

High Antioxidant and Phenolic Contents Related to Antibacterial Activity against Gastrointestinal Pathogenic Bacteria of Some Thai Medicinal Plants

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ABSTRACT

Objective: Thai medicinal plants were used as the traditional medicines and as part of everyday plants diet. Plants contain a mixture of phytochemical and exhibit a lot of functional food and medicinal properties. This project aims to investigate the potential of selected Thai medicinal plants according to the properties of their antioxidant and antibacterial activities. **Methods:** The eighteen plants were extracted using maceration method with 95% ethanol. The antioxidant activity was evaluated by DPPH and FRAP assay. The total phenolic content was evaluated by Folin-Ciocalteu phenol reagent. The antibacterial activity was evaluated by agar disc diffusion method. The extract which exhibited high antioxidant and antibacterial activity was selected to observe morphological changes by the scanning electron microscope (SEM). **Results:** The extract of *Caesalpinia sappan* showed the highest activities on both antioxidant assayed by FRAP method and total phenolic contents, however, exhibited high antioxidant assayed by DPPH compared to *Bauhinia strychnifolia* extract. Moreover, the extract of *C. sappan* showed the excellent antibacterial activities against six pathogenic bacteria in Gastro-intestinal tract. The morphological change by SEM was selected for further investigation antibacterial activities of *C. sappan* extract. The results showed that the inhibitory effect to those bacterial strains could be caused by the disruption of the cell membrane and decrease biofilm formation after treatment with the extract. **Conclusion:** The ethanol extract of *C. sappan* exhibited strong antioxidant and antibacterial activities against the six pathogenic bacteria. This result suggested that *C. sappan* could be applied to use for medicinal purpose and functional products. **Key words:** Antioxidant activity, Antibacterial activity, Total phenolic content, *Caesalpinia sappan*, Scanning electron microscope.

INTRODUCTION

Thailand is in a hot and humid climatic zone which supports a high biodiversity of plants. Medicinal plants are concentrated in tropical forests and Thailand's tropical forests support around 12,000 species of vascular plants.¹ Many of the Thai plants were used as the traditional medicines to treat various illnesses and eaten as the part of everyday meal. Plants contain a mixture of essential, non-essential nutrients and phytochemicals. Their functions as natural antioxidants help prevent oxidative process and delay aging-associated diseases. Essential nutrients such as amino acids, fatty acids, vitamin and minerals are necessary for tissue repair and body's growth. However, they cannot be synthesized by the human body then human can have received those essential nutrients from plant sources.^{2,3}

Phytochemicals are group of compounds that are produced by plants for defending against micro-organisms and for repairing tissue damages. There are several phytochemicals such as phenolic acids, flavonoids, carotenoids, alkaloids and anthocyanins that have therapeutic properties.^{4,5,6} In addition, many compounds

are responsible for plant flavor and aromas.⁷ Since there are many health benefits of essential nutrients and phytochemicals, several plants have been recognized as quality sources for pharmaceutical products and functional ingredients. and functional ingredients. Gastro-intestinal tract or GI tract is an organ system in the human body and animals. The GI tract is not only responsible for food digestion, absorbs nutrients and expels waste materials but also important for protection against pathogenic microorganisms and toxic substances.^{8,9} Gastro-enteritis is inflammation of stomach or intestine and it may be caused by bacteria, virus or parasites. Bacterial infections (e.g. *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, *Helicobacter pylori*, *Staphylococcus aureus* and *Bacillus cereus*) dominate a common cause of gastroenteritis in humans.¹⁰ Their symptoms were included diarrhea, vomiting, nausea, salivation, abdominal pain, weakness and bleeding in GI tract. Synthetic antibiotics have been used for the treat-

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ment of human gastroenteritis. However, those antibiotics are the leading cause of disturbance of the normal gastrointestinal microflora, allergy reactions and antibiotic resistance of bacteria. For these reasons, plants including bioactive compounds and their properties have been investigated to select the new medicinal plants for antibacterial activity in GI tract.^{11,12,13}

Previous studies showed the possibility to use many Thai plants as antioxidants and antibacterial agents. Some Thai plant extracts such as *Cassia siamea*, *Garcinia cowa*, *Limnophila aromatica*, *Polygonum odoratum*, *Dioscorea bulbifera*, *Syzygium cumini* and *Zingiber zerumbet* which have been reported for their antioxidant activities, and these plants also exhibit antibacterial against some pathogenic bacteria.^{14,15,16,17} However, there are many medicinal Thai plants which are not to be evaluated their antioxidant related to antibacterial activities from GI tract. Due to the above-mentioned facts, this project aims to investigate the potential of Thai plants according to the properties of the plant extracts on their antioxidant and antibacterial activities including their effects on morphological changes of selected pathogenic bacteria in GI tract. This information will be used as guide line for development of medicinal

products and functional ingredients for use in novel functional food products.

MATERIALS AND METHODS

Sample collection

Eighteen plant samples were selected based on Thai traditional medicine and their health promoting properties Table 1. Five fresh plants belonged to three varieties of *Pogonatherum crinitum*, *Oryza sativa* and *Belamcanda chinensis* were harvested from a cultivated field in Khon Kaen province, northeastern part of Thailand. The fresh plants were cleaned, air-dried and ground in an electrical blender (HR2061/20, Philips) into powder. Another thirteen powder plant samples were purchased from a traditional medicine shop in Chum-Phae district, Khon Kaen, Thailand.

Extraction

The eighteen plant powders were extracted using maceration method with 95% (v/v) ethanol (Merck, Germany) in the ratio of 1:10 (w/v) for 24 h at 30 °C. The ethanolic extracts were filtrated through Whatman No. 1 filter paper (Sigma-Aldrich, USA) and were evaporated to dryness

Table 1 Plant samples including family names, scientific names, common name in Thai, parts used and ethnomedical claims.

Family name	Scientific name	Common name (Thai)	Part used	Ethnomedical claims
Poaceae	<i>Pogonatherum crinitum</i>	Phai-jeud	Leaf	uses as a diuretic, reduces blood sugar, swelling and motion sickness
	<i>Oryza sativa</i> (R ₁)	Rice berry (R ₁)	Seed	rice oil uses for arthritis pain relief and reducing inflammation of a joint
	<i>Oryza sativa</i> (R ₂)	Germinated rice berry (R ₂)	Seed	
	<i>Oryza sativa</i> (R ₃)	Rice berry grass (R ₃)	Aerial part	
Zingiberaceae	<i>Curcuma aromatica</i>	Wan-nang-kam	Rhizome	regulates menstruation and balance hormone for women, reduces intestinal gas and stomach pain, treats diarrhea
	<i>Curcuma aurantiaca</i>	Wan-en-leung	Rhizome	uses for muscle relaxation, reducing blood sugar
	<i>Curcuma aeruginosa</i>	Wan-maha-mek	Rhizome	reduces fever, nausea and stomach pain, uses to expel intestinal worms
	<i>Curcuma zedoaria</i>	Ka-min-aoi	Rhizome	treat peptic ulcer and reduce gas in the digestive tract
	<i>Kaemferia galanga</i>	Praw-hom	Rhizome	reduces headaches, fever, stress and stomach pain
Fabaceae	<i>Bauhinia strychnifolia</i>	Ya-nang-dang	Stem	helps improve blood circulation, uses as laxatives, detoxifies a poison
	<i>Caesalpinia sappan</i>	Fang	Stem	improves menstrual cycle and complexion, stops bleeding from hugs and chest, treat diarrhea, reduces blood sugar, treat toxic side effects
Iridaceae	<i>Belamcanda chinensis</i>	Wan-hang-chang	Leaf	helps reduces fever, coughing, uses as laxatives, decreases skin rashes
	<i>Eleutherine americana</i>	Wan-hom-dang	Bulb	reduces gas in the digestive tract, uses as a diuretic, treats a cold and hemorrhoids
Menispermaceae	<i>Coscinium fenestratum</i>	Ham	Stem	reduces blood sugar, stomach pain, treats gastroenteritis
	<i>Cissampelos pareira</i>	Ma-noi	Leaf	reduces fever and edema symptom
Euphorbiaceae	<i>Phyllanthus amarus</i>	Luk-tai-bai	Aerial part	reduces blood sugar, detoxifies liver, treats hepatitis symptoms,
Lamiaceae	<i>Orthosiphon aristatus</i>	Ya-nuad-maew	Aerial part	reduces hypertension, treat peptic ulcer and kidney disease uses as a diuretic and laxatives
Rhaminaceae	<i>Ventilago calyculata</i>	Tao-rang-dang	Stem	uses as a laxatives, reduces blood sugar

under vacuum using a rotary evaporator. The extracting yields were calculated in percentage based on plant dry weight. The dry or oily extracts were dissolved in dimethyl sulfoxide (DMSO) to get final concentration of 250 mg/ml before use in all experiments.

Determination of antioxidant activities

DPPH radical scavenging activity assay

The free radical 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was used to determine the free radical scavenging activities of the extracts. DPPH assay was measured according to a modified method of Dawidowicz *et al.*¹⁸ Two milliliter of 0.1 mM DPPH in methanol was added to 0.1 ml sample solution in 0.9 ml deionized water. The reaction mixtures were kept at 25°C in the dark for 30 min. Changes in color were measured at 517 nm using a UV-2501 PC spectrophotometer (Shimadzu, Japan). The antioxidant activities were reported as mg Trolox equivalent per g dry weight of plant sample. The half maximal inhibitory concentration or IC_{50} which refers to the extract concentration to inhibit 50% of the free radicals scavenging activities were determined.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was evaluated according to the modified method of Benzie and Strain.¹⁹ The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v:v:v), respectively. Then, 100 μ l of the extract was mixed with 3 ml of the FRAP reagent and kept at 25 °C for 4 min. The change in color was measured at 593 nm using a UV-2501 PC spectrophotometer (Shimadzu, Japan). The antioxidant activities were recorded as μ mol Fe^{2+} per g dry weight of plant sample.

Determination of total phenolic contents

Total phenolic contents from the extracts were determined using the modified Folin-Ciocalteu method.²⁰ Accordingly, 20 μ l of the sample was mixed with 1.58 ml of deionized water and 100 μ l of Folin-Ciocalteu reagent. After incubation at 25 °C for 5 min, 300 μ l of 20% sodium carbonate solution was added to the reaction mixture. Gallic acid was used as standard and the absorbance of the blue color was measured at 765 nm using a UV-2501 PC spectrophotometer (Shimadzu, Japan). The results were expressed as mg Gallic acid equivalent per g dry weight of sample.

Bacterial culture

Six varieties of pathogenic bacteria, *Bacillus cereus* TISTR687, *Staphylococcus aureus* TISTR1466, *Enterococcus faecalis* TISTR379, *Enterobacter aerogenes* TISTR1540, *Salmonella typhimurium* TISTR292 and *Escherichia coli* TISTR780 which caused infection of the gastrointestinal tract were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. The six bacteria were culture in Tryptic Soy Broth (HiMedia, India) for 18-24 h at 37°C.

Determination of antibacterial activities

The antibacterial activities were determined by using the agar disc diffusion method.²¹ After each pathogenic bacterium was grown to log phase, the culture turbidity was adjusted by comparison with McFarland standard No. 0.5 and then was swabbed on the surface of Muller Hinton agar (MHA) plates. The sterile paper discs with a diameter of 6 mm were soaked in 30 μ l of 250 mg/ml extract solution and were placed on the surface of pre-inoculated testing bacteria plates. The plates were incubated at 37°C for 24 h. The antimicrobial activity of each crude extract

was evaluated by measuring the diameter of inhibition zone. For each extract three replicates measured, and their average value was recorded.

Preparation of the cells for Scanning Electron Microscopy (SEM)

The SEM was performed by the modified method of Daduang *et al.*²² Briefly, the cells of the six bacteria which were nearly or close to the inhibition zones on plates after treated with plant extracts for 24 h were taken and investigated the morphological changes by SEM. The cells were harvested and fixed by 2.5% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2). All tested cells were rinsed with 0.1 M sodium phosphate buffer (pH 7.2) and post-fixed with 1% osmium tetroxide (OsO_4) in the same buffer solution. After rinsed by 0.1 M sodium phosphate buffer (pH 7.2), the cells were dehydrated with a series of 20, 40, 60, 80 and 100% (v/v) ethanol, respectively. Finally, all cells were coated with gold for getting the conductivity. The coating cells were examined using scanning electron microscope (JEOL: JSM-6460 LV, USA).

Statistical analysis

For our statistical analysis three replicates for each experiment were evaluated and the results were expressed as mean \pm SD. Correlation between antioxidant activities and total phenolic contents were analyzed using SPSS software version 19 (IBM), and P-value less than 0.05 was considered to be significant

RESULTS

Antioxidant activities and total phenolic contents

Antioxidant activities and total phenolic contents of the eighteen plant extracts were shown in Table 2. The results indicated that the total phenolic contents of the eighteen extracts exhibited a wide range of 0.27 ± 0.01 to 52.56 ± 0.73 mg GAE/g dry weight. In case of antioxidant activities, the values of FRAP assay varied from 1.38 ± 0.03 to 354.95 ± 4.96 μ mol Fe^{2+} /g dried weight while the DPPH free-radical scavenging activities of the extracts expressed as mg of Trolox equivalent were 0.64 ± 0.02 to 54.28 ± 0.21 mg TE/g dry weight. The IC_{50} of extracts were in range 0.13 to 15.05 mg/ml by DPPH assay. The correlation between total phenolic contents and antioxidant activities of the eighteen plant extracts were statistically analyzed by SPSS 19. The r values of DPPH and FRAP assay were 0.84 and 0.97, respectively (Figure 1). The results of both antioxidant methods expressed well with positive linear correlation with total phenolic contents.

The extract of *C. sappan* exhibited the highest antioxidant activity (354.95 ± 4.96 μ mol Fe^{2+} /g dried weight) and total phenolic contents (52.56 ± 0.73 mg GAE/g dry weight) by FRAP and the Folin-Ciocalteu

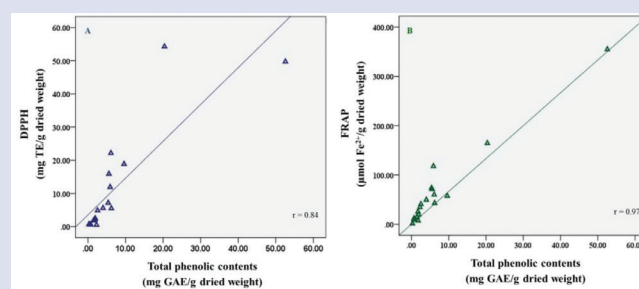


Figure 1: Relationship between total phenolic contents and antioxidant activities of 18 plant extracts analyzed by DPPH assay (A) and FRAP assay (B).

Table 2 Total phenolic contents and antioxidant activity of the eighteen plant extracts

Plant extract	Total phenolic contents (mg GAE/g dry wt)	Antioxidant activities		
		DPPH radical scavenging activity		Ferric reducing activity ($\mu\text{mol Fe}^{2+}$ /g dry wt)
		(mg TE/g dry wt)	IC ₅₀ (mg/ml)	
<i>P. crinitum</i>	5.39±0.09	7.16±0.13	0.49	73.60±1.04
<i>O. sativa</i> (R ₁)	0.70±0.03	0.64±0.02	3.08	12.03±0.21
<i>O. sativa</i> (R ₂)	0.27±0.01	0.70±0.04	11.80	1.38±0.03
<i>O. sativa</i> (R ₃)	1.80±0.03	2.05±0.02	1.89	7.40±0.12
<i>C. aromatica</i>	9.61±0.22	18.86±0.08	0.88	57.69±2.95
<i>C. aurantiaca</i>	6.11±0.16	22.16±0.53	1.18	60.17±2.45
<i>C. aeruginosa</i>	1.94±0.04	2.45±0.05	2.67	19.78±0.18
<i>C. zedoaria</i>	6.22±0.17	5.54±0.09	1.83	43.14±0.32
<i>K. galangal</i>	0.68±0.02	0.64±0.01	15.05	9.61±0.92
<i>B. strychnifolia</i>	20.37±0.40	54.28±0.21	0.13	164.53±0.20
<i>C. sappan</i>	52.56±0.73	49.74±0.99	0.14	354.95±4.96
<i>B. chinensis</i>	1.76±0.03	2.22±0.02	3.05	25.84±1.43
<i>E. americana</i>	2.55±0.05	4.88±0.11	0.63	40.84±0.25
<i>C. fennestratum</i>	2.28±0.10	0.52±0.01	7.21	34.76±0.90
<i>C. pareira</i>	1.47±0.04	1.82±0.06	2.42	15.30±0.55
<i>P. amarus</i>	5.90±0.20	11.94±0.22	0.22	117.95±0.44
<i>O. aristatus</i>	5.57±0.11	15.90±0.44	0.37	71.34±0.62
<i>V. calycalata</i>	4.00±0.02	5.60±0.13	0.74	49.77±0.90

*Note; the results are expressed as mean \pm SD (n = 3)

assays. The extract of *B. strychnifolia* exhibited the highest capacities to neutralize DPPH free-radicals (54.28±0.21 mg TE/g dry weight) with slightly higher than extract of *C. sappan* (49.74±0.99 mg TE/g dry weight). The IC₅₀ of *B. strychnifolia* extract (0.13 mg/ml) slightly differed from *C. sappan* extract (0.14 mg/ml) supporting the high antioxidant activity of both extract of *C. sappan* and *B. strychnifolia* by DPPH assay.

Antibacterial activity

The eighteen crude plant extracts (250 mg/ml in DMSO) were evaluated for their antibacterial activities against the six pathogenic bacteria which were separated into three G-positive bacteria (*Bacillus cereus* TISTR687, *Staphylococcus aureus* TISTR1466 and *Enterococcus faecalis* TISTR379) and three G-negative bacteria (*Enterobacter aerogenes* TISTR1540, *Salmonella typhimurium* TISTR292 and *Escherichia coli* TISTR780) by agar disc diffusion method. The results showed in Table 3.

All eighteen plant extracts could inhibit *B. cereus* (G-positive bacteria) and the inhibition zone was ranging from 6.50±0.00 to 26.83±0.76 mm. The eleven plant extracts which were *C. aromatica*, *C. aurantiaca*, *C. aeruginosa*, *C. zedoaria*, *K. galanga*, *B. strychnifolia*, *C. sappan*, *E. americana*, *C. fennestratum*, *P. amarus* and *V. calycalata* could inhibit *S. aureus* and *E. faecalis* (G-positive bacteria) with the inhibition zone ranging from 6.67±0.29 to 27.50±0.87 mm. However, the seventeen plant extracts could not inhibit the growth of those three G-negative bacteria. Only the *C. sappan* extract from the heart wood showed remarkable broad-spectrum inhibition activity against both G-positive and G-negative bacteria and the zone of inhibition was in range 26.83±0.76 to 27.50±0.87 mm and 10.00±0 to 14.83±0.76 mm, respectively Table 3 and Figure 2. The 100% DMSO was performed as negative control in all experiments and all shown no inhibition.

Scanning Electron Microscopy (SEM)

The morphological changes of G- positive and G-negative bacterial cells after treated with the extract of *C. sappan* were observed under the SEM and the results showed in Figure 3 and Figure 4. Two G-positive bacterial cells of *E. faecalis* TISTR379 and *S. aureus* TISTR1466 treated with *C. sappan* were swollen and some bacterial cells were broken which indicated by the red arrows (Figure 3: A2 and B2) when compared with the untreated cells (Figure 3: A1 and B1). A different result was found in the cells of *B. cereus* TISTR687. The treated cells of *B. cereus* TISTR687 showed different size and some area on the cell surface was cracked (Figure 3: C2), while untreated cells exhibited a normal shape (Figure 3: C1). The G-negative bacterial cells after treated with *C. sappan* extract were shown in the Figure 4. The cells of *S. typhimurium* TISTR292 have shorten and deformed (Figure 4: D2) when compared with the untreated group (Figure 4: D1). In addition, small pores appeared on the cell surfaces of *E. aerogenes* TISTR1540 (Figure 4: E2). However, the size of *E. aerogenes* TISTR1540 cells treated by the extract of *C. sappan* is like the normal cells (Figure 4: E1). The size of cells of *E. coli* TISTR780 after treated with the extract increased and deformed (Figure 4: F2) compared to normal cells (Figure 4: F1).

DISCUSSION

The Thai medicinal plants in this study were selected based on their use in traditional medicine for health promotion. Ethnomedical claims have advantages for selecting plant samples based on their continuous use by humans.²³ So, the bioactive compounds contained in these plants can be safer than the plants with no ethnomedical records. The extracts of selected plants were evaluated according to their antioxidant activity, total phenolic contents and antibacterial activity. The investigation for

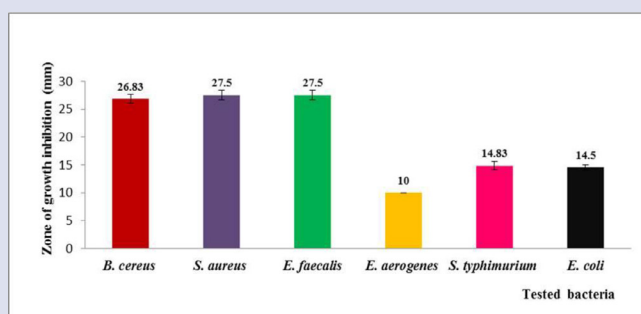


Figure 2: Diameter of inhibition zone of the ethanol extract of *Caesalpinia sappan* on growth of the six pathogenic bacteria.

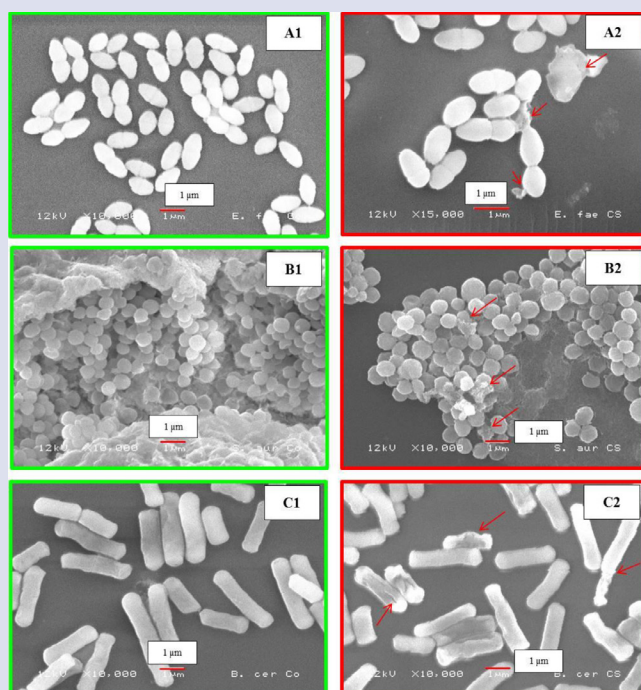


Figure 3: Scanning electron micrographs of Gram-positive cells: *E. faecalis* TISTR379 (A2), *S. aureus* TISTR1466 (B2) and *B. cereus* TISTR687 (C2) treated with the 95% ethanol extract of *Caesalpinia sappan* at 37°C for 24 hours and compared to the untreated cells: *E. faecalis* TISTR379 (A1), *S. aureus* TISTR1466 (B1) and *B. cereus* TISTR687 (C1).

their properties could give us the information on a new potential source for health promotion.

Antioxidant activities and total phenolic contents were evaluated in ethanol extract of eighteen plant sample by using two *in vitro* methods. The FRAP assay was used to evaluate the capacity of extracts to reduce ferric ions (Fe^{3+}) to ferrous ion (Fe^{2+}). This method is rapid for antioxidant determination. DPPH was evaluated only the capacity of extracts to neutralize free radicals by transferring electron or hydrogen atom to DPPH.^{24,25} DPPH assay is the most frequently used for *in vitro* antioxidant determination for plant sample because this method is simple and inexpensive.²⁶ Among all extracts, the extract *Caesalpinia sappan* exhibited the highest antioxidant activity measured by FRAP assay ($354.95 \pm 4.96 \mu\text{mol Fe}^{2+}/\text{g}$ dried weight), while extracts of *B. strychnifolia* and *C. sappan* exhibited the high capacities to neutralize DPPH free-radicals (54.28 ± 0.21 and $49.74 \pm 0.99 \text{ mg TE/g}$

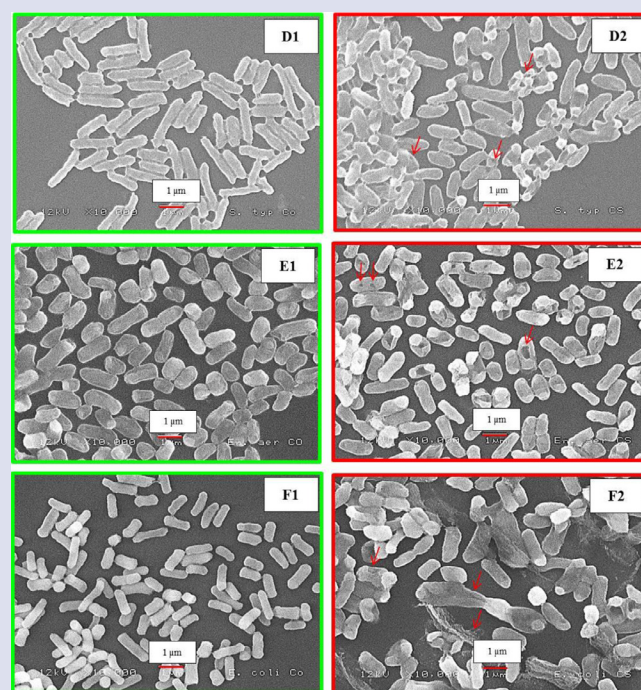


Figure 4: Scanning electron micrographs of Gram-negative cell: *S. typhimurium* TISTR292 (D2), *E. aerogenes* TISTR1540 (E2) and *E. coli* TISTR780 (F2) treated with the 95% ethanol extract of *Caesalpinia sappan* at 37°C for 24 hours and compared to the untreated group: *S. typhimurium* TISTR292 (D1), *E. aerogenes* TISTR1540 (E1) and *E. coli* TISTR780 (F1).

dry weight, respectively) with IC_{50} values of 0.13 and 0.14 mg/ml, respectively. Considering both extracts of *C. sappan* and *B. strychnifolia* also presented capacity to use as antioxidant sources. Correlation between total phenolic contents and antioxidant activities of eighteen plant extracts were further studied using SPSS 19 software. A positive correlation between total phenolic contents and antioxidant activities in all extracts exhibited the significant linear relationship with r values of 0.84 for DPPH assay and 0.97 for FRAP assay. This result indicated that phenolic compounds probably were the dominant antioxidant components in the plant samples.

The results of antibacterial activity indicated that the ethanol extract of *C. sappan* from heart wood showed the wide range of antibacterial activities on the growth of both G-positive bacteria (*Bacillus cereus*) TISTR687, *Staphylococcus aureus* TISTR1466 and *Enterococcus faecalis* TISTR379) and G-negative bacteria (*Enterobacter aerogenes* TISTR1540, *Salmonella typhimurium* TISTR292 and *Escherichia coli* TISTR780). The maximum inhibiting activity of the ethanol extract of *C. sappan* for several microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Enterobacter aerogenes*, *Candida albicans* and *Escherichia coli* have been reported.²⁷ Brazilin (the main bioactive homoisoflavone) and Juglone (5-hydroxy-1, 4-naphthoquinone) were reported as bioactive compounds which presented an antimicrobial property.^{28,29} This information supports our results antimicrobial activity of ethanol extract of *C. sappan* to six pathogenic bacteria in GI tract.

Cells around inhibition zone of the six gastrointestinal pathogenic bacteria after treated by ethanol extract of *Caesalpinia sappan* were further observed using SEM. Considering G-positive bacterial cells, *Staphylococcus aureus* TISTR1466 and *Enterococcus faecalis* TISTR379 were swollen and broken. However, different result was found in the rod-shaped cells

Table 3: Antibacterial activities of plant extracts against the six pathogenic bacteria

Plant extract	Zone of inhibition (mm)					
	Gram-positive bacteria			Gram-negative bacteria		
	<i>B. cereus</i> TISTR687	<i>S. aureus</i> TISTR1466	<i>E. faecalis</i> TISTR379	<i>E. aerogenes</i> TISTR1540	<i>S. typhimurium</i> TISTR292	<i>E. coli</i> TISTR780
<i>P. crinitum</i>	9.00±0.50	7.83±0.04	ni	ni	ni	ni
<i>O. sativa</i> (R_1)	6.50±0.00	ni	ni	ni	ni	ni
<i>O. sativa</i> (R_2)	6.50±0.00	8.33±0.58	ni	ni	ni	ni
<i>O. sativa</i> (R_3)	6.50±0.00	ni	ni	ni	ni	ni
<i>C. aromatica</i>	10.83±0.29	8.17±0.29	7.00±0.00	ni	ni	ni
<i>C. aurantiaca</i>	10.33±0.58	7.17±0.29	7.17±0.29	ni	ni	ni
<i>C. aeruginosa</i>	11.00±0.50	8.83±0.29	8.83±0.29	ni	ni	ni
<i>C. zedoaria</i>	8.83±0.29	6.67±0.29	6.67±0.29	ni	ni	ni
<i>K. galanga</i>	7.00±0.00	7.67±0.29	7.67±0.29	ni	ni	ni
<i>B. strychnifolia</i>	13.00±0.50	12.67±0.29	11.17±0.29	ni	ni	ni
<i>C. sappan</i>	26.83±0.76	27.50±0.87	27.50±0.87	10.00±0.00	14.83±0.76	14.50±0.50
<i>B. chinensis</i>	7.50±0.50	ni	ni	ni	ni	ni
<i>E. americana</i>	17.50±0.50	16.00±0.50	16.00±0.50	ni	ni	ni
<i>C. fennestratum</i>	19.50±0.50	22.33±1.15	11.33±0.58	ni	ni	ni
<i>C. pareira</i>	6.50±0.00	ni	ni	ni	ni	ni
<i>P. amarus</i>	11.33±0.58	11.50±0.50	6.67±0.29	ni	ni	ni
<i>O. aristatus</i>	7.33±0.29	6.50±0.00	ni	ni	ni	ni
<i>V. calycalata</i>	12.17±0.29	11.83±0.76	8.33±0.29	ni	ni	ni
Negative control (DMSO)	ni	ni	ni	ni	ni	ni

*Note; the results are expressed as mean±SD; the concentration of tested extracts is 250 mg/ml (30µl/disc); the negative control is 100% DMSO (30 µl/disc); ni = no inhibition

of *Bacillus cereus* TISTR687 with cell surface was cracked before damaged. Kamonwannasit *et al.*³⁰ have demonstrated that the extract of the *Aquilaria crassna* leaf could inhibit the growth of *Staphylococcus epidermidis*. Scanning electron micrographs also showed the swollen cells after treated by the extract of *A. crassna*. Moreover, biofilm formation of *S. epidermidis* was inhibited by the plant extract. A similar effect has been found in this study which showed that *S. aureus* TISTR1466 exhibited disposing of cells compared to dense cells of untreated cells. It is possible that the biofilm produced by *S. aureus* TISTR1466 was reduced after treatment with the extract of *C. sappan*. The biofilms contribute to most of infectious diseases caused by bacterial and fungal pathogens.³¹ The results from SEM micrograph indicate that the extract of *C. sappan*, which contains the high concentration of phenolic contents, able to inhibit the growth of bacteria and decrease in virulent infection of *Staphylococcus aureus*. In case of G-negative bacteria, cells of *Enterobacter aerogenes* TISTR1540 and *Salmonella typhimurium* TISTR292 after treated

with the extract have shrunk and deformed, while cells of *Escherichia coli* TISTR780 have longer rod and increasing size before broken. Cell surface damage of all tested bacteria was appeared after treatment with the extract and this cause might lead to induced leakage of cells. Previous studies have reported that some phenolic compounds derived from plants had effect on cytoplasmic membrane of bacterial cells and lost membrane integrity.^{32,33,34} These results could be concluded that the *C. sappan* extract has the capacity to interrupt biofilm formation and cell membrane formation, thus can be used as an antimicrobial agent.

CONCLUSION

The ethanol extract of *Caesalpinia sappan* exhibited high antioxidant and phenolic contents related to antibacterial activities against the pathogenic bacteria in GI tract. These results will pave the way to use *C. sappan* as antibacterial in GI tract and beneficial for ingredient of health products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

SEM: Scanning Electron Microscope; **DMSO:** Dimethyl sulfoxide; **DPPH:** 2,2-Diphenyl-1-picryl-hydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **TPTZ:** 2,4,6-triphenylhydrazyl; **MHA:** Muller Hinton Agar; **GI tract:** Gastro-intestinal tract; **IC₅₀:** The half maximal inhibitory concentration; **TISTR:** Thailand Institute of Scientific and Technological Research; **G-positive:** Gram-positive; **G-negative:** Gram-negative; **GAE:** Gallic acid equivalent; **TE:** Trolox equivalent.

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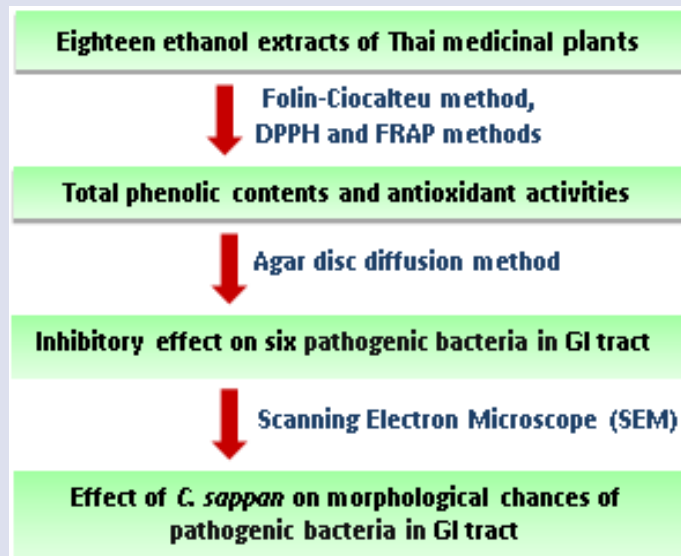


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GRAPHICAL ABSTRACT



SUMMARY

- *Caesalpinia sappan* exhibited strong antioxidant and antibacterial activities against some pathogenic bacteria in GI tract.
- Morphological observation by SEM showed that the extract of *C. sappan* has the capacity to interrupt biofilm formation and cell membrane formation.
- *C. sappan* could be used as antibacterial agent and ingredient of health products.

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