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Antimicrobial activity of Gac fruit (Momordica cochinchinensis)

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Abstract

The aim of this study was to investigate the antimicrobial activity of Gac fruit (Momordica cochinchinensis) extract against gram positive and gram negative bacteria using agar disc diffusion method and lytic activity assay. The parts of seed pulp and flesh of Gac fruit were extracted using 95% ethanol, ether and distilled water as a control. The result showed that the extract of flesh with distilled water inhibited gram positive bacteria more than gram negative bacteria as well as the extract of flesh with ethanol. The extract of flesh with distilled water showed significant inhibition against Staphylococcus aureus, Planococcus sp. and Micrococcus luteus with inhibition zone of 10.0, 9.2 and 9.0 mm, respectively. The maximum antibacterial activities were observed on Micrococcus luteus 745 in ethanolic extract of flesh (20.0 mm). However, the extract of flesh with ether had no antimicrobial activity. Moreover, the extract of seed pulp with ethanol showed strong inhibition against Micrococcus luteus (19.0 mm) which are gram positive bacterial strains. The extract of seed pulp with ether had no antimicrobial activity. For the Lytic activity against Staphylococcus aureus, the extract of seed pulp with ethanol showed the highest value of 22.82%, followed by the extract of seed pulp with distilled water at 22.51%, while the extract of flesh with ethanol was 11.57% and the extract of flesh with distilled water was 10.78%, respectively.

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Keywords: Agar disc diffusion method, Antimicrobial activity, Gac Fruit, Momordica cochinnensis, Lytic activity

1. Introduction

Gac *(Momordica cochinchinensis*, Spreng) is a tropical plant grown in many Asian countries including Viet Nam, Laos, Thailand, China, Bangladesh and India [1-2]. It is botanically classified in the Cucurbitaceae family. The fruit is called Gac in Viet Nam, Mak kao in Loas, Fak kao in Thailand and Bhat kerala in India [2]. The fruit flesh contains red soft and sticky arils covering hard seed as shown in Fig.1. Gac aril, oily, has a palatable and consumed there for dietary as well as medicinal purposes [1]. It was reported that the aril is cooked along with seeds to impart its red color and flavor to rice dish, Xoi Gac, is always served in special ceremonies such as engagement, wedding and lunar New Year in Vietnam [1-3]. While Gac seed are used as a medical purposes

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[2-3]. In Thailand, Gac fruit is consumed in different purposes upon development stage of fruit. Immature Gac (green) is consumed as vegetable while ripe Gac, hard and green, is used as raw material for juice or ice cream processing. Many researches have shown that Gac fruit were excellent sources of carotenoids, lycopene (more than 50 mg/100g) and β-carotene (more than 16 mg/100 g), giving it exceptional antioxidant properties [1-5].

An antimicrobial substance is a substance that can kill or inhibit the growth of microorganism. There are several reports in the literature regarding the antimicrobial activity of plant and fruit extracts [6-12]. A wide range of plant and fruit parts, root stem, flower, sap, leaf, fruit and seed, is used for extracting as raw drugs. In fact, higher plants produce hundreds to thousands of diverse chemical compound with antimicrobial properties. The antibacterial properties of the extracts of *Jatroppha tanjorensis* have been reported [8]. Sajjan et al. [10] reported that the aerial part extracts of *Momordica cymbalaria* showed strongly inhibition the growth of human pathogenic bacteria, both gram negative and gram positive, and fungi. In addition, essential oils produced by plant are known to possess antimicrobial activity. Most of the lycopene is fat-soluble, it is more commonly extracted with organic solvents, such as ethanol, acetone, petroleum ether, hexane, benzene and chloroform. [6-7, 11]. Several studies on antimicrobial activity of Cucurbitaceae species have been reported [13-16]. However, antimicrobial activity of Gac fruit has not been reported. The present study aimed to examine antimicrobial activity against gram positive and gram negative bacterial strains.

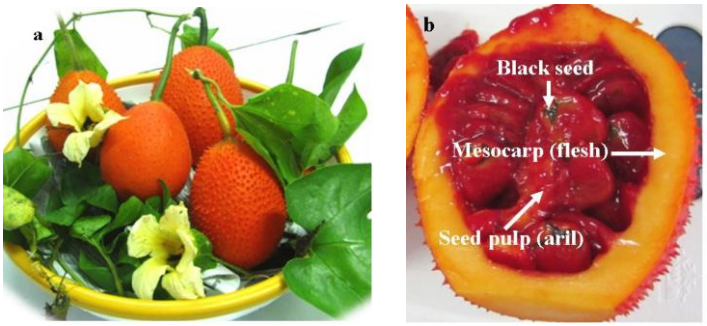

Fig. 1. (a) Ripe Gac fruits; (b) Physical characteristic of ripe Gac fruit

2. Material and methods

2.1 Bacterial strains and stock cultures preparation

Gram positive bacterial strains i.e. *Staphylococcus aureus* TISTR 029, 517, *Leuconostoc mesenteroides* TISTR 053, *Staphylococcus epidermidis* TISTR 518, *Micrococcus luteus* TISTR 745, 884, *Staphylococcus intemedius* TISTR 668, *Pediococcus pentosaceus* TISTR 374, *Staphylococcus simulans* TISTR 669, *Planococcus* sp. TISTR 1408, *Salinicoccus siamensis* TISTR 1562 and gram negative bacterial strains i.e. *Escherichia coli* were collected from Microbiological Laboratory, Kasetsart University, Thailand. All bacterial strains were cultured in nutrient broth at 37°C until the similar optical density of 0.5 McFarland (1.5×10^8 cell/ml) was obtained. These cultures were designated as the working stock, with was used for antimicrobial and lytic activities.

2.2 Preparation of Gac fruit extracts

Gac fruits were harvested in laboratory plant in Nakhon Pathom Prefecture, Thailand. The fully ripe fruits, red soft and sticky arils covering black seed as shown in Fig.1., were collected and transported to the laboratory within 24 h. The fruits were thoroughly cleaned and soaked with 70% alcohol for 15 minutes in order to clean

the fruit. Then, the fruits were cut and separated into seed pulp and flesh. The seed pulp or flesh was blended and the resulting juice was filtered using a cotton membrane. For each extract, the filtered juice was extracted using three solvents including 95% ethanol, ether and distilled water as a control. The samples were extracted with each solvent with the ratio of 1:1 under shaking condition (100 rpm) at 4 °C for 48 h. The supernatant of each sample was collected after centrifugation at a speed of 3000 g for 10 min. Subsequently, the collected samples were evaporated under reduced pressure at 45 °C using rotary evaporation.

2.3 Antimicrobial activity

Antimicrobial activity was carried out by agar disc diffusion method. Nutrient agar plate was used as a medium. The working stock (1 ml) of each bacterial strain was spread over the plate using sterile cotton bud, and sterile discs of 6 mm in diameter were placed upon the surface of the inoculated plated [14]. The 10 µl of each Gac extract was transferred into the discs in the inoculated plated and then incubated at 37°C for 48 h. The diameter of inhibition zone was measured in mm. All determinations were performed five replicates.

2.4 Lytic activity

The 2.88 ml of the *Micrococcus luteus* 745 working suspension was placed in a cuvette, an aliquot (120 µl) of each Gac extract solution was added, followed by repeated inversions for 20 seconds. Lytic activity, expressed as percent of the activities with respect to that of the distilled water, was estimated as the initial velocity of the decrease in turbidity of the cells monitored at 700 nm with a UV-Vis spectrophotometer at 37 °C within 2 minutes.

2.5 Statistical Analysis

Data were expressed as Mean±Standard Deviation. The obtained data were subjected to ANOVA test to analyze whether there was significant difference between each extract.

3. Results and discussion

3.1 Antimicrobial activity

The seed pulp and flesh of Gac fruit were extracted using 95% ethanol, ether and distilled water as a control. Antimicrobial activity of Gac fruit extracts was determined against twelve bacterial strains and the summarized results were shown in Table 1. It is very clear that the flesh extract with distilled water has shown antimicrobial activity against *S. aureus* (10 mm), *M. luteus* (9.0 mm) and *Planococcus* sp (9.2 mm). The maximum antibacterial activities were observed in ethanolic extract of flesh against both *M. luteus* 745 (20.0 mm) and *M. luteus* 884 (18.5 mm). However, the extract of flesh with ether had no inhibition effect on bacterial strains tested. For the seed pulp extracts, only one inhibition effect of ethanolic extract on *M. luteus* 745 was observed with the inhibition zone of 19.0 mm. A comparison of the susceptibility of the extracts toward bacterial strains, including gram positive and negative strains, showed that *M. luteus* 745 appeared to be more susceptible to flesh extracts than other strains tested. This possibly means that the different compounds in cell wall of each culture responsible for the different antimicrobial activity. The cell wall of gram positive bacterium is composed of peptidoglycan layers combined with teichoic acid molecules. In gram negative cell wall, the peptidoglycan layer is much thinner, and there is no teichoic acid. Moreover, an outer membrane closely overlies the peptidoglycan layer so that the membrane and layer comprise the cell wall. [17].

Rojas et al. [9] reported that the solvents, 95% ethanol and dimethyl sulfoxide, used for dissolving the crude extracts always gave negative results, showing that they did not influence in the antimicrobial activities of the plant extracts. However, in this study, the ethanolic and aqueous extracts seemed to have effective compounds

more than the ether extracts. The antimicrobial activities of other species of Cucurbitaceae have been reported [13-16]. The crude methanol extract of cucumber showed antifungal and antibacterial activities [13]. Kumar and Kamaraj [14] indicated that *Cucumis anguria* fruit extract with ethanol can be used as antimicrobial activity. The stem, leaves and fruits extracts of *Momordica cymbalaria* exhibited strong antibacterial activity especially, gram negative (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) pathogens [10].

3.2 Lytic activity

The extracts both with aqueous and ethanol solvents presented significantly effect on *M. luteus* 745 as compare to the control (distilled water) (Fig. 2). Lytic activities of the seed pulp extracts with distilled water (22.51%) and ethanol (22.82%) were observed, as well as the flesh extracts with distilled water (10.78%) and ethanol (11.57%). However, the flesh extracts showed lower activity than those of seed pulp extracts. These results agree with the antimicrobial activity described above that the seed pulp and flesh extracts produced inhibition effects against *M. luteus*. Although, mode of action was different, the antimicrobial activity of the Gac fruit extracts would be expected to relate to their components such as phenolic compounds, carotenoids, lycopene, β-carotene and some proteins. It is well known that lysozyme, egg white protein, is excellent enzyme presenting lytic activity against *Micrococcus* sp. Due to, the lysozyme catalyzes the hydrolysis of the beta-1,4-glycosidic linkage of the peptidoglycan in the bacterial cell wall [18]. In case of the Gac fruit extracts, the mode of action has not been demonstrated. To the best of our knowledge, there are no studies to date regarding the mode of action of Gac fruit against microbial strains.

Table 1. Antimicrobial activity of Gac fruit extracts

Microorganisms	Diameter of inhibition zone in mm Mean±SD					
	Flesh			Seed pulp		
	Distilled water extract	Ethanol extract	Ether extract	Distilled water extract	Ethanol extract	Ether extract
Staphylococcus aureus 029	ND	ND	ND	ND	ND	ND
Staphylococcus aureus 517	10.0±1.4^{a}	ND	ND	ND	ND	ND
Leuconostoc mesenteroides 053	ND	ND	ND	ND	ND	ND
Staphylococcus epidermidis 518	ND	ND	ND	ND	ND	ND
Micrococcus luteus 745	9.0±1.2 ^{aB}	20.0±3.1 ^{aA}	ND	ND	19.0±3.1 ^{A}	ND
Micrococcus luteus 884	9.0±1.4 ^{aB}	18.5±3.1 ^{aA}	ND	ND	ND	ND
Staphylococcus intemedius 668	ND	ND	ND	ND	ND	ND
Pediococcus pentosaceus 374	ND	ND	ND	ND	ND	ND
Staphylococcus simulans 669	ND	ND	ND	ND	ND	ND
Planococcus sp. 1408	9.2±1.89 ^{a}	ND	ND	ND	ND	ND
Salinicoccus siamensis 1562	ND	ND	ND	ND	ND	ND
Escherichia coli	ND	ND	ND	ND	ND	ND

ND means not detected. The values in the same column followed by different small letters are significantly different. The values in the same row followed by different capital letters are significantly different.

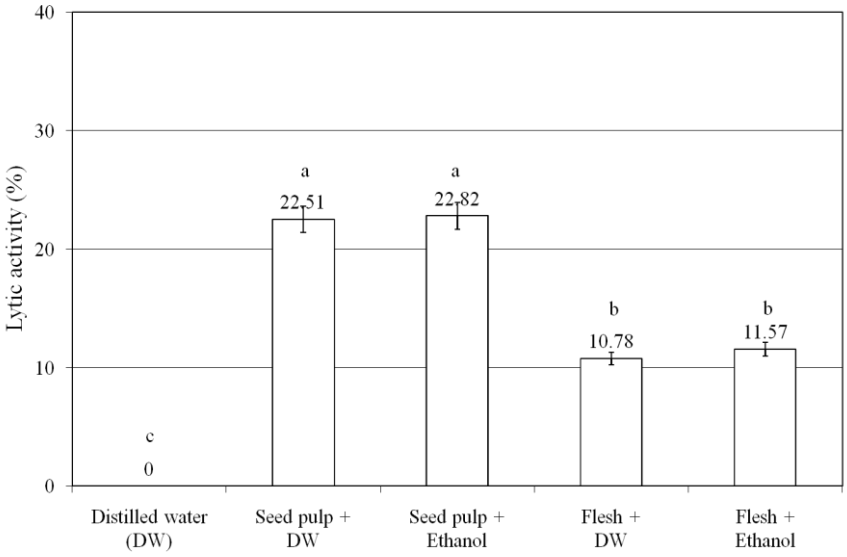

Fig. 2. Lytic activity of the Guc fruits extracts against *M. luteus* 745. The different letters are significantly different

4. Conclusion

This study has revealed the antimicrobial activity of the Gac fruit extracts with different solvents. The results lend credence to the used of Gac fruit as a functional food to promote a healthy lifestyle. The present study supports the idea that Gac fruit could be promising source of antimicrobial agents for food industry.

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