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Antibacterial Effect of Leaf Extract from *Lithocarpus falconeri* (Kurz) Rehder on Gram-Negative Bacteria

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Abstract— Plants have been the most important sources of leading antimicrobials and are utilized in traditional medicine. The present study focused on exploring the antimicrobial properties and total phenolic contents from *Lithocarpus falconeri* (Kurz) Rehder leaves. Antibacterial activity of the 95% ethanol extract was investigated using the disk diffusion method and the minimal inhibitory concentration (MIC) was determined by the broth dilution technique. Total phenolic compounds were evaluated using the Folin-Ciocalteu colorimetric method. The result suggested that the extract had a strong antibacterial potential against *Escherichia coli* and *Salmonella* spp. but it had shown no activity against *Staphylococcus aureus.* The average inhibition zones of *E. coli* and *Salmonella* spp. were 24.83 ± 0.68 and 24.33 ± 0.52 mm, respectively. The minimal inhibitory concentration (MIC) values of the extract were 312.5 and 625 mg/mL, respectively. In addition, the extract showed a high content of total phenolic compounds which contained 257.31 ± 1.55 mg gallic acid equivalent/g extract. This result indicates that it is possible to use the extract as a natural antibacterial agent in the future.

Keywords: *Lithocarpus*; Fagaceae; Antimicrobial, Extract; Traditional medicine

INTRODUCTION

Plants are well-known sources of bioactive natural products called secondary metabolites. The amount and diversity of chemical nature and metabolites composition can vary among plant species [1, 2, 3]. Secondary metabolites are synthesized from primary metabolites including small molecules such as carbohydrates, lipids, proteins, amino acids, and nucleosides that are produced by higher plants [4]. The major function of secondary metabolites plays an important role in plants' protection from predators and microbial pathogens [4, 5]. They are interesting for pharmaceuticals, antibacterial or antifungal agents, anticancer drugs, herbicides, diagnostics, and tools for research [3, 4, 6]. There are three major classes of secondary metabolites in plants, consisting of terpenoids, phenolic compounds, nitrogen-containing compounds or sulfur-containing compounds [4, 5]. These phytochemicals are produced through several metabolic pathways including glycolysis, Krebs cycle, pentose phosphate pathway, aliphatic and aromatic amino acids, and shikimate pathway [7]. Nowadays, antibiotic resistance is a *growing problem and rising to be dangerous to health.* For a long time, Medicinal plants have been a valuable source of natural products used for therapeutic purposes [8].

Lithocarpus falconeri (Kurz) Rehder is one of the *Lithocarpus* genera in the family Fagaceae. The *Lithocarpus* is the second largest genus, with approximately 341 species. There are approximately 57 species reported in Thailand. The genus *Lithocarpus* is commonly known as Stone Oaks. The geographic distribution of all species within the genus are widely distributed throughout the East to Southeast Asia. *Lithocarpus* plants were reported to have anti-tumor, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic and antioxidant activities. Recent studies indicate that *Lithocarpus* plants are rich in a variety of bioactive compounds, including triterpenes, steroids, chalcones, and various phenolic compounds. The objective of this present study was to determine the antimicrobial potential of ethanol extracts obtained from *L. falconeri* (Kurz) Rehder leaves against pathogenic bacteria. This is the first study to report the antibacterial properties of the extract from *L. falconeri* (Kurz) Rehder leaves.

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MATERIALS AND METHODS

Materials

Chemicals Folin-Ciocalteu reagent and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Mueller-Hinton broth, Mueller-Hinton agar and nutrient broth were purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

Methods

Preparation of plant extracts

Fresh leaves of *Lithocarpus falconeri* (Kurz) Rehder were collected from Phuket province, Thailand. The leaves were washed and air-dried for 7 days. After drying, air-dried plant leaves were ground into powder and immersed in 95% ethanol for 5 days. The extract was filtered with Whatman No.1 filter papers before the solvent was evaporated by rotary evaporator under a partial vacuum at 40 °C. The crude ethanol extract was kept at -20°C for future analysis.

Antibacterial activity assay

The antibacterial activity of the extract was evaluated by a disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Briefly, three pathogenic bacterial samples, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. were grown in nutrient broth at 35°C for 18 h. Each bacterial suspension was adjusted for turbidity to achieve a turbidity of 0.5 McFarland standard. A sterile cotton swab was dipped into bacterial suspension and pressed the swab against the wall of the tube to remove excess inoculum, and then the swab was spread on the surface of Mueller-Hinton Agar (MHA) plates. Sterile filter paper discs (6 mm in diameter) impregnated with 50 µL of extract in 10 % DMSO (100 mg/mL) were placed on the surface of each inoculated MHA plate. After incubation at 35° C for 24 h, the inhibition zone was measured in mm. Ampicillin (10 mg) was used as positive control, while 10 % DMSO was used as negative control.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was performed following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The potential extract was determined by a two-fold serial broth dilution technique. About 100 mg of the extract was dissolved in 10 % DMSO and two-fold serially diluted with Mueller-Hinton broth to a concentration ranging from 9.76-5,000 mg/mL. Then, a diluted series of the extract was added to the test tube. The tested bacterial suspensions with 100 mL of 0.5 McFarland standard were transferred into each tube. After 24 h of incubation at 35°C, the lowest concentration that showed no visible growth was considered the MIC. All the tests were carried out in triplicates.

Determination of Total Phenolic Content

Total Phenolic Content of *L. falconeri* (Kurz) Rehder extract was analyzed by using the Folin-Ciocalteu reagent method described previously with some modification. The extract (100 mL) was mixed with 10% Folin-ciocalteu reagent and 7.5% Na_2CO_3 was added to the reaction mixture. After 45 min, the absorbance of the mixture was measured at 765 nm. The total phenolic content of the extract was calculated using the linear equation that was obtained based on the standard gallic acid calibration curve and expressed as mg of gallic acid equivalents per g of dry weight of the extract.

RESULTS AND DISCUSSION

Antibacterial activity of *Lithocarpus falconeri* (Kurz) Rehder 95% ethanol extract was determined by using a disk diffusion method. The disk diffusion method is the most common technique used to evaluate the antimicrobial activity of plant extracts. The extract was tested against some pathogenic bacterial strains, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. The result indicated that the extract had a strong effect on the growth of *E. coli* and *Salmonella* spp. (Fig. 1A and 1B) with the inhibition zones of 24.83 ± 0.68 and 24.33 ± 0.52 mm, respectively (Table 1). However, no antibacterial effect was observed against *S. aureus* (Fig. 1C). Ampicillin, used as a positive control, exhibited inhibitory activity against *E. coli*, *S. aureus* and *Salmonella* spp. (Fig. 1) with inhibition zones of 22.33 ± 0.52, 22.17 ± 0.68 and 20.83 ± 0.93 mm, respectively (Table 1). The negative control of 10% DMSO showed no inhibitory effect against all tested bacterial strains (Fig. 1).

In determining the minimal inhibitory concentration (MIC) of the extract, the strongest antibacterial activity of the extract was found against *E. coli* with an MIC value of 312.5 g/mL followed by *Salmonella* spp. with an MIC value of 625 g/mL (Table 1). Therefore, the extract could be more effective against *E. coli* than *Salmonella* spp.

The total phenolic content of *L. falconeri* (Kurz) Rehder extract was carried out using the Folin-Ciocalteu assay. The concentration of total phenolic content represented in milligram of gallic acid equivalent per g of the extract (mg GAE/g of extract), using the following equation of the linear regression of the calibration curve of gallic acid ($y = 0.0088x - 0.0111$, $R^2 = 0.9992$) (Fig. 2). The result showed that the concentration of total phenolic content of the extract was 257.31 ± 1.55 mg gallic/mL extract. In the present study, the potential of antibacterial of the 95% ethanol extract against gram-negative bacteria including *E. coli* and *Salmonella* spp. with high activity. When compared with previous report, the 95% ethanol extract of *L. falconeri* (Kurz) Rehder was more effective against *E. coli* and *Salmonella* than n-hexane, dichloromethane and ethyl acetate

leave extract of *Calpurnia aurea* (Ait.) Benth. The difference in the inhibitory effect may be due to the polarity of solvent and attributed to differences of bioactive compounds among species, which may have antibacterial activity. Moreover, the 95% ethanol extract of *L. falconeri* (Kurz) Rehder showed a high content of total phenolic compounds, which is probably associated with the better antimicrobial activity shown by the extract. This finding is in agreement with previously reported results concerning the inhibition effect of phenolic compounds against *E. coli* and *Salmonella*. The antimicrobial properties of phenolic compounds depended on their chemical structures, hydroxyl phenol groups, which shown to be important for antimicrobial activity through various mechanisms such as destruction of bacterial cell membrane and inhibiting nucleic acid or specific enzyme synthesis. Moreover, inhibitory effects of the extract may be involved in damage or structural change of the outer membrane of gram-negative bacteria, leading to loss of permeability of the outer membrane. However, the bioactive compounds of the extract should be isolated and purified to clarify the antibacterial properties for pharmaceutical applications.

Table 1. Antimicrobial activity and Minimum Inhibitory Concentration (MIC) of plant extracts against microorganisms.

Sample Tests	**Inhibition zone (mm)**			**MIC (mg/mL)**		
	E. coli	***S. aureus***	***Salmonella* spp.**	***E. coli***	***S. aureus***	***Salmonella* spp.**
L. falconeri	24.83 ± 0.68	-	24.33 ± 0.52	312.5	ND	625
Ampicillin	22.33 ± 0.52	22.17 ± 0.68	20.83 ± 0.93	ND	ND	ND

- : No inhibition zone; ND: Not Detected

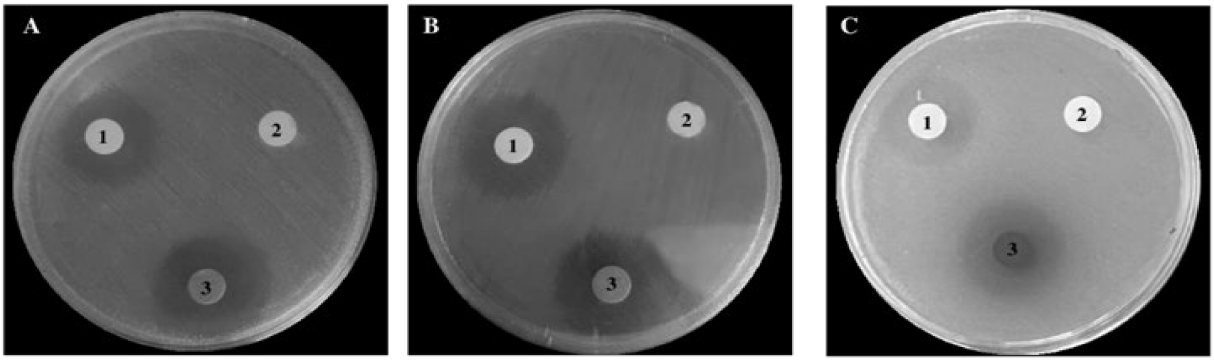

Fig. 1. Inhibition zone (mm) of *Lithocarpus falconeri* (Kurz) Rehder extract against *Escherichia coli* (A), *Salmonella spp.* (B) and *Staphylococcus aureus* (C), at the concentration of 50 mg/mL. Number 1 represents the positive control (ampicillin), number 2 represents negative control (10% DMSO), and number 3 represents the extract.

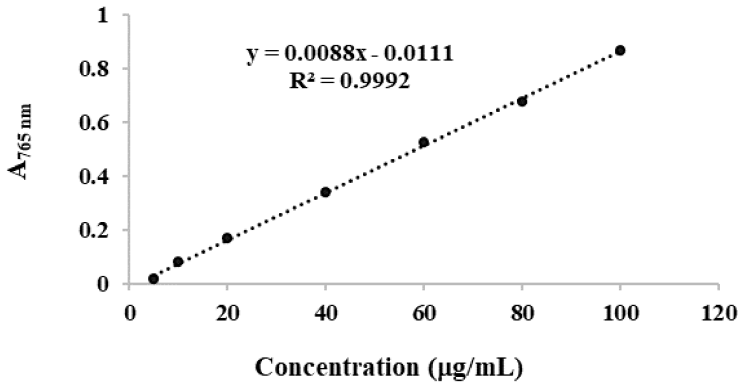

Fig. 2. Standard curve of gallic acid for total phenol content assay.

CONCLUSIONS

This study showed that the *Lithocarpus falconeri* (Kurz) Rehder ethanol extract had a strong antibacterial ability against *E. coli* and *Salmonella* spp. Additionally, the extract contained a high amount of total phenolic content. The findings of this study could be important for further isolation and purification to elucidate the bioactive compounds that are responsible for antibacterial activity and possible applications for natural antibacterial agents.

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