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Antibacterial Activity of Ethanol Extract from Some Thai Medicinal Plants against *Campylobacter Jejuni*

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***Abstract*—**In this study, the forty Thai medicinal plants were used to screen the antibacterial activity against *Campylobacter jejuni*. Crude 95% ethanolic extracts of each plant were prepared. Antibacterial activity was investigated by the disc diffusion assay, and MICs and MBCs were determined by broth microdilution. The results of antibacterial screening showed that five plants have activity against *C.jejuni* including *Adenanthera pavonina* L., *Moringa oleifera* Lam., *Annona squamosa* L., *Hibiscus sabdariffa* L. and *Eupotorium odortum* L. The extraction of *A. pavonina* L. and *A. squamosa* L. produced an outstanding against *C. jejuni*, inhibiting growth at 62.5-125 and 250-500 µg/mL, respectively. The MBCs of two extracts were just 4-fold higher than MICs against *C. jejuni*, suggesting the extracts are bactericidal against this species. These results indicate that *A. pavonina* and *A. squamosa* could potentially be used in modern applications aimed at treatment or prevention of foodborne disease from *C. jejuni*.

***Keywords*—**Antibacterial activity, Thai medicinal plants, *Campylobacter jejuni*

I. Introduction

CAMPYLOBACTER JEJUNI is recognized as the most common bacterial cause of foodborne human gastroenteritis throughout the world [1]. *C. jejuni* is currently estimated to cause 5-14% of diarrhea world wide, which translates into 400-500 million cases per year [2]. This is also recognized as the most identifiable infection preceding Guliiain-Barré syndrome (GBS), which lead to immune-mediated disorder and acute flaccid paralysis [3]. While outbreaks of *Campylobacter* can be transferred to humans by direct contact with contaminated animals, or indirectly through ingestion of contaminated food [4] such as undercooked, meat and poultry products [5, 6], lamb kebabs and unpasteurized dairy [7]. Therefore, some process or treatments were used to prevent or eliminate the population of *Campylobacter* on raw poultry products such as adopting chlorine, trisodium- prosphate and hydrogen peroxide [8]. However, consumers are increasingly avoiding foods prepared with preservative of chemical origin, and natural alternatives are therefore needed to achieve sufficiently long shelf life of foods and high degree of safety with respect to foodborne pathogenic microorganisms [9, 10].

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For centuries, many infectious diseases have been treated with medicinal plants. The use of medicinal plants as complementary and alternative medicine has increased dramatically in the last 20-25 years [11, 12]. According to a WHO report, 80% of the world's inhabitants depend on traditional medicines as their main source of health care [13]. Over 100 chemical substances that are considered to be important drugs and are either currently in use or have been widely used in one or more countries have been derived from a little under 100 plant species. Approximately 75% of these substances were discovered as a direct result of chemical studies focusing on the isolation of active substances from plants used in folk medicine [14]. Thailand is a tropical country with an abundance of diverse plant resources [15]. Many of these have been used medicinally (e.g. ginger) or as food additives (e.g. garlic) to treat or prevent foodborne illness [16]. Medicinal plants are still widely and legally used in traditional Thai medicine and hold great potential for researchers seeking to identify and develop new antibacterial agents [17].

However, few of Thai plants have been investigated for their activity against *C. jejuni*. Then, the aims of this study were to screen the forty Thai medicinal plants for anti-*C. jejuni* and also examined the inhibitory activity of the three plant extract which showed the high anti-*C. jejuni*.

II. Materials And Methods

A. Bacteria and media

C. jejuni ATCC 29428 was obtained from the American Type Culture Collection. Prior to testing, this strain was cultured on brucella agar supplemented with 62.5 mg/L each of ferrous sulphate, sodium metabisulfite and sodium pyruvate and incubated at 42°C for 48 h in microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). Inocula were prepared by suspending bacterial colonies in 0.1% (w/v) sterile peptone water and adjusting to the required cell density using enumeration graphs.

B. Plant collection and extraction

The plants (Table 1) were collected in Walairukavej Botanical Research Institute, Maha Sarakham (Thailand). All plants were rinsed, sliced, dried (50°C for 24 h), ground and sieved (80 mesh), with the resulting powder being added [1:10 (w/v)] to 95% (v/v) ethanol. The suspension was shaken at 120 rev min^{-1} for 24 h (room temperature) and filtered. Excess solvent was removed by rotary evaporation (50°C), and the sample was stored in tightly sealed, foil-wrapped bottles at 4°C until required.

C. Antibacterial assay

Antibacterial activity was assessed against *C. jejuni* strain using the the disc diffusion assay [18] . In brief, pour plates were prepared containing 20 mL agar seeded with 10^6 CFU/mL of strain. Solutions of 50 mg/mL plant extract were prepared by dissolving in 95% ethanol. Sterile 6 mm discs (Whatman) were impregnated with 100 µL of the plant extract solutions, dried at 40ºC. The final mass of extracts on each disc was 5 mg. all disc were added to the agar surface. Negative control discs were prepared with ethanol only. Standard discs of the antibiotic erythromycin discs (10 µg/disc) were served as the positive controls [19]. After 48 h incubation at 42ºC, the diameters of the zones of inhibition were measured. Extracts and antibiotics were tested in triplicate and each experiment was performed twice.

D. MIC and MBC assay

MICs were determined using the broth microdilution method [20]. Assays were performed in 96-well microtitre trays with an inoculum density of $5x10^5$ CFU/mL, in a final volume of 100 μL broth. Crude of the three plant extract which showed the high antibacterial activity were dissolved in DMSO [the final concentration being 2% (v/v) DMSO]. Mueller-Hinton broth supplemented with 5% (v/v) laked sheep blood was used for testing. Positive controls were prepared by inoculating broth containing DMSO only. Negative controls consisted of an uninoculated dilution series of the test agent in broth. Erythromycin was used as a reference standard. MICs were determined after 48 h incubation at 42°C for *C. jejuni*. MBCs were determined using the microtitre trays from the MIC assay [21]. The entire volume of liquid (~100 μL) was aspirated from wells with no visible growth, and streaked across the surface of fresh agar plates. After incubation, colonies were counted and the endpoint was determined by identifying the lowest concentration to cause a 99.9% decrease in CFU numbers. All experiments were repeated to verify the reproducibility of results.

III. Results and Discussion

The inhibitory activities of ethanolic extract of forty Thai medicinal plants obtained using disc diffusion assay against *C. jejuni* are showed in Table I. Values presented are the mean diameter sizes of bacterial tested zones of inhibition. The reference standards erythromycin disc showed inhibitory activity against this bacterium. The 95% ethanol was used as negative control, and did not show any activity.

The five kinds of plant extracts including *Adenanthera pavonina* L., *Moringa oleifera* Lam., *Annona sguamosa* L., *Hibiscus sabdariffa* L. and *Eupotorium odortum* L. , demonstrated antibacterial activity against *C.jejuni.* Different results concerning the antibacterial activity against *C. jejuni* might be due to different secondary metabolites of each plant [22], different geographic sources of the plant used, different types of strains used, and different assay methods [23-25]. The extract exhibiting the greatest activity (as determined by the diameters of zones of inhibition) was the one derived from the leaflet of *A. pavonina* L., with a zone of inhibition greater than 34.5 mm in diameter. The *A. pavonina* extract is reported to contain saponins, alkaloids, tannins and flavonoids [26, 27]. These compounds could be responsible for its antibacterial activity [22]. In these results correspond with the MIC results

In MIC and MBC assay, the three plants (*A. pavonina, M. oleifera* and *A. squamosa*) which showed the high activity against *C. jejuni* are shown in Table II. Of the three plants, *A. pavonina* had the most potent inhibitory activity against *C. jejuni*, with MICs of 62.5-125 µg/mL. *A. squamosa* inhibited this species at the slightly concentration of 250-500 µg/mL. These values compare favourably with those proposed by Ríos and Recio [12] for evaluating the antibacterial activity of medicinal plants. In case of MICs determined for the *M. oleifera* extract were 1,000 µg/mL or higher, indicating this plant has negligible inhibitory activity against *C. jejuni* [12]. Results in Table II suggest that *A. pavonina* and *A. squamosa* extracts may have bactericidal activity. By definition, bactericidal agents have MBC values no more than four times the MIC [1, 28],

IV. Conclusion

The screenings of crude extracts made from Thai medicinal plants have shown that five of the forty plant extracts have antibacterial activity against *C. jejuni*. In this study, the activity of *A. pavonina* and *A. squamosa* extracts possess bactericidal activity against this species. Then, these two plant extracts could be a source of new antibiotic compounds for treating or preventing of foodborne infection from *C. jejuni*. Further investigation is warranted to identify the optimal extraction method, to isolate the secondary metabolites and to fractionate the most active extract.

TABLE I
LOCAL AND SCIENTIFIC NAMES OF PLANT SPECIES SCREENED, PARTS EXTRACTED AND RESULTS OF ANTI-*CAMPYLOBACTER JEJUNI*

Code	Local name	Scientific name	Part of use	Inhibited zone (mm.)
1.	Bai Kut	*Diplazium esculentum* SW.	leaf	-
2.	Bai Mi (Mu Men)	*Thunbergia fragrans* Lour.	leaf	-
3.	Bai Muk	*Wrightia tomentosa* Roem.	leaf	-
4.	Bai Phlu	*Piper betle* L.	leaf	-
5.	Bo ra phet	*Tinospora crispa* L.	leaf	-
6.	Bua bok	*Centella asiatica* L.	leaf	-
7.	Bua bok Nam	*Centella asiatica* L.	leaf	-
8.	Cha phlu	*Piper sarmentosum* Roxb.	leaf	-
9.	Cham ma liang	*Lepisanthes fruticosa* Leenh.	leaf	-
10.	Fa rang khi nok	*Psidium guajava* L.	leaf	-
11.	Kha	*Alpinia galangal* L.	rhizome	-
12.	Khae Dok Khao	*Dolichandrone serrulata* L.	leaf	-
13.	Kra chai Dam	*Boesenbergia pandurata* Roxb.	rhizome	-
14.	Kra chiap Daeng	*Hibiscus sabdariffa* L.	leaf	8.67±0.58
15.	Kra Thin Pa	*Acacia mangium* Willd	leaf	-
16.	Lin Fa (Phe ka)	*Oroxylum indicum* L.	leaf	-
17.	Ma klam	*Adenanthera pavonina* L.	leaflet	34.67±0.58
18.	Ma krut	*Citrus hystri* L.	leaf	-
19.	Ma Mao Pa	*Antidesma velutinosum* Blume.	leaf	-
20.	Ma rum	*Moringa oleifera* Lam.	leaf	20.67±2.31
21.	Maeng lak	*Ocimum basilicum* L.	leaf	-
22.	Mak	*Areca eatechu* L.	seed	-
23.	Met Ma Mao Pa	*Antidesma velutinosum* Blume.	seed	-
24.	Noi na	*Annona sguamosa* L.	leaf	13.67±1.15
25.	Non si	*Peltophorum pterocarpum* D.C.	leaf	-
26.	Pae Tam Pueng	*Gynura divaricata* D.C.	leaf	-
27.	Phak Kan Trong	*Colubrina asiatica* L.	leaf	-
28.	Phak Kha yaeng	*Limnophila aromatica* Merr.	leaf	-
29.	Phet sang khat	*Cissus Quadrangularis* L.	leaf	-
30.	Pla lai phueak	*Eurycoma longifolia* Jack.	leaf	-
31.	Rak Nguang Sum	*Getonia floribunda* Lark.	leaf	-
32.	Rang chuet	*Thunbergia lauriflolia* L.	leaf	-
33.	Sa let phang phon	*Barleria luputina* Lindi.	leaf	-
34.	Sab sua	*Eupotorium odortum L.*	leaf	7.33±0.58
35.	Ta khrai	*Cymbopogon citratus* Stapf.	All part	-
36.	Thao Boraphet	*Tinospora crispa* L.	stem	-
37.	thong Phan Chang	*Rhinacanthus nasutus* L.	leaf	-
38.	Ton Kha Non	*Pouzolzia pentandra* Benn.	leaf	-
39.	Ya Dok Khao	*Leptochloa chinensis* L.	leaf	-
40.	Yi ra	*Ocimum gratissimum* L.	seed	-
		Positive control (erythromycin)		24.33±0.58
		Negative control (95% ethanol)		-

Data presented is the mean diameter size of zone of inhibition ± SD (mm) for six replicates. -, no activity detected.

TABLE II
THE MIC AND MBC (MG/ML) OF THREE PLANT EXTRACT AGAINST *C. JEJUNI*

Samples	MIC (µg/ml)	MBC (µg/ml)
A. pavonina L.	0.625-125	250
Moringa oleifera Lam.	1,000-2,000	4,000
Annona sguamosa L	250-500	≥2,000
Erythromycin	4	4

Note: MICs were tested up to a maximum concentration of 2,000 µg/ml and MBCs were tested up to a maximum concentration of 8xMIC

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REFERENCES

[1] Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology. 6th edition. Philadelphia: Elsevier Mosby; 2009.

[2] Friedman C, Neimann J, Wegener H, Tauxe R. Epidemiology of *Campylobacter jejuni* Infections in the United States and Other Industrialized Nations *Campylobacter*. 2 ed: ASM Press; 2000: p. 121 - 38.

[3] Näther G, Alter T, Martin A, Ellerbroek L. Analysis of Risk Factors for *Campylobacter* Species Infection in Broiler Flocks. Poult Sci. 2009 June 1, 2009;88(6): 1299-305.

[4] Figueroa G, Troncoso M, Lopez C, Rivas P, Toro M. Occurrence and Enumeration of *Campylobacter* spp. During the Processing of Chilean Broilers. BMC Microbiology. 2009;9(1): 94.

[5] Habib I, Sampers I, Uyttendaele M, Berkvens D, De Zutter L. Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. Appl Environ Microb. 2008 September 1, 2008;74(17): 5483-9.

[6] Mor-Mur M, Yuste J. Emerging Bacterial Pathogens in Meat and Poultry: an Overview. Food and Bioprocess Tech. 2010;3(1): 24-35.

[7] Centers for Disease Control and Prevention. Outbreak of *Campylobacter jejuni* Infections Associated with Drinking Unpasteurized Milk

Produced Through a Cow-Leasing Program---Wisconsin, 2001. MMWR weekly. 2002 June, 28;Sect. 548-9.
[8] Lee C-F, Han C-K, Tsau J-L. In vitro inhibitory activity of Chinese leek extract against *Campylobacter* species. Int J Food Microbiol. 2004;94(2): 169-74.
[9] Ramadan MF, Selim Asker MM. Antimicrobical and antivirial impact of novel quercetin-enriched lecithin. J Food Biochem. 2009;33(4): 557-71.
[10] Rauha J-P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 2000;56(1): 3-12.
[11] Kaur GJ, Arora DS. Antibacterial and Phytochemical Screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC complementary and alternative medicine. 2009 August, 6: 1-10.
[12] Ríos JL, Recio MC. Medicinal Plants and Antimicrobial Activity. J Ethnopharmacol. 2005;100(1-2): 80-4.
[13] Ballabh B, Chaurasia OP. Traditional Medicinal Plants of Cold Desert Ladakh--Used in Treatment of Cold, Cough and Fever. J Ethnopharmacol. 2007;112(2): 341-9.
[14] Grabley S, Thiericke R. Drug discovery from nature. Berlin: Springer-Verlag; 2000.
[15] Thailand Public Relations Department. Thailand Declares 2010 the Year of Diodiversity. Thailand: Thai Government; 2009 December, 22.
[16] Craig W. Ginger - Useful for More Than Just Candy. *Vegetarianism & Vegetarian Nutrition* 2003.
[17] Saralamp P, Chukul W, Temsiririrkkul R, Clayton T. Medicinal Plants in Thailand Volume I. Bangkok: Faculty of Pharmacy, Mahidol University; 1996.
[18] Ratnam KV, Raju RRV. *In vitro* Antimicrobial Screening of the Fruit Extracts of two *Syzygium* Species (Myrtaceae). Advances in Biological Research. 2008;2(1-2): 17-20.
[19] Philip K, Malek SNA, Sani W, Shin SK, Kumar S, Lai HS, et al. Antimicrobial activity of some medicinal plants from Malaysia. Am J Appl Sci. 2009;6(8): 1613-7.
[20] CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Guideline (M7-M5). Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2000.
[21] CLSI. Methods for Determining Bactericidal Activity of Antimicrobial Agents: Approved Guideline (M26-A). Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 1999.
[22] Cowan MM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev. 1999 October 1, 1999;12(4): 564-82.
[23] Al-Bayati F, Al-Mola H. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. Journal of Zhejiang University - Science B. 2008;9(2): 154-9.
[24] Ncube NS, Afolayan AJ, Okoh AI. Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends. Afr J Biotechnol. 2008;7(14): 1797-806.
[25] Nostro Aea. Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. Lett Appl Microbiol. 2000;30: 379-84.
[26] Adedapo ADA, Osude YO, Adedapo AA. Blood Pressure Lowering Effect of *Adenanthera pavonina* Seed Extract on Normotensive Rats Aduragbenro. Records of Natural Products. 2009;3(2): 82-9.
[27] Ara A, Saleh-e-In MM, Ahmed NU, Ahmed M, Hashem A, Bachar SC. Phytochemical Screening, Analgesic, Antimicrobial and Anti-Oxidant Activities of Bark Extracts of *Adenanthera pavonina L*. (Fabaceae). Adv Nat Appl Sci. 2010;4(3): 352-60.
[28] Prescott LM, Harley JP, Klein DA. Microbiology. 6th edition. New York: Mc Graw-Hill; 2005.