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Research article

Microwave-Assisted Extraction of Phenolic Compounds from *Smilax ovalifolia* Roxb. and Chemical Compositions

Jarurat Panyo[1*] and Sappasith Klomklao[2]

[1] Faculty of Agro and Bio-Industry, Thaksin University, Phatthalung, 93210, Thailand; jarurat.p@tsu.ac.th
[2] Faculty of Agro and Bio-Industry, Thaksin University, Phatthalung, 93210, Thailand; sappasith@tsu.ac.th
* Correspondence: e-mail: jarurat.p@tsu.ac.th

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Abstract: *Smilax ovalifolia* Roxb. is a medicinal herb in southern Thailand. However, there are not many studies on active compounds from this plant. This study aimed to optimize extraction conditions for total phenolic content (TPC) from *S. ovalifolia* root using microwave-assisted extraction (MAE) and to determine the phytochemical compositions and antioxidant activity of the extract. The result showed that the optimal conditions of phenolic compounds extraction include ethanol concentration of 85% v/v, a solid-to-solvent ratio of 1:30 (g/mL), microwave power of 450 W, and extraction time of 150 s. The phenolic-rich extract exhibited strong antioxidant activity with IC_{50} of 6.31 ± 0.05 µg/mL. LC-MS was used to fingerprint analysis of the extract. The result revealed the presence of 18 bioactive compounds. The main components of *S. ovalifolia* root extract were some flavonoids, including catechin, epicatechin, procyanidin B2, and quercetin.

Keywords: *Smilax ovalifolia* Roxb., microwave-assisted extraction, chemical compositions

1. Introduction

Microwave-assisted extraction (MAE) is a green extraction technology. Microwaves can convert some of the absorbed electromagnetic energy to heat energy, which are waves of frequency between 300 MHz to 300 GHz. The heating mechanism under microwave depends on the conduction of ions and dipole rotation, which enhances the solvent penetration into the sample matrix [1]. Generally, MAE is used with polar solvents for extracting organic components from dried plant samples. It has been widely used for extracting bioactive compounds from many plants due to the use of nontoxic solvents. The advantages of MAE are time reduction, reduced solvent usage, and provided high extraction yield [2]. Many reports presented the optimal MAE conditions for phenolic compounds extraction from plants were the mixture of ethanol and water (42-95%), 1:10 to 1:32 (g/mL) solid to liquid ratio, 62 s to 5 min extraction time, and 100 to 500 W microwave power [2-5]. These conditions gave high polyphenol content extracted from various plants.

Smilax ovalifolia Roxb. belongs to the Smilacaceae family, locally known as Hau-Ai Lek (Figure 1). It is grown in the southern regions of Thailand, especially in Phatthalung and Satun provinces. Hau-Ai Lek roots have been used in Thai folklore medicine for treating and preventing diseases in Kongra District, Phatthalung Province, Thailand. The rhizome of *S. ovalifolia* is used as traditional medicine in northern Thailand for treating aphthous ulcers, scars, body pain,

cancer, hypertension, and diabetes [6]. Moreover, Shah's study also showed that *S. ovalifolia* roots could be used in various diseases such as sexual diseases, uterine diseases, rheumatism, skin diseases, and wound healing. The chemical analysis of root extract showed that carbohydrates, steroids, flavonoids, tannins, and phenolic compounds were composed of the extract [7]. However, there have been few reports on the bioactivity and chemical composition of this plant. There was a report of antioxidant activity in a methanol root extract from *S. zeylanica* [8]. Moreover, steroidal saponins and polyphenols were isolated from the root of *S. china* and showed anti-inflammatory activity and anti-tumor activities [9,10]. Our preliminary study found that an ethanol extract from the roots of *S. ovalifolia* exhibited strong antioxidant activity. There have been no previous reports on LC-MS study of the root of this plant. Therefore, this study investigated the optimization of MAE parameters (Microwave power, extraction time, ethanol concentration, and ratio of dried plant material to solvent) for extracting phenolic compounds from *S. ovalifolia* root and determining the antioxidant activity of the phenolic-rich extract. In addition, this study also intended to determine the chemical compositions of the root extract.

Figure 1. The fresh *Smilax ovalifolia* Roxb. roots

2. Materials and Methods

2.1 Chemicals

Sodium carbonate (Na_2CO_3) and gallic acid were purchased from Sisco Research Laboratories (India). Folin-Ciocalteu' s phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), and quercetin were purchased from Loba Chemie (India). Absolute ethanol (99.9%) was purchased from RCI Labscan (Thailand).

2.2 Plant material

The roots of *S. ovalifolia* Roxb. were purchased from Kong Ra District, Phatthalung Province, Thailand. The plant was identified according to Smitinand and Larsen [11]. Fresh roots were cut and dried at 60 °C for 48 h in a hot air oven and then ground using a grinder. The dried powder was stored in a desiccator at room temperature.

2.3 Microwave-assisted extraction

Dried root powders were extracted using a domestic microwave oven (Samsung model, MS23F300EEK/ST). The extraction conditions were: ethanol concentration (65, 75, 85, and 95% v/v), a ratio of dried plant material (g) to solvent (mL) (1:10, 1:20, and 1:30), microwave power (100, 300 and 450 W), and extraction time (90, 120 and 150 s). After extraction, the root extracts were filtered through a Whatman No. 4 filter paper and the solvent was evaporated under reduced pressure at 60 °C using a rotary evaporator. The MAE extracts obtained from each condition were analyzed for the total phenolic content (TPC).

2.4 Determination of total phenolic content (TPC)

The total phenolic content of root extracts was determined using the Folin-Ciocalteu method previously reported by Lovric et al. with a slight modification [2]. A volume of 500 µL of the extract was mixed with 500 µL of 10% (v/v) Folin-Ciocalteu reagent and 9.5 mL of distilled water, and after 5 min, 2 mL of 10% (w/v) Na_2CO_3 was added. This mixture was incubated at room temperature for 30 min. The absorbance was measured at 760 nm using a spectrophotometer (model GENESYS 180, Thermo Scientific, USA). The TPC was calculated according to the gallic acid calibration curve and expressed in mg of gallic acid equivalents (GAE) per g of extract. Each sample was analyzed in triplicate.

2.5 Determination of antioxidant activity

The phenolic-rich extract obtained from optimal MAE conditions (microwave power of 450 W, extraction time of 150 s, a solid-to-solvent ratio of 1:30, and ethanol concentration of 85%) was performed with an antioxidant activity using the DPPH method [12]. Briefly, a volume of 1 mL of the sample was mixed with 1 mL of 0.2 mM DPPH. This mixture was incubated at room temperature (25 °C) for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Quercetin was used as a positive control. The DPPH radical scavenging activity of the extract was calculated as follows:

$$\% \text{ scavenged} = (1\text{-}As/Ac) \times 100$$

Where: As = the absorbance of the sample

Ac = the absorbance of the control

The half-maximal inhibitory concentration (IC_{50}) value of each sample was determined using a calibration curve.

2.6 LC-MS analysis

The chemical composition of *S. ovalifolia* root extract was evaluated by liquid chromatography-mass spectrometry (LC-MS/QTOF) method (model X500R QTOF, SCIEX). The analysis was performed on a Kinetex® C-18 column (150 mm × 3 mm × 2.6 µm) with 40 °C. The gradient mobile phase was a mixture of 0.5% acetic acid and methanol at a 0.5 mL/min flow rate. Mass spectra were recorded with electron ionization (EI) mode. The ion source temperature was 350 °C. The constituents were identified by comparing their retention time and mass spectral data with the National Institute of Standards and Technology (NIST) library.

2.7 Statistical analysis

The results are expressed as the mean ± standard deviation (SD). The statistical comparisons were evaluated with the Kruskal-Wallis test.

3. Results and Discussion

3.1 Optimization of MAE

This study established the effect of MAE processing parameters including microwave power, extraction time, ethanol concentration and solid/solvent ratio, to extract polyphenols from the root of *S. ovalifolia*. The results are shown in Figure 2. The results indicated that the optimal microwave power for phenolic compounds extraction from *S. ovalifolia* root was 450 W, the most effective extraction time was 150 s with ethanol 85% (v/v), and the ratio of dried plant material (g) to solvent (mL) was 1:30. These conditions obtained higher TPC than other extractions. These results are related to that reported by Zaki et al., in which the authors studied the effect of MAE on TPC and antioxidant activity of the leaf of *Pandanus amaryllifolius* extract. Their results showed that the highest TPC was 1.557 mg/g GAE and DPPH scavenging activity was 77.7%, respectively, with an ethanol concentration of 75% v/v, microwave power of 450 W, extraction time of 10 min, and the solid to-solvent ratio of 3:100 (g/mL) [13]. In the extraction of *Centella asiatica* leaves with MAE, the optimal conditions were 75% ethanol concentration, 450 W microwave power, and 10 min irradiation time [14]. Moreover, Singh et al.'s study also showed that the highest values for the total phenolic content (83.53 mg GAE/g) and flavonoid content (18.98 mg QAE/g) were reached when MAE at 320 W during 3 min

extraction time and ethanol concentration of 50% (v/v) [15]. These data suggest that applying the optimal microwave power and extraction time may effectively extract phenolic compounds under MAE. If the extraction time is too long, the microwave energy will degrade unstable compounds, but if the irradiation is too short, microwave energy may be insufficient [16]. In the same way as microwave power, when the power of the microwave is too high, the extraction yield decreases, and the degradation of phenolic compounds at high temperatures may cause a decrease. There is evidence supporting this hypothesis. The TPC is highest at an extraction temperature of 60-80 °C [17]. This study found that when the intermittent microwave-assisted extraction time was 150 s and microwave power of 450 W, the polyphenol content was highest (221.65 ± 1.30 mg GAE/g extract). Moreover, 85% (v/v) ethanol concentration in water and the ratio of solvent to plant material 30 mL/g were considered to be optimal for the extraction of the phenolic from *S. ovalifolia* root because ethanol-water mixtures have different polarity, as a result, obtained the desired properties of the solvent and the extraction capability of the mixture was higher than a single solvent [4]. In addition, Rostagno and Prado's study found that if the solvent used for extraction is too small, the plant material does not absorb enough solvent during extraction. On the other hand, the higher the solvent content, the more significant the time needed to achieve the required temperature for extraction [18]. Our study showed that the highest TPC (195.64 ± 1.48 mg GAE/g extract) with an ethanol concentration of 85% and the solid-to-solvent ratio of 1:30. When the ethanol concentration was 95%, the content of phenolics was decreased may be caused by some polar compounds are insoluble in this mixture.

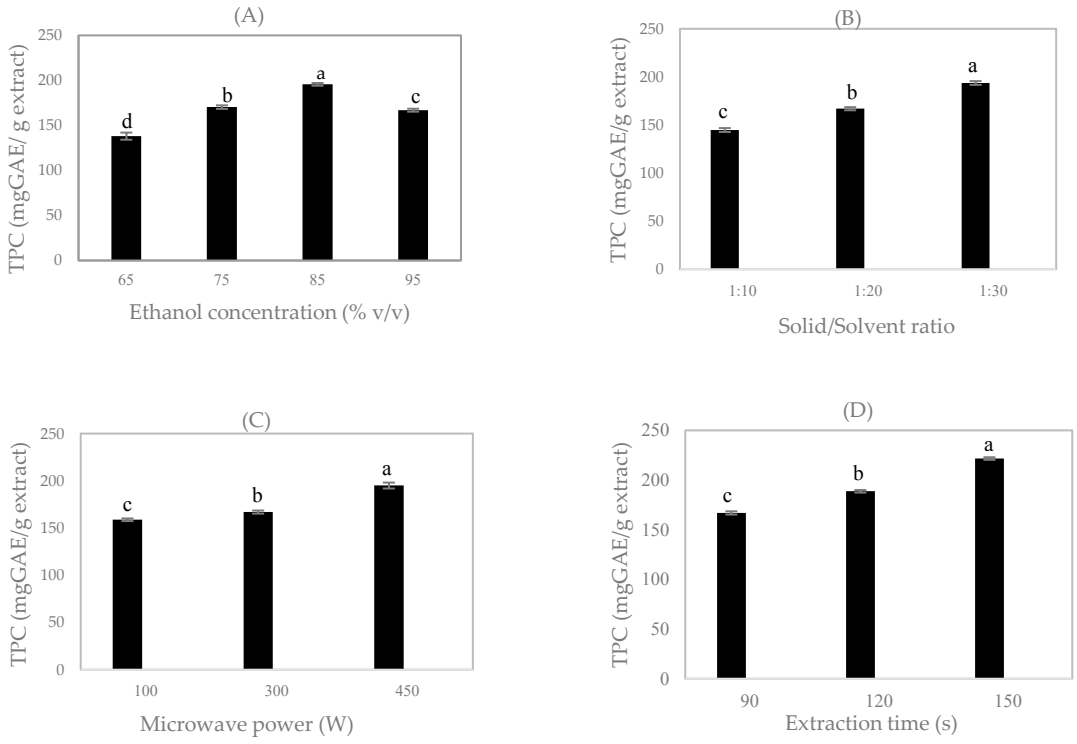

Figure 2. Influence of extraction parameters on the total phenolic content (TPC); (A): ethanol concentration, (B): solid/solvent ratio, (C): microwave power, (D): extraction time. The means presented by the various alphabets differ significantly at $p < 0.05$.

Table 1. The DPPH radical scavenging activity of phenolic-rich extract from *S. ovalifolia* root.

Extract	IC_{50} (μg/mL)
Phenolic-rich extract	6.31 ± 0.05
Quercetin (positive control)	6.79 ± 0.01

3.2 Chemical compositions and antioxidant activity of *S. ovalifolia* root extract

Chemical compositions of *S. ovalifolia* root extract were identified by liquid chromatography-mass spectrometry (LC-MS) analysis (Figure 3). The main component of *S. ovalifolia* root extract was flavonoids, including catechin, procyanidin B2, epicatechin, quercetin, quercetin glycosides (hyperoside, guaijaverin), diosmetin, morin, nepetin 7-O-glucoside, isorhamnetin 3-O-glucoside and orientin (Table 2). Because of these flavonoids (flavanols, flavonols, flavones, anthocyanidins), the phenolic-rich extract from *S. ovalifolia* root showed strong antioxidant activity with IC_{50} of 6.31 ± 0.05 μg/mL as same as quercetin (Table 1). Many reports showed that catechin, epicatechin, and quercetin have some biological activities, such as antioxidant, anticancer, antibacterial, antiviral, and anti-inflammatory activity [19-20]. Paneru and Rajbhandari showed the presence of carbohydrates, phenolics, and flavonoids in the methanolic *S. ovalifolia* leaf extract, which showed antimicrobial and antioxidant activities [21]. Moreover, Divya et al. reported that ethanol, as well as aqueous extracts of *S. ovalifolia* root, exhibited potent anti-inflammatory activity in Wistar albino rats because the root extract contained alkaloids, glycosides, flavonoids, and saponins [22].

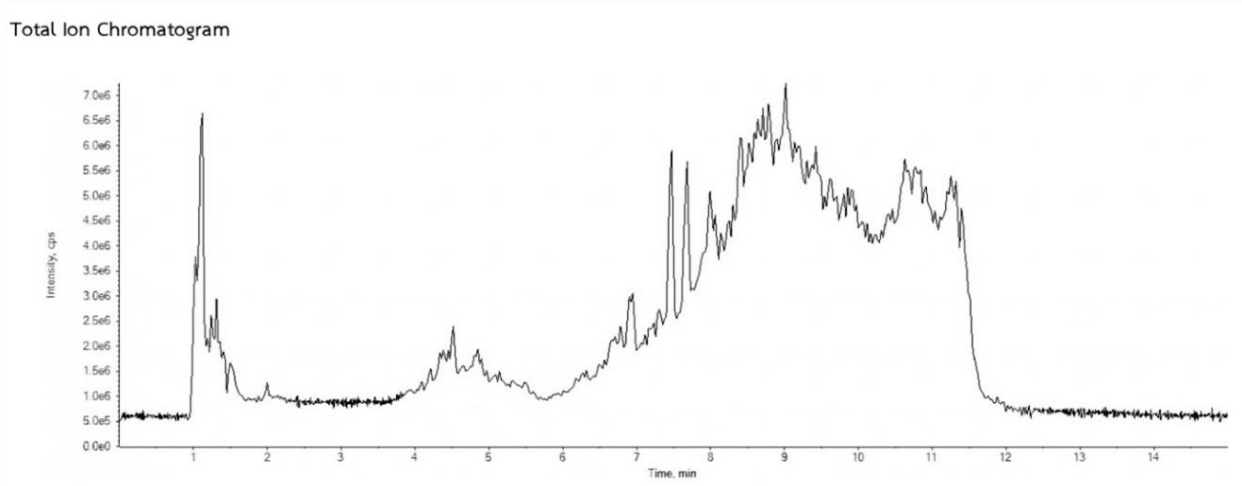

Figure 3. LC-MS chromatogram of *S. ovalifolia* root extract.

4. Conclusions

The results of this study indicated that the MAE was an efficient technique for phenolic compounds extraction from the root of *S. ovalifolia*. Optimal MAE conditions for extracting phenolic compounds were obtained with a microwave power of 450 W, an extraction time of 150 s, a solid-to-solvent ratio of 1:30 (g/mL), and an ethanol concentration of 85% (v/v). The LC-MS analysis revealed 18 bioactive components in *S. ovalifolia* root extract with catechin as the main compound. The major active compound from this plant was flavonoids. Results show that the root of *S. ovalifolia* has considerable potential as a source of natural bioactive components for healthcare products.

Table 2. Chemical compositions of *S. ovalifolia* root extract.

Compound Number	Retention time	Compound Name	Molecular formula	Molecular weight	Peak area (V)	Library Score
1	4.85	(-)-Catechin	$C_{15}H_{14}O_6$	291.08	3.194e+06	97.1
2	4.50	Procyanidin B2	$C_{30}H_{26}O_{12}$	579.13	2.112e+06	96.4
3	4.85	(-)-Epicatechin	$C_{15}H_{14}O_6$	291.08	1.506e+06	97.6
4	6.93	Quercetin	$C_{15}H_{10}O_7$	303.04	1.480e+06	97.5
5	6.93	Hyperoside	$C_{21}H_{20}O_{12}$	465.09	8.619e+05	97.6
7	1.31	Betaine	$C_5H_{11}NO_2$	118.08	2.596e+05	95.2
8	7.42	Diosmetin	$C_{16}H_{12}O_6$	301.06	2.582e+05	92.8
9	1.24	Stachydrine	$C_7H_{13}NO_2$	144.09	2.393e+05	96.7
10	7.83	Morin	$C_{15}H_{10}O_7$	303.04	2.163e+05	90.0
11	1.29	Salsolinol	$C_{10}H_{13}NO_2$	180.09	1.857e+05	93.1
12	6.65	Nepetin 7-glucoside	$C_{16}H_{12}O_7$	316.26	1.760e+05	95.2
13	7.14	Guaijaverin	$C_{20}H_{18}O_{11}$	435.08	1.522e+05	94.6
14	6.51	Emodin	$C_{15}H_{10}O_5$	271.05	1.203e+05	91.0
15	9.01	13-Keto-9z,11E-octadecadienoic acid	$C_{18}H_{30}O_3$	295.22	8.759e+04	93.7
16	1.54	Niacinamide	$C_6H_6N_2O$	123.05	2.904e+04	96.8
17	7.35	Isorhamnetin 3-O-glucoside	$C_{22}H_{22}O_{12}$	479.10	2.715e+04	97.5
18	6.40	Orientin	$C_{21}H_{20}O_{11}$	449.10	2.311e+04	96.4

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Conflicts of Interest: The authors declare no conflict of interest.

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