

ภาคผนวก

ผลงานวิจัยที่เกี่ยวข้องกับการทำโครงการวิจัยและได้รับการตีพิมพ์เผยแพร่

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Anti-pathogenic Activity of *Momordica Cochinchinesis* (Lour.) Spreng by Hexane Extraction

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Abstract: *Momordica cochinchinensis* (Lour.) Spreng or Gac is Thai medicinal plants widely use as food and relief therapy such as inflammation, swelling, eczema, ringworm sores, bruises, hemorrhoids cure diarrhea, rashes and skin infections, etc. The hexane extracts of root, stem, fruit and leaf of *M. cochinchinensis* were performed by maceration method for primarily screen of anti-bacterial activity. The concentration of 100 mg/ml of hexane extracts against four pathogenic bacterial strains including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Acinetobacter baumannii* were tested by using the disk-agar diffusion method. The stem crude extract exhibited inhibition zones against all four bacterial strains with 7.0±0.5, 8.1±0.5, 8.1±0.5 and 8.2±0.5 mm respectively while the extracts of root and leaf showed the resistant activity only on *Staphylococcus aureus*, *Enterococcus faecalis* and *Acinetobacter baumannii* with the inhibition zone of 6.5±0.5 to 8.8±0.5 mm in which the activity of root extract presented bigger clear zone than leaf extract. Fascinatingly, the fruit extract showed anti bacterial activity against *Enterococcus faecalis* only with the inhibition zone of 8.0±0.5 mm. This result may useful for drug development of Southeast Asia herb in the future.

Keywords: *Momordica cochinchinensis*; hexane; crude extract; antibacterial activity; agar diffusion test.

Introduction: *Momordica cochinchinensis* (Lour.) Spreng or Gac while in Thailand known as “Fak Khaao”, is typical plan in Southeast Asia including Thailand. It belongs to the melon family (Cucurbitaceae) [1]. This plant was reported as the medicinal plan which native using as food and medicinal treatment such as, swelling, eczema, ringworm sores, bruises, hemorrhoids cure diarrhea, rashes and skin infections, etc [2,3]. It has been reported that Fak Khaao has very high lycopene and carotenoid content compare to other fruit such as tomato, papaya, rosehip etc. [4,5]. Because of the abundant high quantity of lycopene and carotenoid in Fak Khaao fruit (from the seed membrane (seed pulp or aril) of the ripe fruit), it has been continually research of the effective of these nutrition on blindness in children. In Vietnamese preschool children, they were given “Xoi Gac” (rice mixed with Gac seed membrane or red rice) for 30 days. It is showed significantly improved plasma levels of retinol, alpha- and betacarotenes, and lycopene in these pre-school children [6]. However, other nutritional compound are highly identified in Fak Khaao such as vitamin E [7,8], fatty acid [8,9]. However, most researches related to Fak Khaao, were focused on the nutritional composition but rarely found the information on clinical research or disease treatment. Whereas, the traditional use of Fak Khaao has also been reported for infectious diseases treatment as mention earlier, the scientific study of the treatment is still poor information. To earn more understanding of diseases treatment caused by photogenic bacteria using Fak Khaao, we focused on anti-bacterial activity of different tissues of Fak Khaao including root, stem, fruit and leaf to future develop as an antibiotic use for medicinal treatment.

Materials and Methods:

Plant Material: The plant materials used in the study consisted of root, stem, fruit and leaf of *M. cochinchinensis* which collected from Chonburi and Songkhla provinces of Thailand.

Bacterial Strains and Culture Medium: Pathogenic bacteria which were used in this study, was supported by Department of Microbiology, Prince of Songkla University, Thailand including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Acinetobacter baumannii*. These strains were cultured in Nutrient medium for propagation and anti-bacterial assay.

Preparation of Crude Extracts: The medicinal herbs, Fak Khaao ; root, stem, fruit and leaf used in this study were dried in Hot air oven at 75°C then chopped in small pieces and grounded. The 503.20, 500.34, 538.23 g of dried root, stem and fruit and 210.03 g of dried leaf were extracted by maceration in 3 liters of hexane solvent with the mixing everyday 3 times a day for 7 days. The extracts were filtered using 3 layers of filter cloth. The solvent was then removed under reduced pressure in a rotary evaporator at 65°C and the extracts were completely evaporated to dryness at 70°C in water bath for 15 hrs to get constant weight. All dried crude extracts were stored at 4°C until required for testing. The extracts were dissolved in DMSO before use [10,11].

Preparation of bacteria and agar plates: Nutrient broth and agar was prepared in the usual fashion by autoclaving, and nutrient agar was plated into Petri dishes allowed the agar began to set. Bacterial glycerol stocks of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Acinetobacter baumannii*, were plated on ice to allow the defrost cell. The 10 µl of stock cells was streaked on nutrient agar and incubate at 37°C overnight to get single colony.

Screening of Antibacterial Activity by the Agar Diffusion Method: The disk-agar method which was modified following the method standardized by the National Committee for Clinical Laboratory Standards [12], was used for antibacterial assay of four different hexane extracts of root, stem, fruit and leaf of Fak Khaao. The 2-3 single colonies of each bacterial strain were inoculated in 5 ml nutrient broth then incubated at 37°C for 3 hrs. Each cell culture was diluted with sterile 0.85% NaCl to get the cell concentration of 10⁵ cell/ml and 500 µl of diluted cultures was spread over the surface of nutrient agar. Six mm-diameter paper discs were bored in the agar plates and 5 µl of each herbal extract reconstituted in DMSO to a concentration of 100 µg/ml was dispensed into the discs. Antibacterial activity was evaluated after incubation at 37°C overnight by measuring diameters of inhibition zone. DMSO and hexane were served as negative controls. Each test was performed triplicate for statistic evaluation.

Results and Discussion

Extraction efficiency of hexane: The dried weight of crude hexane extracts of root, stem, fruit and leaf of *M. cochinchinensis* (Lour.) Spreng were quantified at the constant weight after completely solvent evaporating. The result showed that from 503.20, 500.34, 538.23 and 210.03 g of dried root, stem, fruit and leaf, only 2.03, 2.05, 19.53 and 1.98 g of dried crude extract were gathered (figure 1). The productivity of hexane extraction was calculated. It showed only 0.40, 0.40, 3.62 and 0.94 percentages of extraction products of dried root, stem, fruit and leaf respectively (table 1).

Antibacterial activity: The total of 5 ng of hexane crude extracts on each paper disc were tested for antibacterial activity. The results exhibited that stem crude extract inhibited all four bacterial strains including *S. aureus*, *E. faecalis*, *E. coli* and *A. baumannii* with inhibition zones of 7.0±0.5, 8.1±0.5, 8.1±0.5 and 8.2±0.5 mm respectively while the extracts of root and leaf showed the inhibitory zone only on *S. aureus*, *E. faecalis* and *A. baumannii* with 6.8±0.5, 8.8±0.5, 8.3±0.5 and 6.5±0.5, 7.0±0.5, 6.7±0.5 mm in which the activity of root extract presented bigger clear zone than leaf extract (figure 2). Interestingly, the hexane extracts of root and leaf had no activity on *E. coli*. Similar observation was reported by Sutabhaha and Khantawa (2011) [13] on hexane extract of Fak Khaao leaf, they found that anti-bacterial activity was observed against *S. aureus* with MIC value of 625.0 mg/ml and no activity was found against *E. coli*. Fascinatingly, the fruit extract showed anti-bacterial activity

against only *E. faecalis* with the inhibition zone of 8.0 ± 0.5 mm (figure 2). This result was the first report of anti-bacterial activity on different tissues from *M. cochinchinensis*.

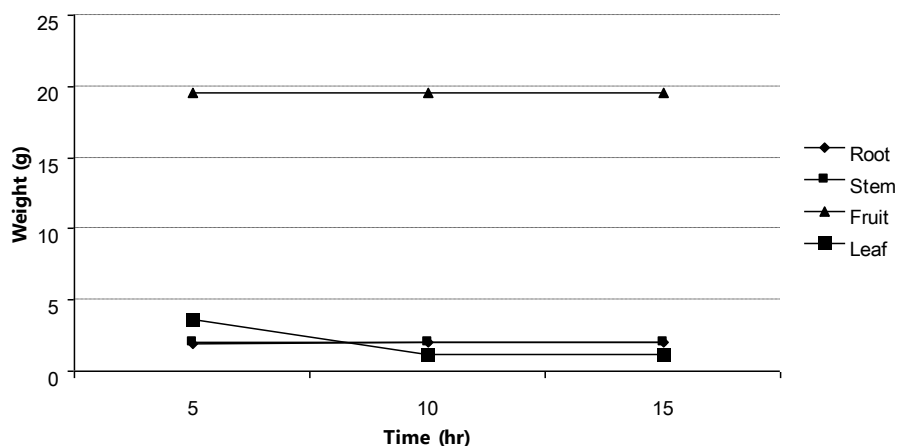


Figure 1. The dry weight determination of crude extracts of of root, stem, fruit and leaf of *M. cochinchinensis* (Lour.) Spreng

Table 1. The efficiency of the hexane extraction of different tissues of *M. cochinchinensis* (Lour.) Spreng

Component	Dry weight (g)	Dried crude extract (g)	Extraction efficiency (%)
Root	503.20	2.03	0.40
Stem	500.34	2.05	0.40
Fruit	538.23	19.53	3.62
Leaf	210.03	1.98	0.94

However, most researches on *M. cochinchinensis* were focused on the nutritional values or antioxidant activity of *M. cochinchinensis* fruit. Lately, Kubola and Siriamornpun (2011) [14] reported the phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac. They found that the aril had the highest contents for both lycopene and beta-carotene while peel (yellow) contained the highest amount of lutein in which lycopene, beta-carotene and lutein are grouped in phytochemicals and antioxidant activity was found in all fractions. Also, the anti-inflammatory and free radical scavenging activities of *M. cochinchinensis* seed extract, common name (“Bolengguazi”) in the Tibetan medicine, was presented [15]. Their study supports the claims by the traditional Tibetan medicine practitioners about the use of *M. cochinchinensis* seeds in inflammatory diseases, such as “Chiba”. Combine with our results, it is strongly supported that many tissues compost of root, stem, fruit and leaf of *M. cochinchinensis* can significantly use for medicinal treatment maintaining the Asian traditional use and also promoting for world wide use. For traditional use, *M. cochinchinensis* was reported as the medicinal plan for treatment some symptoms such as swelling, eczema, ringworm sores, bruises, hemorrhoids cure diarrhea, rashes and skin infections as mention earlier [2,3]. Significantly, our result showed the supporting of traditional use such as diarrhea which cause by some bacteria infection such as *E. coli* or *E. faecalis*. The results showed that stem extract has shown the antibacterial activity against *E. coli* while all extracts showed antibacterial activity against *E. faecalis*. As well as the treatment of skin infections which cause by *S. aureus*, our result revealed that stem, root and leaf extracts showed antibacterial activity against this type of bacteria. Importantly, the stem, root and leaf extracts of this plant presented antibacterial activity against *A.baumannii* which is drug resistant bacterial strain in hospital. It will be very practical for future application for this treatment. Hopefully, this research maybe useful for drug development from *Momordica cochinchinensis* (Lour.) Spreng in the future.

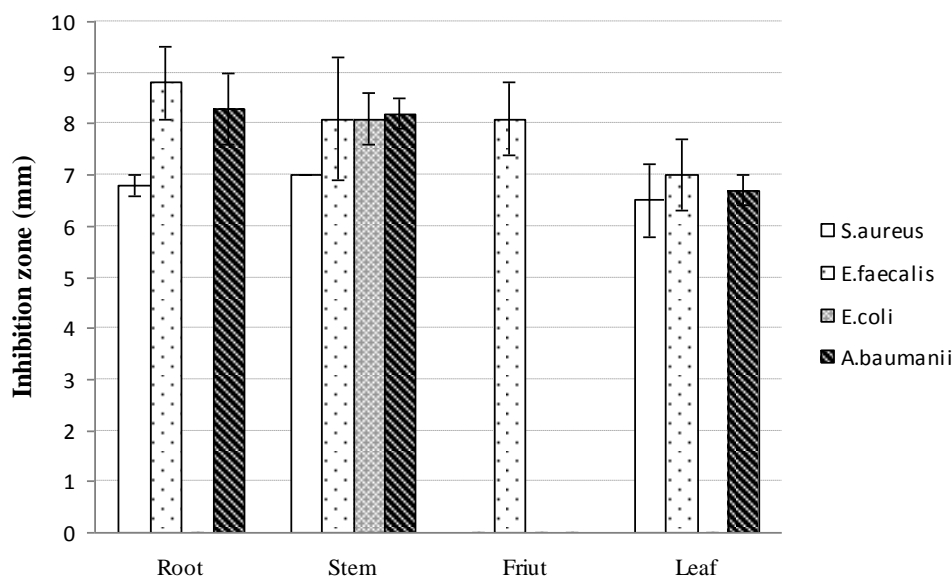


Figure 2. The inhibition zone of crude extracts of different tissues of *M. cochinchinensis* (Lour.) Spreng

Conclusions: Antibacterial activity of *Momordica cochinchinensis* (Lour.) Spreng or Gac was examined in this study. It revealed that hexane extracts of root, stem, fruit and leaf of this plant performed by maceration method showed anti-bacterial activity against both gram positive and gram-negative bacteria. This result supported the traditional use of *Momordica cochinchinensis* as medicinal plant and hopefully useful for drug development in the future.

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ข้อมูลประวัติคณะผู้วิจัย

ประวัติส่วนตัว

ชื่อ-สกุล

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ประวัติการศึกษา

ชื่อย่อปริญญา	สาขา	สถาบันที่จบ	ปีที่จบ
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วท.บ.	เทคโนโลยีชีวภาพ	มหาวิทยาลัยสงขลานครินทร์	2546

สาขาวิจัยที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา)

ชีววิทยาโมเลกุล, อนุพันธุศาสตร์, วิศวกรรมชีวเคมี

ทุนการศึกษาและทุนวิจัยที่เคยได้รับ

ปี พ.ศ.	ทุนการศึกษาและทุนวิจัย	สถาบันที่ให้
2555	การศึกษาชนิดของจุลินทรีย์ในการบำบัดตะกอนของเสียจากบ่อเลี้ยงกุ้งทะเลแหล่ง	คณะวิศวกรรมศาสตร์ มหาวิทยาลัยบูรพา
2554	การศึกษาคุณสมบัติทางชีวเคมีของสารสกัดสมุนไพรไทยพื้นบ้าน	คณะวิศวกรรมศาสตร์ มหาวิทยาลัยบูรพา
2553	การศึกษาเอ็นและกลไกการทำงานของเอ็นที่เกี่ยวข้องกับการเจริญของกล้ามเนื้อกุ้งตระกูล Penaeus	คณะวิศวกรรมศาสตร์ มหาวิทยาลัยบูรพา

ผลงานวิจัย/งานสร้างสรรค์ที่ตีพิมพ์เผยแพร่ (ระดับชาติและนานาชาติ)

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การเสนอผลงานวิชาการ

- International conference on chemical and Biochemical , Paris, France, July 20-22, 2015
“Enhancing Biogas Production from Shrimp Pond Sediment with additive”
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