ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimicrobial Studies of *Sonneratia caseolaris* Using Different Agar Diffusion Method

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ABSTRACT

Sonneratia caseolaris (cork tree or berembang) is one of the lead plants of mangrove forest that can be generally found in Asia such as Malaysia, Philippines and also Thailand. Cork tree is used in varies of applications including corks or floats for fishing nets, food and medicine. Antioxidant activity of the cork tree extract has been reported previously. The active substances that found in cork tree are phenolic compound such as gallic acid and two flavoniods luteolin and luteolin 7-O-β-glycoside. Moreover some previous studies were reported about its antimicrobial activities which are interesting. Three different agar-based assays (spread plate cup diffusion, swab plate cup diffusion and pour plate cup diffusion) were used in this study. The methanolic cork tree seed extract and gallic acid were tested. The results showed that this extract could inhibit *Staphylococcus aureus* and *Candida albicans* but could not inhibit *Escherichia coli* whereas gallic acid showed the activity against only *S. aureus*. All methods exhibited the results of antimicrobial activity were not different. Subsequently the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid were tested for antibacterial activity using swab plate cub diffusion method against three species (*Streptococcus mutans*, *Propionibacterium acnes* and anaerobic bacteria). All tested extracts exhibited antibacterial activity against only *P. acnes* while gallic acid had the antibacterial activity against *S. mutans*, *P. acnes* and anaerobic bacteria.

Keywords: Antimicrobial activity, Agar diffusion, *Sonneratia caseolaris*, Gallic acid

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ISSN: 0975-8585

INTRODUCTION

Sonneratia caseolaris (cork tree or berembang) is a mangrove plant in family Sonneratiaceae [1,2]. It can be found in Malaysia, Indonesia, Philippines, Singapore, Sri Lanka, Viet Nam and Thailand [3]. It is used in varies of applications including corks or floats for fishing nets, food and medicine [4]. It is traditionally used as an astringent and antiseptic [5]. The fermented juices of cork tree can be used to treat haemorrhage whereas the half-ripe fruit can be used to treat coughs [6,7]. Antioxidant activity of the cork tree extract has been reported previously. The active substances that found in cork tree are phenolic compound such as gallic acid and two flavoniods luteolin and luteolin 7-O-β-glycoside [8,9]. Moreover some previous studies were reported about its antimicrobial activities. *Sonnerratia alba* had antimicrobial activity against *S. aureus*, *V. cholerae* and *P. aeruginosa* [10]. Gallic acid was claimed to have the antibacterial property against *E. coli, S. aureus*, *P. aeruginosa* and especially *K. pneumoniae* [11].

In this paper, the antimicrobial activities of the methanolic *Sonneratia caseolaris* seed extract against *S. aureus*, *E. coli* and *C. albicans* were evaluated using different agar diffusion method (spread plate diffusion, swab plate diffusion and pour plate diffusion). Subsequently, the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid were tested for antimicrobial activity against anaerobic microbe (*S. mutans*, *P. acnes* and anaerobic bacteria).

MATERAILS AND METHODS

Materials

The methanolic extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid (batch no.48630, Fluka) were prepared. Luteolin and Luteolin-7-O-glucoside were purchased from Extrasynthese, Genay, France.

Methods

Preparation of the extract cork tree

The methanolic cork tree seed extract (1 %w/v) was dissolved in 0.2% dimethyl sulfoxide (DMSO). Ampicillin disc (10 µg) was used as positive control for this study.

Microbial strains and preparation of inoculums

The microorganism used in this study included *Staphylococcus aureus* (ATCC 6538P), *Escherichia coli* (ATCC 25922), *Candida albicans*, *Streptococcus mutans* (ATCC 27175), *Propionibacterium acnes* (ATCC 11827) *and* anaerobic bacteria. Bacteria were grown in broth culture in aerobic condition (for *S. aureus*, *E. coli* and *C. albicans*) and anaerobic condition (for *S. mutans*, *P. acnes* and anaerobic bacteria) at 37°C for 24-48 h.

Antimicrobial testing methods

Microbes were inoculated in Mueller-Hinton Broth (MHB) and compared the turbidity with that of the standard 0.5 McFarland solution (for *S. aureus* and *E. coli*) and 1.0 McFarland solution (for *C. albicans*). Mueller-Hinton Agar (MHA) plates were prepared for each experiment. Antimicrobial testing were studied using three different agar-based assays (spread plate diffusion, swab plate diffusion and pour plate diffusion).

Spread plate diffusion (SPPD)

One hundred microliters of inoculum was then pipette onto the MHA. A bent glass rod was used to inoculate the MHA plate by spreading over the surface with rotation to ensure even distribution of the inoculum.

Swab plate diffusion (SWPD)

One hundred microliters of inoculum was then pipette onto the MHA. The adjusted inoculum was swabbed on the MHA plates by a sterile cotton swab.

Pour plate diffusion (PPD)

One hundred microliters of inoculum was mixed with 5 ml of MHB and poured onto the MHA plate. After 15 minutes, the plates were altered to solidify.

Subsequently, the cylinder cups were placed onto the top layer of the MHA plates. The samples were poured into the cups. Plates were incubated in incubators at 37°C for 24 h (*S. aureus* and *E. coli*) and for 48 h (*C. albicans*). The inhibition zones were measured in millimeters.

Antimicrobial test against anaerobic microbe

The antimicrobial activity of the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid was determined using the agar diffusion method. The microorganisms used in this study included *Streptococcus mutans*, *Propionibacterium acnes* and anaerobic bacteria). The desired bacteria were inoculated and prepared by adjusting the turbidity of an actively growing broth culture in the brain heart infusion (BHI) broth to an optical density at 540 nm equivalent to $1x10^6$ cfu/ml. The microbial lawn was prepared on agar plates using a sterile cotton swab. The cylinder cups were placed onto the top layer of the BHI agar plates. All samples (2.5% w/w) were prepared and poured into the cups. The plates of bacteria were then incubated in anaerobic condition at 37 °C for 48 h. All tests were performed in triplicate and inhibition zone were measured using a millimeter scale.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Antimicrobial activity of *Sonneratia caseolaris* was studied using different agar diffusion method (spread plate diffusion, swab plate diffusion and pour plates diffusion). The methanolic cork tree seed extract and gallic acid were evaluated. The cork tree extract could inhibit *Staphylococcus aureus* and *Candida albicans* but could not inhibit *Escherichia coli* (Table 1-3). Previous studies showed *Sonnerratia alba* had antimicrobial activity against *S. aureus* but could not inhibit *E. coli* [10]. The methanolic cork tree seed extract exhibited the results of all agar-based assay were not different. Gallic acid had the antimicrobial activity against only *S. aureus* both spread plate diffusion and swab plate diffusion method but was not found in the pour plate diffusion method as shown in Table 2. The antimicrobial activity against *S. aureus* and *E. coli* of ampicillin are shown in Table 1 and 2. Ampicillin was not found the antimicrobial activity against *C. albicans* (Table 3).

Table 1 Antimicrobial activities of the methanolic cork tree seed extract, gallic acid and ampicillin against *S. aureus* in three different agar-based assays.

Methods	Sample / Inhibition diameter (mm)		
	Seed extract	Gallic acid	Ampicillin
Spread plate diffusion	12.0 ± 0.0	9.0 ± 0.0	29.0 ± 1.0
Swab plate diffusion	14.0 ± 1.0	11.0 ± 1.0	35.0 ± 1.0
Pour plate diffusion	12.0 ± 0.0	0.0 ± 0.0	35.0 ± 1.0

Table 2 Antimicrobial activities of the methanolic cork tree seed extract, gallic acid and ampicillin against *E. coli* in three different agar-based assays.

Methods	Sample / Inhibition diameter (mm)		
	Seed extract	Gallic acid	Ampicillin
Spread plate diffusion	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 1.0
Swab plate diffusion	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 1.0
Pour plate diffusion	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 3 Antimicrobial activities of the methanolic cork tree seed extract, gallic acid and ampicillin against *C. albicans* in three different agar-based assays.

Methods	Sample / Inhibition diameter (mm)		
	Seed extract	Gallic acid	Ampicillin
Spread plate diffusion	18.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Swab plate diffusion	17.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Pour plate diffusion	17.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Subsequently the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid were tested for antibacterial activity using swab plate diffusion method. Antimicrobial activity against three species anaerobic bacteria (*Streptococcus mutans*, *Propionibacterium acnes* and anaerobic bacteria) are shown in Fig. 1-3. All tested extracts exhibited antibacterial activity against only *P. acnes*. Gallic acid and two flavoniods luteolin and luteolin 7-O-β-glycoside were the phenolic

ISSN: 0975-8585

compounds in cork tree. Antimicrobial activities of the phenolic compounds were evaluated and compared with the cork tree extracts. Gallic acid had antimicrobial activity against *S. mutans*, *P. acnes* and aerobic bacteria while luteolin 7-O-β-glycoside showed antimicrobial activity against only *P. acnes*. However, luteolin could not inhibit *S. mutans*, *P. acnes* and anaerobic bacteria.

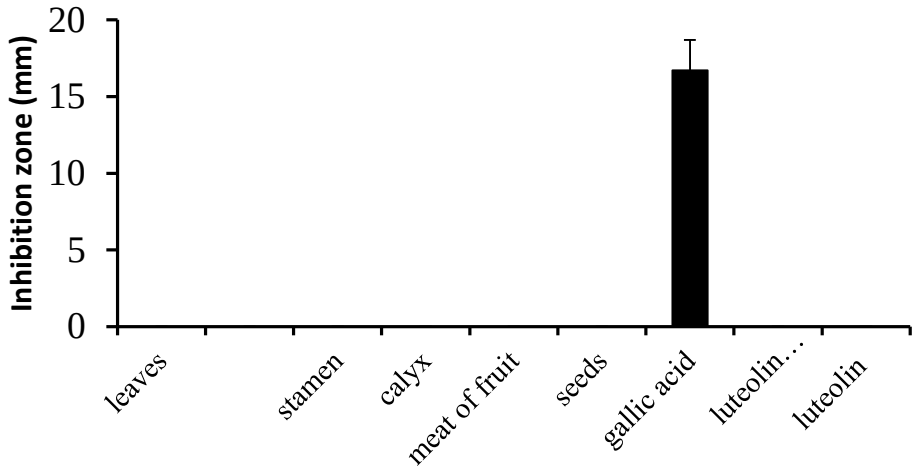

Fig. 1 Antimicrobial activities of the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid against *S. mutans.*

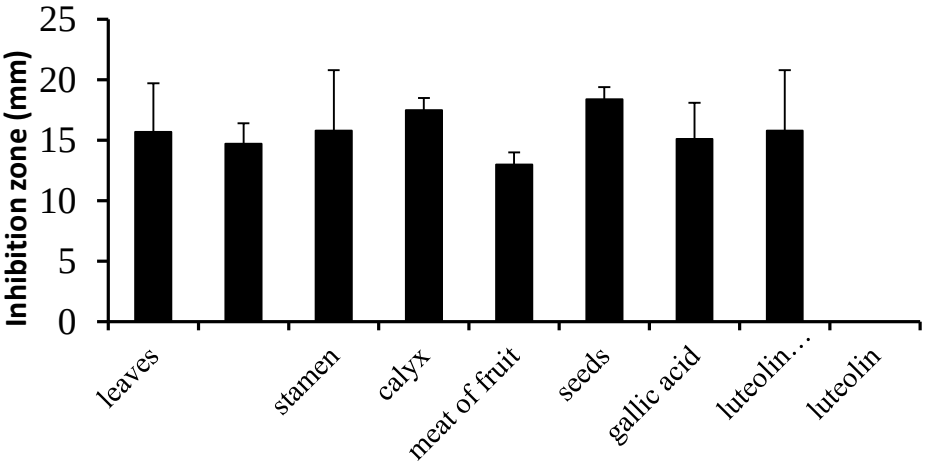

Fig. 2 Antimicrobial activities of the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid against *P. acnes*.

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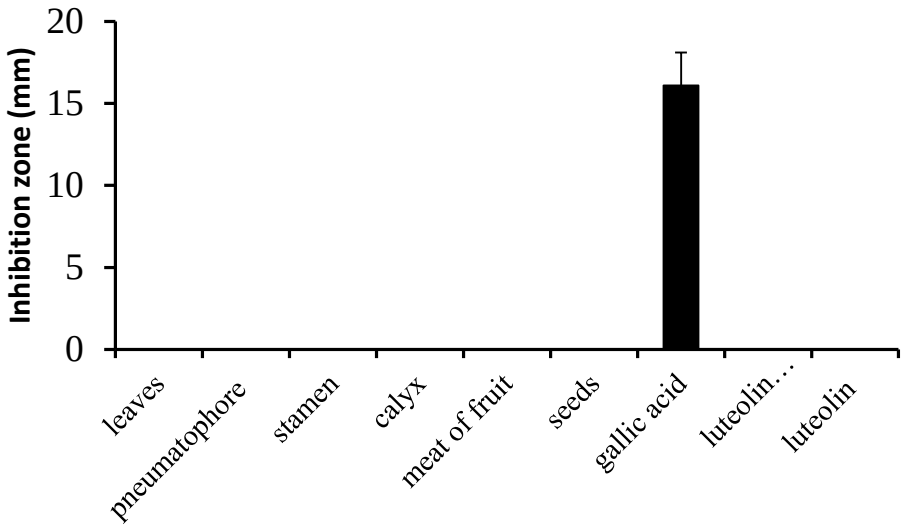

Fig. 3 Antimicrobial activities of the extracts of the leaves, pneumatophore and different parts of the flower or fruit (stamen, calyx, meat of fruit and seeds) and gallic acid against anaerobic bacteria.

CONCLUSION

In this study, we attempted to evaluate the antimicrobial activity of *Sonneratia caseolaris* (cork tree) against *S. aureus*, *E. coli* and *C. albicans* using three different agar-based assays. The most results showed that the different agar-based assay did not affect on antimicrobial activity, but some pour plate diffusion assay showed the antimicrobial activity was differ from the other assays. For antimicrobial activities against anaerobic microbes, the cork tree extract had the antimicrobial activity against only *P. acnes*.

ACKNOWLEDGEMENTS

The authors express their sincerest thanks for the support from Research and Development Institute of Silpakorn University and Faculty of Pharmacy, Silpakorn University.

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