ANTIBACTERIAL ACTIVITIES OF RAMBUTAN PEEL EXTRACT

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ABSTRACT: The antimicrobial activities of the phenolic component containing rambutan (*Nephelium lappaceum* L.) extract are determined. The extract of rambutan pericarp possesses bactericidal activity against *Staphylococcus aureus* ATCC6538, methicillin-resistant *Staphylococcus aureus* (MRSA) DMST20645, and *Streptococcus mutans* ATCC25175T whilst no activity was found on gram negative bacteria *Escherichia coli* ATCC25922 and fungus *Candida albicans* ATCC10231 by agar disc diffusion assay. Due the rambutan pericarp extract displayed antimicrobial activity against *S. aureus* ATCC6538 and MRSA DMST20645 at diameter of the inhibition zone > 10 mm, tests of the minimal inhibitory concentration (MIC) of rambutan pericarp extract against *S. aureus* ATCC6538 and MRSA DMST20645 were further determined by broth microdilution assay resulting in 2 and 0.4 mg/ml, respectively.

Keywords: antimicrobial, rambutan, tannins, phenolic components

INTRODUCTION

Infections caused by bacteria resisting multiple antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly found worldwide in hospitals [1] and leads to the discovery of novel antimicrobial agents. Natural products are the sources of biologically active compounds including antimicrobial agents [2]. Reports of antimicrobial activity from many kinds of fruit pericarps were available [3]. One is rambutan pericarp extract which exhibited many interesting biological activities such as antioxidation and antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* which derived from polyphenolic component found in its pericarp [3]. The antibacterial activity against MRSA of polyphenolic compounds has been reported [4, 5]. Therefore, our interest is stressing on exploring the antimicrobial activity of rambutan pericarp extract.

MATERIALS AND METHODS

Plant materials

The fruits of *N. lappaceum* L., plant in the family Sapindaceae, were collected from Rayong Province, Thailand during June-July, 2007.

Extraction

The pulverized dried peels of *N. lappaceum* L. (950.40 g) were successively extracted with methanol (3×10 L). The filtrates were pooled and evaporated under reduced pressure at the temperature not exceeding 40°C to give the methanol extract (153.21 g, 16.12 % w/w dry weight of plant material).

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Antimicrobial assay

Test organisms

The bacterial strains used were the gram positive bacteria, *Staphylococcus aureus* ATCC6538, methicillin-resistant *Staphylococcus aureus* (MRSA) DMST20645, and *Streptococcus mutans* ATCC25175^T, and the gram negative bacteria, *Escherichia coli* ATCC25922. The fungus used was *Candida albicans* ATCC10231. All of the pathogens were obtained from Thailand National Institutes of Health.

All bacteria were cultured in Tryptic soy broth or agar at 37°C for 24 h prior to use while the fungus was cultured on Sabouraud's dextrose broth at 37°C prior to use.

Chemicals

Tryptic soy broth, Tryptic soy agar, Sabouraud's dextrose broth, and Sabouraud's dextrose agar were purchased from Difco (USA). Amoxicillin and clotrimazole were purchased from Sigma Chemical CO (USA). Dimethylsulfoxide (DMSO) and methanol analytical grade were purchased from Merck (Germany). Folin-Ciocalteu and reagent and aluminium trichloride hydrate were purchased from Carlo-Erba (Italy). Gallic acid, quercetin dihydrate, and sodium carbonate anhydrous were purchased from Fluka (Switzerland).

Agar disc diffusion method

The antimicrobial assay was performed by the agar disc diffusion method as described in Tadtong [6]. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition against the test microorganisms. Three independent experiments were performed and each experiment was run in triplicate.

Microdilution method

The minimal inhibitory concentration (MIC) values were determined only for the microbial strains being sensitive to the extracts in disc diffusion assay by broth microdilution method as described in Tadtong [6]. The extract was dissolved in 2% DMSO in appropriated medium and then diluted to make the stock solutions at concentration of 4, 0.8, 0.16, 0.032, and 0.0064 mg/ml, respectively. Microbial growth was determined by turbidity being observed under daylight. The lowest concentration exhibiting no turbidity is the MIC. Three independent experiments were performed and each experiment was run in triplicate.

The minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) values were determined by applying 20 µl of the mixture of extract, pathogen, and culture medium which was the MIC and the higher concentration(s) into the 180 µl Tryptic soy broth or Sabouraud's dextrose broth. The 96-well plate was incubated at 37°C for 24 h. Microbial growth was determined by turbidity being observed under daylight. The lowest concentration that showed no turbidity is the MBC or MFC. Three independent experiments were performed and each experiment was run in triplicate.

Analysis of total phenolics content

The total phenolics content was performed by a modified Folin-Ciocalteu method of Miliauskas [7]. Mix 1 ml of sample solution with 1 ml each of Folin-Ciocalteu reagent and (7.5% w/v) sodium carbonate, then diluted with methanol to 10 ml, shaken vigorously, and kept in dark for 90 min. For the standard curve of gallic acid solutions were prepared under the same condition. Absorption of reaction and a prepared blank were measured at 725 nm using spectrophotometer (UV-Vis model 1601, Shimadzu). Each assay was performed in tripicate. The total phenolic was present in gallic acid equivalent in milligrams per gram (mg/g) dry plant extract.

Analysis of total flavonoids content

The total flavonoids content was performed by colorimetric method reported by Miliauskas [7]. One ml of plant extract in methanol was mixed with 1 ml (2.0% w/v) aluminium trichloride and diluted with methanol to 25 ml. The standard quercetin solutions were prepared under the same condition. The absorption at 415 nm was measured after kept in dark for 40 min by using spectrophotometer (UV-Vis model 1601, Shimadzu). Each assay was performed in tripicate. The total flavonoid in plant extract was present in quercetin equivalent in milligrams per gram (mg/g) dry plant extract.

RESULTS AND DISCUSSION: Screening for antimicrobial activity of rambutan pericarp extract by agar disc diffusion method revealed that the rambutan pericarp extract displayed antimicrobial activity against *S. aureus* ATCC6538, MRSA DMST20645, and *S. mutans* ATCC25175^T whilst no activity was found on gram negative bacteria *E. coli* ATCC25922 and fungus *C. albicans* ATCC10231 as shown in Table 1. The rambutan pericarp extract displayed antibacterial activity against *S. mutans* at < 10 mm in diameter of inhibition zone, therefore, the extract was further investigated for MIC and MBC against *S. aureus* and MRSA.

Table 1 Antimicrobial activity of rambutan pericarp extract determined by agar disc diffusion method

Pathogens	Inhibition zone (mm) ± SD
S. aureus ATCC6538	20.2 ± 0.12
MRSA DMST20645	19.2 ± 0.11
S. mutans ATCC25175^T	8.5 ± 0.07
E. coli ATCC25922	-
C. albicans ATCC10231	-

ND= not determined

The rambutan pericarp extract exhibited MIC against *S. aureus* and MRSA at concentration of 2 and 0.4 mg/ml, respectively and the MBC against *S. aureus* and MRSA at the same concentration of 2 mg/ml.

In 2008, Thitilertdecha reported that rambutan pericarp contained high amount of phenolic components and reported that methanolic rambutan pericarp extract displayed antibacterial activity against gram positive bacteria, *S. epidermidis*, *S. aureus*, *Enterococcus faecalis*, and gram negative bacteria *Pseudomonas aeruginosa* and *Vibrio cholera* [3]. Thus, total phenolic contents of our rambutan pericarp extract was evaluated. The amount of total phenolic component was found at 10.7% w/w of the extract equivalent to gallic acid.

Flavonoids and tannins are the remarkable biologically active members of phenolic compounds [4]. Flavonoid exhibited variety of biological activities including antimicrobial activity [4]. However, the flavonoid content in the methanol extract of rambutan pericarp was found only at 1.9% w/w equivalent to quercetin.

Thus, it can be deduced that the antimicrobial activity of rambutan pericarp extract was a result of phenolic compounds contained in the extract as reported by Thitilertdecha [3]. Recently, Thitilertdecha reported that the phenolic components found in the rambutan pericarp are ellagic acid, corilagin, and geraniin [3, 8]. Therefore, it can be concluded that the antimicrobial activity of the extract should be the effect of these ellagitannins.

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