Research Article

Composition of phenolic compounds and antibacterial activities of *Paederia pilifera* Hook. f. leaf extract

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Abstract

In this study, phenolic compounds were extracted from *Paederia pilifera* Hook. f. leaves. The phenolic compositions of crude extract were determined by Liquid Chromatography - Mass Spectrometry (LC-MS). Phenolic acids and flavonoids were identified as the major phenolic compound compositions in the crude extract. Furthermore, crude extract was performed to evaluate the antibacterial activities against 3 human pathogenic strains: *Staphylococcus aureus* DMST 8840, *Staphylococcus epidermidis* DMST 15505 and Methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651. The results revealed that *P. pilifera* crude extract exhibited antibacterial activities against all tested bacterial strains with a minimal inhibitory concentration (MIC) value of 25.60 mg/mL and minimal bactericidal concentration (MBC) value of 51.20 mg/mL for *S. aureus* and MRSA, and 25.60 mg/mL for *S. epidermidis*.

Keywords: Antibacterial activity, Flavonoids, *Paederia pilifera*, Phenolic compounds

Introduction

Recently, plant-derived phenolic compounds have received considerable interest due to their beneficial health effects. Numerous studies have been carried out on the biological activities and applications of plant phenolics in replacing the use of synthetic chemicals [1-3]. This is because plant phenolics possess various pharmacological properties. They are also safer and milder than the synthetic chemicals used for health promotion. Further, they are not associated with side effects and have greater acceptance for use as antimicrobial agents against antibiotic-resistant bacteria [4]. Plant phenolics are the major secondary metabolites produced by plants, consisting of structurally heterogeneous groups ranging from simple phenolic acids, flavonoids, and coumarins, to more complex structures such as tannins. They are the main groups found in natural products and exhibit diverse bioactivities such as antioxidants, radical scavenging potentials, and antibacterial activities, etc. At present, these compounds are widely used in the food, pharmaceutical, and cosmetics industries.

Paederia pilifera Hook. f., belonging to the family Rubiaceae, is a perennial climbing shrub. This plant can be found in deciduous forests and tropical rainforests throughout India and Southeast Asia, including Thailand. In Thai herbal pharmacopoeias,

this medicinal plant is used for the treatment of gastrointestinal disorders including diarrhoea, food poisoning, dyspepsia, gastritis, jaundice, and hyperbilirubinemia [5]. It has been reported that the ethanolic extract of *P. pilifera* leaves have the highest total phenolic content of 12.99 mg GAE/100g DW and show significant scavenging activities compared to the reference antioxidants [6].

This research aimed to extract the phenolic bioactive compounds from *P. pilifera* leaves by ultrasound-assisted solvent extraction. The chemical compositions of the crude extract were analysed using a Liquid Chromatography-Electrospray Ionisation-Mass Spectrometer (LC-ESI-MS). Antibacterial activities against 3 human pathogenic bacterial strains were also investigated.

Materials and method

Chemicals

Muller Hilton agar and Muller Hilton broth were provided by Himedia (India). Analytical grade of acetonitrile, dimethyl sulfoxide (DMSO), ethanol and glacial acetic acid were obtained from Carlo Erba (France). Antibiotic erythromycin, resazurin, chlorogenic acid and rutin were purchased from Sigma-Aldrich (USA).

Raw material

Paederia pilifera Hook. f. leaves were collected from a plantation in Chom Bueng District, Ratchaburi Province, Thailand. The leaf sample was cleaned, cut into smaller pieces, and then oven-dried at 40 ºC until the moisture content of the sample was less than 10%. The dried sample was further ground and stored in an airtight container for further analysis.

Phenolics extraction by UAE

Phenolic compounds were extracted from *P. pilifera* leaf powder using an ultrasonic cleaning bath (Bandelin sonorex digitec, DT 510 H, 35 kHz, 16 W). The sample was extracted with 70% (aq) ethanol using the ratio for solvent-to-sample of 100 (v/w). UAE was conducted at 50 °C for 60 minutes according to the method recommended by Siramon & Wongsheree (2019) [6]. The mixture was then filtered through filter paper and the filtrate evaporated to dryness under vacuum on a rotary evaporator.

Analysis of the chemical composition of crude extract by LC-ESI-MS

The chemical compositions of *P. pilifera* crude extract were analysed by a Liquid Chromatography-Electrospray Ionisation-Mass Spectrometer (LC-ESI-MS) (Agilent Technologies 6420 Triple Quad) in a negative ionisation mode. A ZORBAX Eclipse Plus C18 analytical column (4.6×100 mm, 3.5µm; Agilent) was used for LC separation. Solvent gradient HPLC analysis was applied using the modified method of Lee et al. (2008) [7]. The mobile phase consisted of solvents A and B. Solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. The solvent flow rate was 0.5 mL/min, and the detector was a photodiode array (PDA) set at 254 nm and 280 nm. The injection volume was 20 µL of the sample. The linear gradient of HPLC solvent was as follows: B was increased from 8 to 10% for 2 min, then from 10 to 30% for 25 min, from 30 to 90% for 23 min, from 90 to 100% for 10 min, and kept at 100% for 5 min, before being returned to the initiation state. Quercetin-3-O-rutinoside (Rutin) and Chlorogenic acid were used as the authentic standards to confirm the fragmentation patterns of the sample. The full mass spectra were recorded in the 100-1,500 *m*/*z* range.

Antibacterial activity evaluation

Preparation of extract solution

The extract solution in dimethyl sulfoxide (DMSO) at the concentration of 204.80 mg/mL was prepared. The extract solution was sterilised by passing through a 0.45 µm membrane filter.

Microbial strains

The three human pathogenic bacterial strains used in this study were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand, namely *Staphylococcus aureus* DMST 8840, *Staphylococcus epidermidis* DMST 15505, and Methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651. The bacterial strains were grown and maintained on nutrient agar slant at 37 ºC for 24 hours. The inoculum size of each test strain was 10^8 bacteria/mL.

Determination of MIC and MBC values

The minimum inhibitory concentration (MIC) of the extracts was determined according to the method of Rahman et al. (2004) [8] using the two-fold serial microdilution method. The tested extracts were added to a sterile Mueller Hinton broth and put onto microtiter plates before the diluted bacterial suspension was added. Each extract was assayed in triplicate. The bacterial suspensions were used as the positive control, while the extracts in broth were used as the negative control. The minimum bactericidal concentration (MBC) was determined according to Basri & Fan (2005) [9] by a subculture of the well showing no apparent growth in a sterile agar plate. The lowest concentration showing no visible growth on agar subculture was taken as the MBC value. Antibiotic erythromycin was used as the standard.

Results and discussion

Identification of chemical compositions

The total ion chromatogram of the *P. pilifera* crude leaf extract is shown in Figure 1. The analysis of mass spectra in negative ionisation mode is shown in Figure 2 (a-e). Five peaks were identified as follows:

Peak 1 (Fig. 2a) was identified as Chlorogenic acid ($C_{16}H_{18}O_9$, molecular weight 354) showing $[M - H]^-$ ion of *m*/*z* 352.8, which yielded a fragment at *m*/*z* 190.8 (deprotonated Quinic acid) [10]. Chlorogenic acid dimer was also found at *m*/*z* 706.8.

Peak 2 (Fig. 2b) was identified as Quercetin hexose malic acid derivatives (molecular weight 742) showing $[M - H]^-$ ion of *m*/*z* 740.8 (neutral loss of a hexose-malic acid moiety: 278 amu), and found the product ion of Quercetin-3-O-glucoside at *m*/*z* 462.9 [11-14].

Peak 3 (Fig. 2c) was identified as Quercetin-3-O-rutinoside (Rutin) ($C_{27}H_{30}O_{16}$, molecular weight 610) showing $[M - H]^-$ at *m*/*z* 608.8 (loss of a Rhamnose moiety: 146 amu) [15,16]

Peak 4 (Fig. 2d) was identified as Chlorogenic acid derivatives (molecular weight 452) showing $[M - H]^-$ at *m*/*z* 450.9, which yielded a fragment at *m*/*z* 352.6 (Chlorogenic acid) [11]

Peak.5 (Fig. 2e) was identified as Tri-caffeoylquinic acid (molecular weight 712) showing $[M - H]^-$ at *m*/*z* 711, which yielded a fragment at *m*/*z* 676.9 [17,18]

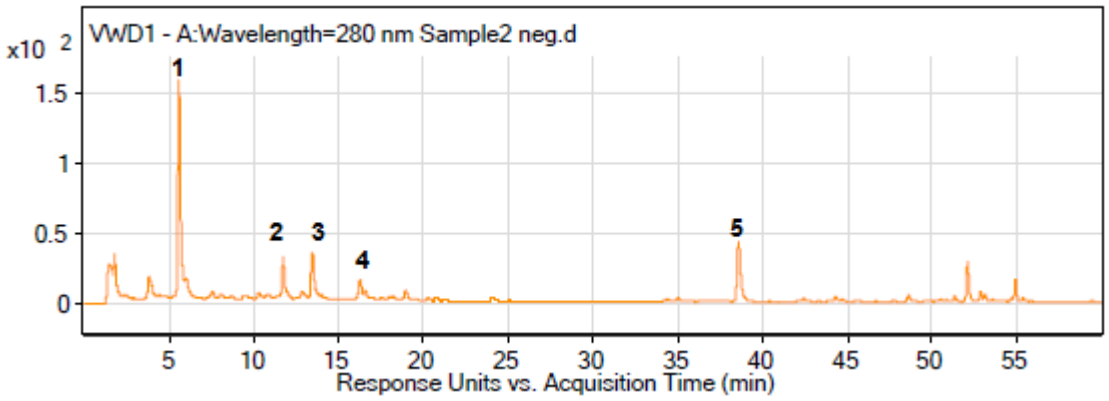
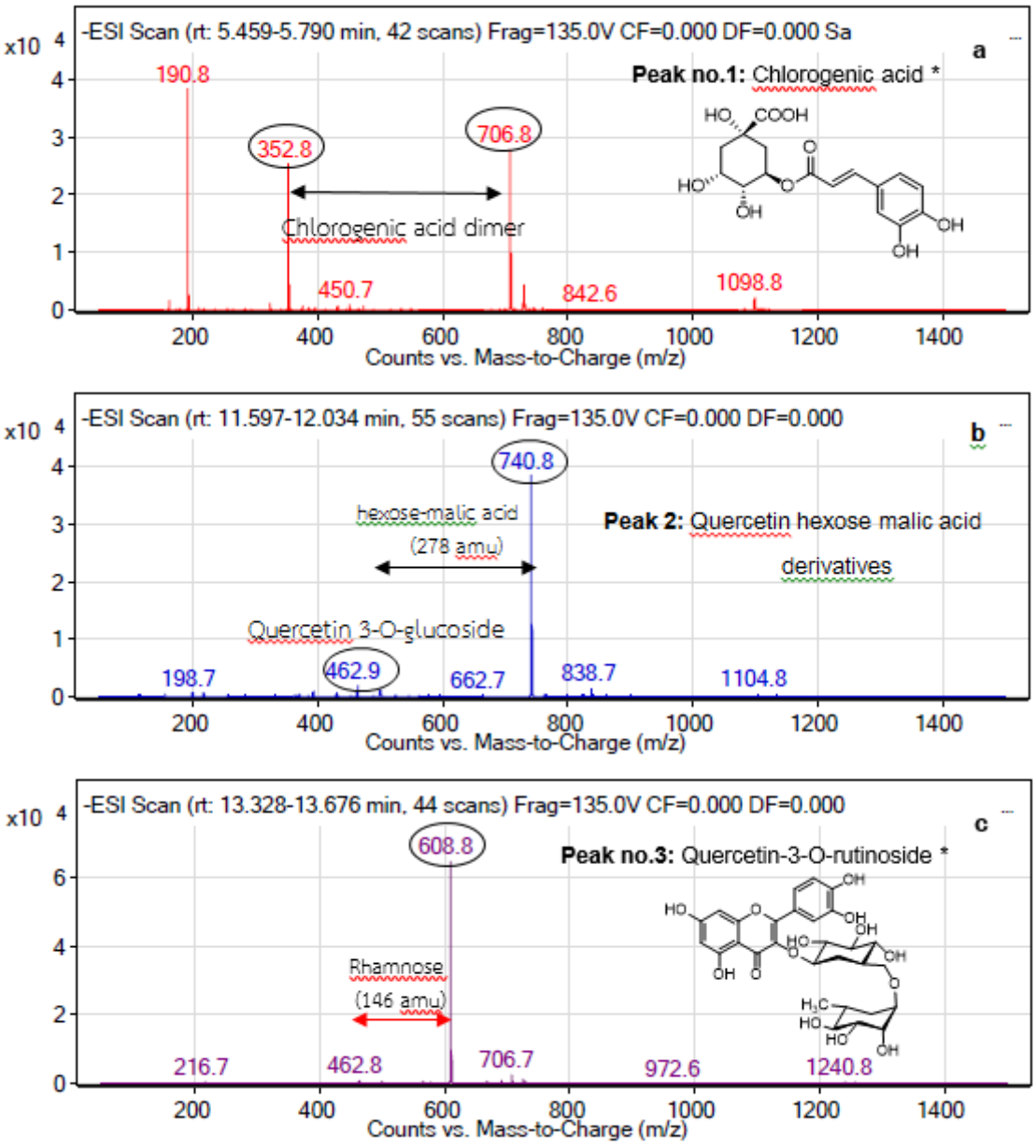


Figure 1. Total ion chromatogram of the *P. pilifera* crude leaf extract



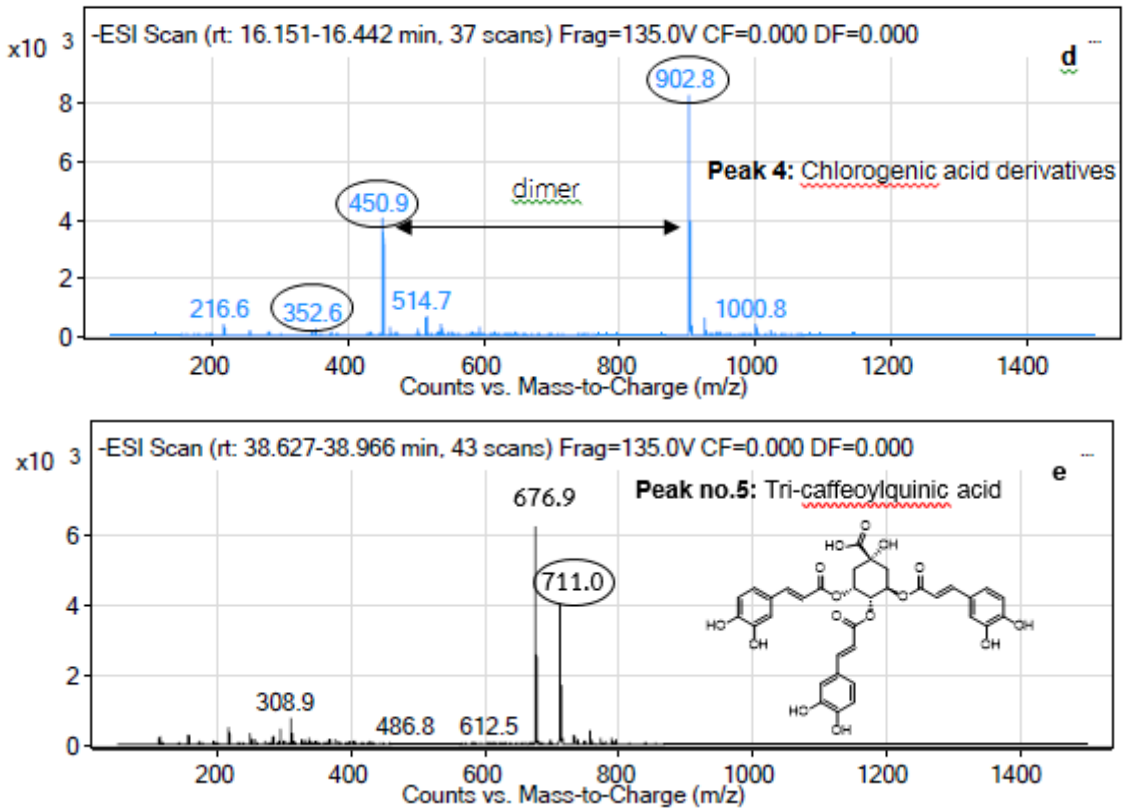


Figure 2. Mass spectra of the identified peaks in the *P. pilifera* crude leaf extract

Note: *Identity confirmation with authentic standards

Determination of antimicrobial activities

The antibacterial activities of the crude extract were tested against 3 human pathogenic strains: *S. aureus* DMST 8840, *S. epidermidis* DMST 15505, and MRSA DMST 20651. These test strains involved gram-positive bacteria, which cause dermal infections and can pose severe clinical as well as public health concerns. MRSA is a *S. aureus* that is resistant to Methicillin and ß-lactams [19]. The tested results in Table 1 show that *P. pilifera* crude leaf extract exhibited antibacterial activities against all tested bacterial strains with a minimal inhibitory concentration (MIC) value of 25.60 mg/mL and minimal bactericidal concentration (MBC) value of 51.20 mg/mL for *S. aureus* and MRSA, and 25.60 mg/mL for *S. epidermidis*.

Table 1 Minimal inhibitory concentration (MIC, mg/mL) and minimal bactericidal concentration (MBC, mg/mL) for the crude extract and standard Erythromycin

Bacterial strains	***S. aureus***		***S. aureus* (MRSA)**		***S. epidermidis***	
Concentration	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Crude extract	25.60	51.20	25.60	51.20	25.60	25.60
Erythromycin	0.04	0.04	0	0	0.02	0.02

Note: 0 = no inhibition

These biological properties were related to the presence of phenolic bioactive compounds, namely Chlorogenic acid, Quercetin hexose malic acid derivatives, Rutin, Chlorogenic acid derivatives and Tri-caffeoylquinic acid. It has been reported that the antibacterial action of Quercetin hexose malic acid derivatives against *S. aureus* was mainly due to the inhibition of D-Ala-D-Ala ligase activity, thus interfering with bacterial cell wall growth [20]. Rutin was reported to exert antibacterial activity by the inhibition of DNA isomerase IV [21], and the hydroxyl group on its structure exerted rapid bactericidal action by penetrating into the lipid bilayer of the membrane, resulting in membrane damage, leakage of intracellular compounds, and protein coagulation [22]. Normally, bacteria use membrane-bound efflux transporters to remove cytotoxic compounds or drugs as a mechanism of drug resistance. The efflux pump systems reduced the intracellular concentrations of antimicrobial drugs to make bacteria more resistant and hard to treat. In addition, Chlorogenic acid and Tri-caffeoylquinic acid have been reported for their role as efflux pump inhibitors (EPI) in the major facilitator super family (MFS) of the drug resistant bacterium *S. aureus* [23,24]. In this study, *P. pilifera* leaf extract was found to exert multiple antibacterial functions and exhibit antibacterial activities against 3 human pathogenic strains including *S. aureus*, *S. epidermidis*, and drug-resistant MRSA. Therefore, *P. pilifera* leaf extract is an attractive alternative to antibiotics that could be useful in the treatment of infections caused by drug-resistant bacteria.

Conclusions

From the analysis of the chemical compositions of *P. pilifera* leaf extract, it was revealed that phenolic compounds including (1) phenolic acids and their derivatives (major group): Chlorogenic acid, Chlorogenic acid derivatives, Tri-caffeoylquinic acid, and (2) flavonoids: Quercetin hexose malic acid derivatives, Rutin were detected. The crude extract also exhibited antibacterial activity against all tested bacterial strains with a minimal inhibitory concentration (MIC) value of 25.60 mg/mL and a minimal bactericidal concentration (MBC) value of 51.20 mg/mL for *S. aureus* and MRSA, and 25.60 mg/mL for *S. epidermidis*. From the test results, it could be concluded that *P. pilifera* leaves, a natural source of phenolics, exhibited significant antibacterial properties, making them an interesting alternative antibacterial agent applied in various healthcare products.

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