ประสิทธิภาพของสารสกัดจากเปลือกมังคุดในการยับยั้งเชื้อ *Streptococcus agalactiae* ที่เป็นแบคทีเรียก่อโรคในปลา

EFFICACY OF MANGOSTEEN PEEL (*Garcinia mangostana* Linn.) EXTRACT ON INHIBITION OF *Streptococcus agalactiae*, A FISH PATHOGENIC BACTERIUM

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บทคัดย่อ

การศึกษาประสิทธิภาพของสารสกัดจากเปลือกมังคุดในการยับยั้งเชื้อ *Streptococcus agalactiae* ที่เป็นแบคทีเรียก่อโรคในปลา มีวัตถุประสงค์เพื่อทดสอบความสามารถของผงจากเปลือกมังคุดที่สกัดด้วยตัวทำละลายต่างกัน 5 ชนิด ได้แก่ เฮกเซน เมทานอล เอทธิลแอลกอฮอล์เข้มข้น 95% เอทธิลอะซิเตต และน้ำกลั่น ที่สามารถยับยั้งการเจริญของเชื้อ *S. agalactiae* และหาค่าความเข้มข้นที่น้อยที่สุด (Minimum inhibitory concentration ; MIC) ในการยับยั้งเชื้อ *S. agalactiae* จากเปลือกมังคุด ด้วยวิธี Agar disc diffusion ผลการศึกษาพบว่าสารสกัดจากเปลือกมังคุดที่สกัดด้วยตัวทำละลายเอทธิลแอลกอฮอล์เข้มข้น 95% สามารถยับยั้งเชื้อ *S. agalactiae* ได้มากที่สุด รองลงมา คือ เปลือกมังคุดที่สกัดด้วยเฮกเซน เมทานอล น้ำกลั่น และเอทธิลอะ - ซิเตต มีค่า inhibition zone เท่ากับ 17.00±0.50, 16.40 ±0.54, 15.80±0.83, 13.80±0.83 และ 6.80±1.09 มิลลิเมตร ตามลำดับ สำหรับค่า MIC ของสารสกัดจากเปลือกมังคุดที่สกัดด้วยตัวทำละลายเฮกเซนมีปริมาณความเข้มข้นที่น้อยที่สุดในการยับยั้งเชื้อ เท่ากับ 0.0038 ไมโครกรัมต่อมิลลิลิตร รองลงมาคือ เปลือกมังคุดที่สกัดด้วยเมทานอล เอทธิลแอลกอฮอล์เข้มข้น 95% เอทธิลอะซิเตต และน้ำกลั่น ซึ่งมีค่า MIC เท่ากับ 0.5800, 6.5480, 7.500 และ 1,170.00 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ

คำสำคัญ : สารสกัดจากเปลือกมังคุด, *Streptococcus agalactiae*, ปริมาณความเข้มข้นที่น้อยที่สุดในการยับยั้งเชื้อ (MIC)

ABSTRACT

The study was conducted to evaluate the efficacy of mangosteen peel powder on inhibition of *Streptococcus agalactiae,* a fish pathogenic bacterium. The herb was extracted using five different solvents including hexane, methanol, 95% ethanol, ethyl acetate and distilled water. By using agar disc diffusion assay, it was found that 95% ethanol extract had the highest antimicrobial activity followed by hexane, methanol, distilled water and ethyl acetate extract with the inhibition zone of 17.00±0.50, 16.40 ±0.54, 15.80±0.83, 13.80±0.83 and 6.80±1.09 mm, respectively. For the determination of the minimum inhibitory concentration (MIC), hexane extract was found to have the lowest MIC with the value of 0.0038 µg/ml, follow by methanol, 95% ethanol, ethyl acetate and distilled water extract had the MIC values of 0.5800, 6.5480, 7.500 and 1,170.00 µg/ml, respectively.

Keywords: Mangosteen peel extract, *Streptococcus agalactiae*, antimicrobial activity, minimum inhibitory concentration (MIC)

INTRODUCTION

Streptococcus agalactiae is a group B *Streptococcus* causing significant morbidity and mortality among a variety of freshwater and saltwater fish species throughout the world (Robinson and Meyer, 1966; Evans *et al*., 2002). It has been considered to be a major problem leading to economical losses in aquaculture. Outbreaks of Streptococcosis were found in many fish around the world. The first outbreak of Streptococcosis was recorded in rainbow trout (*Oncorhynchus mykiss*) culture in Japan. It was later reported in the USA in freshwater fish, the golden shiner (*Notemigonus crysoleucas*) and in sea-water fish, such as striped mullet (*Mugil cephalus*), menhaden (*Brevoortia patronus*), sea catfish (*Arius felis*). Other outbreaks of disease have been reported in rainbow trout cultured in South Africa and Spain (Austin and Austin, 1999).

Antibiotics have been used to control disease in aquaculture for a long time. Although they are effective, they result in adverse effects in animals, consumers and environment. Their improper use can also lead to the development of drug resistance in microorganisms. Therefore, many antibiotics such as chloramphenicol, enrofloxacin, rifampin and spectinomycin are banned to use in food-producing animals and animal-feed products. More antibiotics are expected to be banned in the future. Recently, many efforts have been put to find safer alternatives to treat fish disease. Because herbs and spices are natural products and considered to be safe, many of them have been investigated to be used to replace antibiotics.

Many herbs have been reported to possess antimicrobial activity against pathogens causing disease in animal and human. Guava leaf (*Psidium guajava*) powder was used to treat on diarrhea in dairy calves (Kromna *et al*, 2006). The antimicrobial properties of the Mayan pharmacopoeia revealed that tissues of *Capsicum* species (Solanaceae) are included in number of herbal remedies for a variety of ailments of probable microbial origin (Cichewicz and Thorpe, 1996). Prophylactic and therapeutic efficacy of a combination of *C. frutescens* (red pepper), *Citrus limon* (lemon), and *Opuntia vulgaris* (prickly pear) against Newcastle disease in domestic fowl were evaluated (Mtambo, 1999). In aquaculture, some herbs have been found to be able to inhibit fish pathogens. The use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in *Oreochromis* sp. (tilapia) was described (Abutbu *et al*, 2004). In additional, Chinese herbs (*Astragalus radix* and *Scutellaria radix*) were need to treat disease in tilapia, *Oreochromis niloticus* (Yin *et al*, 2006). These plants have potentials to be used in aquaculture to control fish diseases.

This study focused on investigation of antimicrobial activity of mangosteen peel (*Garcinia mangostana* Linn.) extract against *S. agalactiae*. It is the first step towards the finding of proper herbs to treat fish disease caused by the pathogen.

MATERIALS AND METHODS

Microorganism

The microorganism was *Streptococcus agalactiae*, given by Asst. Prof. Dr. Nilubon Kitancharoen, the Department of Fisheries, Faculty of Agriculture, Khonkhan University, Thailand. It was propagated in Brain Heart Infusion (BHI, Difco, MD, USA) broth at 37 ℃ for 24 h. The bacterial culture stock was kept in BHI broth supplemented with 20 % glycerol (V/V) at -20 ℃.

Herbs and preparation of herb extracts

Mangosteen peels were collected in Ubon Ratchathani Market, Thailand, between April and June 2008. Mangosteen peels were used to prepare extract. The herb was oven-dried (70 ℃ for 72 h) (Figure 1) and powdered (Figure 2) before extracted with five different solvents including hexane, methanol, 95% ethanol, ethyl acetate and distilled water. One gram of herb was added to 10 ml of each solvent. After stirred for 24 h at 25 ℃, the extracts were then centrifuged at 10,000 rpm 15 min at room temperature (Sorvall Instruments Dupont RC5C, NYR) and supernatant were collected and stored at 4 ℃ until use. Dry weight of each extract was determined after drying 500 µl of the extract by Speed Vac Plus (Sc210A, Instruments, Sorvant).

Screening of antibacterial activity by disc diffusion method

The antibacterial activity was tested using the disc method (Alderman and Smith, 2001) with some modifications. The surface of a Brain Heart Infusion (BHI) agar plate was inoculated with *S. agalactiae* suspension ($1x10^8$ CFU/ml). Sterile, six mm-diameter paper disks were placed on the inoculated agar plate and 25 µl of herbal extracts were put on to the disks. For control, the corresponding solvents were similarly tested. Plates were incubated at 37 ℃ for 24 h. After that, antibacterial activity was evaluated by measuring inhibition zone diameters. The diameters of growth inhibition zones in control (Figure 3) treatments were subtracted (in case of no growth inhibition, the diameter of the disk only was subtracted) and divided by two, giving the sizes of growth inhibition zones beyond the paper disk (showed the diameter in table 1).

Determination of minimum inhibitory concentration (MIC)

An agar dilution method (Alderman and Smith, 2001) with slight modifications was used to determine the MIC of the mangosteen peel (*Garcinia mangostana* Linn.) by solution different solvents extract. MIC was defined as the lowest concentration of the extract that completely suppressed colony growth.

RESULTS AND DISCUSSIONS

Preliminary screening of herbs extracts

The results of the disk diffusion test showed that 95% ethanol extract had the highest antimicrobial activity with the diameter of inhibition zone of 17.00±0.50 mm followed by hexane, methanol, distilled water and ethyl acetate extract with the values of 16.40 ±0.54, 15.80±0.83, 13.80±0.83 and 6.80±1.09 mm, respectively. (Figure 4). The lowest antimicrobial activity was found in ethyl acetate extract. (Table 1).

Minimum inhibitory concentration

The results showed that the hexane extract had the MIC with the values of 0.0038 µg/ml (Table 2).

Table 1 Inhibitory activity of mangosteen peel (*Garcinia mangostana* Linn.) extracts with different solvents against *Streptococcus agalactiae*

Solvents					
Diameter of the inhibition zone(mm ; mean ±SD)					
Herbs	Hexane extract	Methanol extract	95% ethanol extract	Ethyl acetate extract	Distilled water extract
Garcinia mangostana Linn.	16.40±0.54	31.20±0.83	33.60±0.50	18.80±1.09	13.80±0.83
Dry weight(gm)/25 µl	0.050±0.07	0.930±0.42	0.655±0.27	0.375±0.05	0.585±0.51
Control (solvent only)	NC	15.40±0.89	16.60±1.81	12.00±1.87	NC
Net of inhibited zone	16.40±0.54	15.80±0.83	17.00±0.50	6.80±1.09	13.80±0.83

NC = No Clear Zone

Table 2 Minimum inhibitory concentrations (MIC; µg/ml) of different solvents extract of mangosteen peel (*Garcinia mangostana* Linn.) against of *Streptococcus agalactiae*

Herbs	Hexane extract	Methanol extract	95% ethanol extract	Ethyl acetate extract	Distilled water extract
Garcinia mangostana Linn.	0.0038	0.5800	6.5480	7.5000	1170.00

Although some previous was studies on applications of mangosteen products in pathogen inhibition had been investigated. Such as bioassay-guided fractionation of solvents extract of mangosteen peel (*Garcinia mangostana* Linn.) extracts, a complete reported by Mahabusarakam *et al.*(1986) were inhibition of growth of *Staphylococcus aureus* both normal and penicillin-resistant strains, and moderate activities against *Trichophyton mentagrophytes* and *Microsporum gypseum*. Suksamrarn *et al.*(2003) reported the prenylated xanthones, isolated from the fruit hulls and edible arils and seeds of *Garcinia mangostana*, were tested for their antituberculosis potential, α- and β-Mangostins and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the minimum inhibitory concentration (MIC) value of 6.25 µg/ml

In the present study, it is interesting to note that mangosteen peel extracts was active specifically against fish pathogenic bacteria as well. The data suggests that all extracts of mangosteen peels contained some active components which possessed antibacterial properties. The findings of this study, probably the first attempt provides some scientific basis for the bacterial inhibitory activities of this herb on aquatic animal pathogen.

CONCLUSION

Crude 95% ethanol extract of mangosteen peel (*Garcinia mangostana* Linn.) had the highest antimicrobial activity with the diameter of inhibition zone, and hexane extract had the MIC with the value of 0.0038 µg/ml showed lowest inhibitory action against *S. agalactiae* was done. Isolation and identification of active compounds attributed to antimicrobial activities should be further studied to establish the connection between the antimicrobial activity and chemical of mangosteen peel (*Garcinia mangostana* Linn.) composition of the plant.

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Figure 1. Mangosteen peel oven-dried (70 ℃ for72 h).

Figure 2. Powdered of mangosteen peel

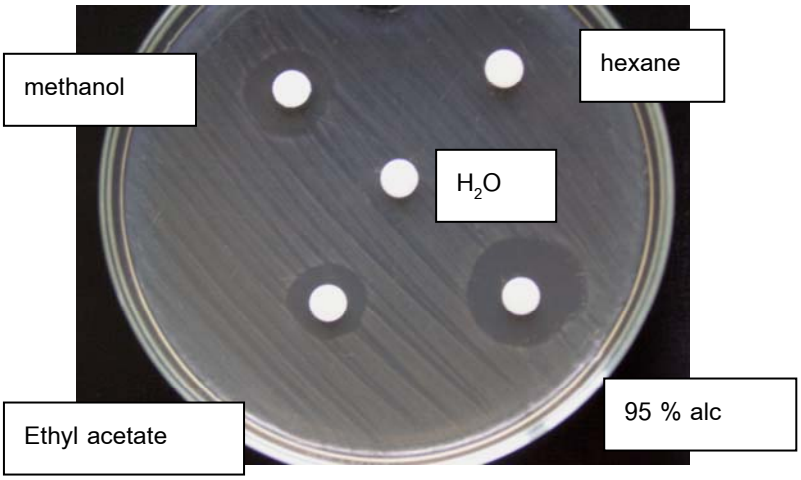

Figure 3. Inhibition zone of *S. agalactiae* with different solvents (control)

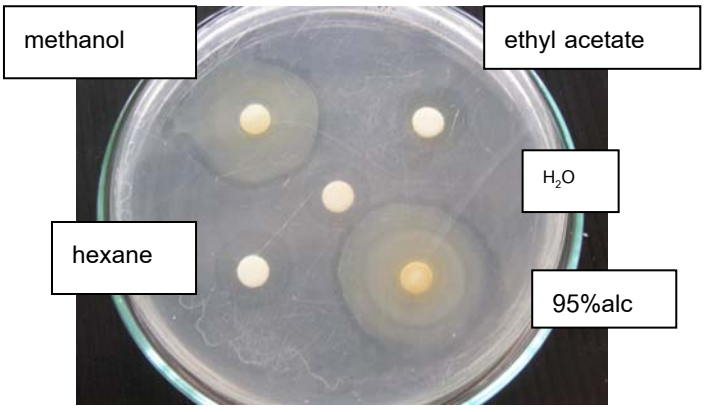

Figure 4. Inhibition zone of *S. agalactiae* by mangosteen peel extract with