Effect of solvents on total phenolic compounds and antibacterial activity of *Ceriops tagal* extracts

Luksamee Vittaya*[a], Chakhriya Chalad[a], Thunwadee Ritthiwigrom[b]
[a]Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang, Thailand 92150
[b]Chemistry Department, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 50200

*nokluksamee@hotmail.com phone +66-74204064 fax +66-74204064

ABSTRACT

Ceriops tagal is a mangrove plant in RHIZOPHORACEAE family which plays an importance role in coastal ecological balance and exhibit versatile pharmacological area. In this study, leaves and barks were collected from Rajamangala beach, Rajamangala University of Technology Srivijaya, Trang province and were dried to successively extract with hexane, ethyl acetate and methanol. The total phenolic content was evaluated by Folin-ciocaltue method. The results showed that methanolic barks extracts exhibited the highest total phenolic content values 6.063 ± 0.205 mg GAE/g extract. The antimicrobial activities against *Bacillus cereus* TISTR 687, *Escherichia coli* TISTR 780, *Staphylococcus aureus* TISTR 1466, and *Samolnella typhi* TISTR 292 of the leaf and bark extracts were determined using paper disk diffusion method. The methanol extracts from the bark exhibited the best activity. None of the extracts showed antibacterial activity against *E*.coli.

Keywords: Total phenolic, Antibacterial, Mangrove, Extracts, Disk diffusion, *Ceriops tagal*

1. INTRODUCTION

Thailand has several kinds of medicinal plants which have been used to treat many diseases for a long time. Among these potential Thai plants, *Ceriops tagal* are medicinal mangrove plants which widely use as Thai traditional medicine for therapy. It is traditional Thai plants from Rhizophorceae family and generally dominant interface ecosystems between the land and the sea in the tropical forest. It is important in the economy of those regions in terms of mangrove-linked fisheries and forestry. Almost parts of both plants can be used in traditional medicine for treatment of various ailments, for example, anti-inflammability [1], anticancer activity [2-3], induced antidiabetes in rats from leaves extract [4]. However, there are quite a few data on antimicrobial activity of *C. tagal* [5]. Therefore, we have investigated the antimicrobial activity of hexane, ethyl acetate and methanolic extracts from leaf and barks of *C. tagal* against *Bacillus cereus* TISTR 687, *Escherichia coli* TISTR 780 *Staphylococcus aureus* TISTR 1466 and *Samolnella typhi* TISTR 292. The correlation between antimicrobial and total phenolic compounds was also study.

2. MATERIALS AND METHODS

Plant identification

C. tagal were collected from Rajamangala beach, Rajamangala University of Technology Srivijaya, Trang province which located in the southern part of Thailand in September, 2012.

Plant material and extraction procedures

Both dried parts of *C. tagal* was collected and were successively extracted in solvents by increasing polarity organic solvents as hexane, ethyl acetate, and methanol over a period of seven days each at room temperature. Crude extracts were acquired by concentrating the extract under reduced pressure.

Determination of total phenolic content

The content of total phenolic compounds of leaf and barks crude extracts were determined using the Follin-Ciocalteau method which is modified by Miliauskas *et al* [6]. A standard curve was plotted using gallic acid as standard. Ten milligram of each samples and standard was diluted with methanol to the final volume of 0.5 mL. Then, 0.25 mL of the Follin-Ciocalteau reagent and 1.25 mL of Na_2CO_3 solution (20%) were added in each tube, respectively. The tubes were vortexed and the absorbance of all samples and standard were measured at 725 nm using a UV-vis spectrophotometer after 40 min. Total phenolic content were expressed as gallic acid equivalent (GAE) calculated from the calibration curve.

Antimicrobial screening

Antimicrobial activity of leaf and barks of both plants were performed using a paper disk diffusion method which modified from National Committee for Clinical Laboratory Standards (NCCLS) [7]. *Bacillus cereus* TISTR 687 *Escherichia coli* TISTR 780 *Staphylococcus aureus* TISTR 1466 and *Samolnella typhi* TISTR 292 obtained from the National Center for Genetic Engineering and Biotechnology, Thailand, were used for antibacterial study. The bacterial culture media (Nutrient agar) was autoclaved for 20 min at 121°C and at 15 lb pressure before inoculation and then it was poured into a plate and allowed to solidify. The bacteria were incubated in Nutrient broth (NB) at 36°C for 24 h. Turbidity was adjusted at 0.5 McFarland standard (10^8 CFU/ml)and was swabbed over the surface of media using a sterile cotton swab to ensure even growth of the organisms. The tested crude extracts were dissolved in dimethylformamide (DMSO) to give stock solutions of 100 mg mL^{-1}. Sterile filter paper discs were measured as 6 mm in diameter (Whatman No. 1 filter paper), before placed on the solidified nutrient agar medium and 10 μL of tested crude extracts were then placed on sterile filter paper discs. After overnight incubation for 24 h at 37°C, the zones of inhibition were measured in mm and compared with standard antibiotics (gentamicin and penicillin). All experiments were repeated three times and the average values are presented.

3. RESULTS

Sample of leaf and bark of this plant were died and successively extract with hexane, ethyl acetate and methanol to obtain six extracts. Total phenolic content of extracts was determined by Folin-Ciocalteu method and the data was shown in Table 1. Total phenols of *C. tagal* extracts were calculated according to the equation $y = 0.005x + 0.008$ ($R^2 = 0.992$) as gallic acid equivalent (GAE, mg/g dry material).

Table 1. Tatal phenolic content of crude extracts of *C. tagal*.

Parts	*C. tagal* extracts (mg of GAE/g)		
	Hexane extract	Ethy acetate extract	Methanol extract
Leaf	0.227±0.033	0.385±0.031	1.130±0.039
Bark	0.301±0.034	4.681±0.051	6.063±0.205

Total phenolic content show value between 0.227 to 6.063 mg GAE/g of the dried material in various extracts. In regard to amount of phenolic in leaf and bark of *C. tagal* crude extract, methanolic extract contained the higher content of phenolics (1.130 and 6.063 mg QE/g of the dried material), compared to ethyl acetate extracts (0.385 and 4.681 mg QE/g of the dried material) and hexane extract (0.227 and 0.301 mg QE/g of the dried material). It is hypothesized that tannin present in leaf and bark of *C. tagal* play an important role in the biological activity [8]. Moreover, the difference in the antibacterial activity of extracts may be attributed to the difference in the total phenolic content. Antibacterial activities in various solvents of leaf and barks of *C. tagal* were carried out (Table 2) and represented in Figure 1.

Table 2. The antimicrobial activities of crude extracts of *C. tagal*.

Part used	solvents	Zone of inhibition diameter (mm)			
		B. cereus	*E. coli*	*S. aureus*	*Salmonella*
leaf	H	8.66 ± 0.14	-	8.76 ± 0.85	6.27 ± 0.13
	E	8.33 ± 0.43	-	8.73 ± 0.69	7.78 ± 0.36
	M	9.78 ± 0.32	-	9.25 ± 0.84	9.47 ± 0.07
bark	H	10.61 ± 0.95	-	10.50 ± 0.38	10.58 ± 0.80
	E	11.89 ± 0.99	-	12.80 ± 0.68	11.70 ± 0.27
	M	12.39 ± 0.55	-	13.37 ± 0.97	12.14 ± 0.63

Values are mean ± SD; n = 3
H = hexane, E = ethyl acetate, M = methanol

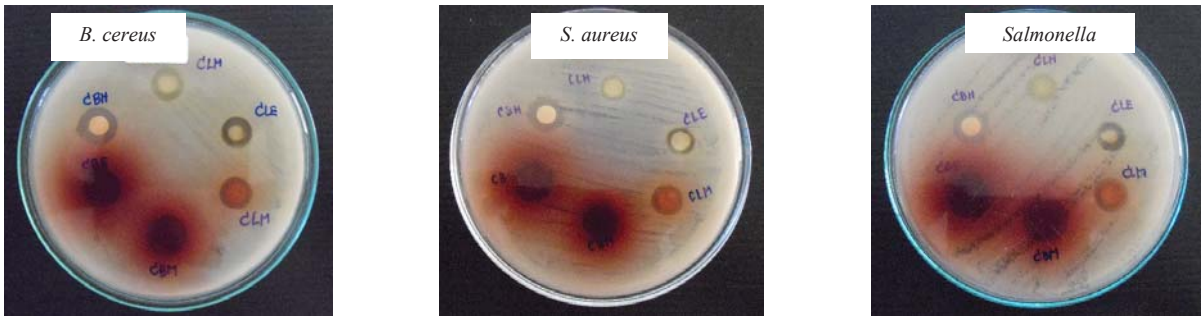

Figure 1. Antimicrobial activity of crude extracts to *B. cereus* (left) *S. aureus* (middle) and *Salmonella* (Right). CBH, CBE and CBM are bark of *C. tagal* extracted by hexane, ethyl acetate and methanol, respective. CLH, CLE, CLM are leaves of *C. tagal* extracted by hexane, ethyl acetate and methanol, respective. The technique were performed by disk diffusion method

The results revealed that the extracts were active against *B. cereus, S. aureus*, *Samonella typhi* exceptionally *E. coli* with their inhibition zones ranged 6.27-13.37 mm. In addition, the results indicated that barks of hexane, ethyl acetate, and methanolic extracts have greater antibacterial activities against the tested microorganisms compared to all leaf extracts. Moreover, the crude extracts of *C. tagal* leaves and bark active against only *B. cereus, S. aureus* and *Samonella typhi* and did not show any active for *E. coli*. This is explained that *E. coli* has outer membrance and periplasmic space having lopopolysaccharide which put up extracts across permeable membrance [9].

4. CONCLUSIONS

The results obtained from this study showed that the leaf and barks crude extracts of *C. tagal* inhibitory effect against *B. cereus* TISTR 687*, S. aureus* TISTR 1466 and *Salmonella* except *E. coli* TISTR 780. Therefore, this mangrove plant may has potential as antibacterial activity. In addition, the determination of total phenolic content related to antimicrobial activity of *C. tagal* leaf and barks in hexane, ethyl acetate, and methanol solvents.

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