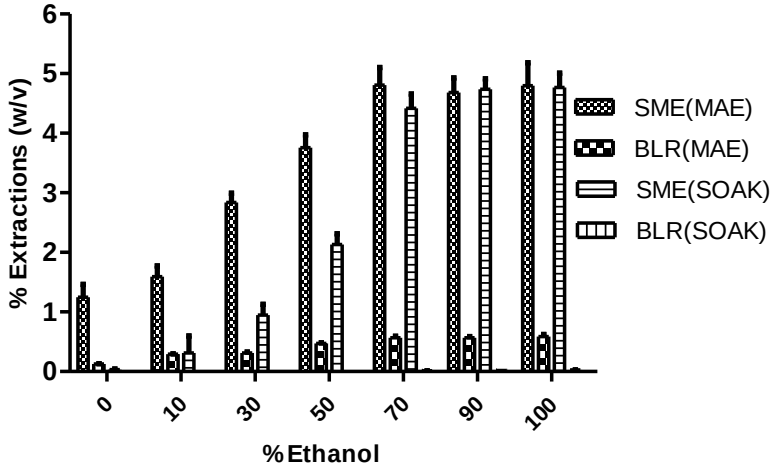


Figure 4. The effect of difference ethanol concentration in water

The influence of microwave irradiation power on extraction efficiency is presented in Table 3 and Figure 5. The extraction efficiencies of SME and BLR increased with the rise in microwave irradiation power from 100 W to 600 W. This phenomenon can be attributed to the higher extraction temperature

resulting from increased microwave power, which enhances extraction efficiency. The extraction efficiencies of SME and BLR at 600 W are comparable to those at 450 W therefore, we selected a microwave irradiation power of 450 W for the extraction of *B. prionitis* L.

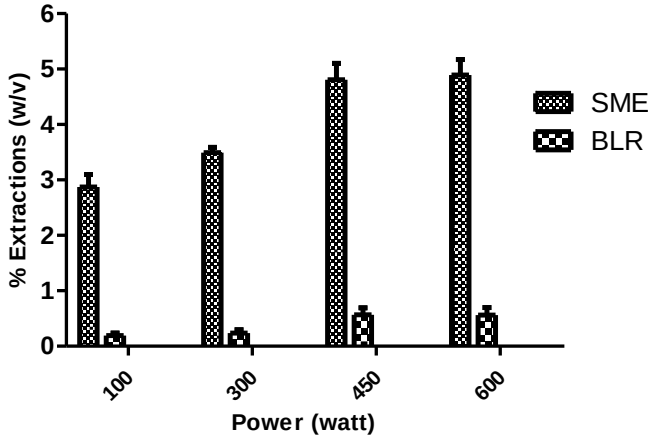


Figure 5. The effect of difference microwave power (watt) and yields of SME and BLR in sample

The impact of microwave irradiation duration on extraction efficiency is presented in Table 4 and Figure 6. The extraction efficiencies of SME and BLR improved with an increase in microwave irradiation time from 1 second to 5 seconds. When the MAE time exceeded 5 minutes, the extraction percentages of SME and BLR declined as MAE time increased. Figure 6 illustrates the impact of MAE duration on the yields of SME and BLR. The findings demonstrated that the yield of SME and BLR rose with an increase in MAE time during the initial phase of extraction. The yield may attain a maximum of 4% within 5 minutes during the MAE process. Consequently, 5 minutes were identified as the optimal duration for MAE to achieve the highest yield. The investigation did not include longer extraction times, as such durations may not yield additional benefits and could potentially lead to negative outcomes due to the degradation or conversion of the analytes.

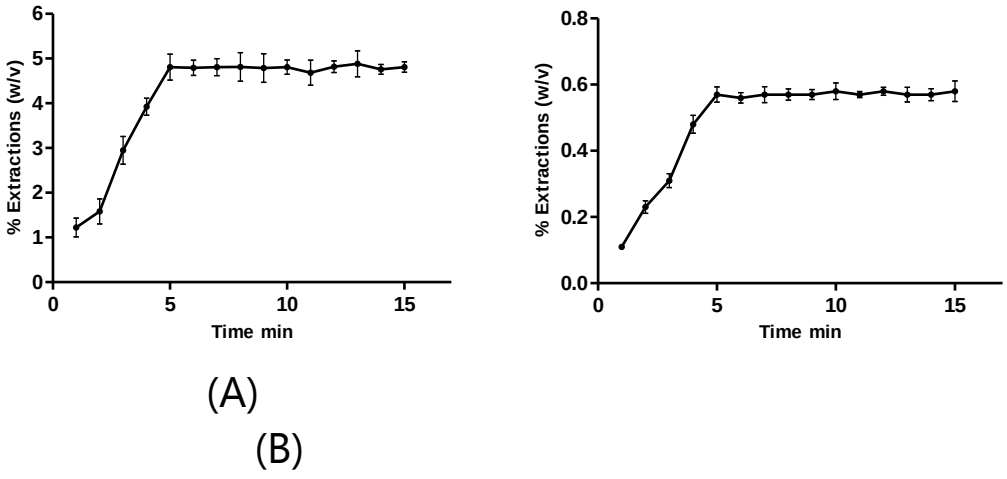


Figure 6. The effect of microwave irradiation times 1-15 min and yields of SME (A) and BLR (B) in sample

4. Conclusions

This study investigated the microwave-assisted extraction of Barlerin and Shanzhiside Methyl Ester from *B. prionitis* L. The impact of extraction conditions, including solvent concentration, microwave irradiation power, and extraction duration. The optimal conditions for MAE were determined to be 70% ethanol as the solvent, a microwave irradiation power of 450 W, and a duration of 5 minutes, resulting in the highest yield.

Acknowledgements

This work was supported by King Mongkut' s University of Technology Thonburi and Herbal and Cannabis Science Program, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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