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Efficacy of Thai Herbal Extract for Growth Inhibition of *Xanthomonas axonopodis* pv. *citri* , the Bacterial Canker of Citrus

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ABSTRACT

The efficacy test of Thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. *citri*, the causal agent of bacterial canker of citrus, was studied. The experiment was conducted by Paper disc diffusion method on double layer NGA. The 23 kinds of Thai herbal extracted by 95% ethyl alcohal at the concentration of 100,000 ppm were tested. Five kinds of those could inhibit the growth of bacteria on culture media and showed the typical inhibition zone. Those were guava leaf, beleric myrobalan fruit, pomegranate fruit peel, nut gall fruit and myrobalan wood fruit. But the other 18 kinds showed no inhibition zone. The 5 kinds of extract that showed high efficacy were diluted to 5 concentration lavels of 100,000, 50,000, 10,000, 5,000, and 1,000 ppm and tested by paper disc diffusion method again. It was founded that the guava leaf extract could inhibit the growth of bacteria at 50,000 ppm, the myrobalan wood fruit extract could inhibit the growth of bacteria at 10,000 ppm, the extract of beleric myrobalan fruit, nut gall fruit and pomegranate fruit peel could inhibit the growth of bacteria at all concentration levels started at 1,000 ppm.

Key words: bacterial canker, citrus, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas campestris* pv. *citri*, Thai herbal extract

INTRODUCTION

Bacterial canker of citrus caused by *Xanthomonas axonopodis* pv. *citri* is one of the most serious citrus diseases, affecting all type of important citrus crops. It causes necrotic lesions on fruit, leaves, and twigs. Losses are caused by reduced fruit quality and quantity and premature fruit drop. All citrus-producing countries without canker maintain a strict prohibition on import of citrus plants and fruit from non-canker-free countries. The symptom are appear on young leaves, twigs, and fruits. The lesions at first is small, slightly raised, round, light green spots. Later, they become graynish white, rupture, and appear corky with brown, sunken centers. The margins of the lesions are often surrounded by a yellowish halo. The size of the lesions varies from 1 to 9 mm in diameter on leaves and up to 1 cm in diameter or length on fruits and twigs. Severe infections of leaves, twigs, and branches debilitate the tree, while severely infected fruit appear scabbed and deformed. The casual agent was *Xanthomonas axonopodis* pv. *citri* (formerly *Xanthomonas campestris* pv. *citri*), the Gram negative bacteria, straight rods 0.5-0.75×1.5-2.0 micrometer, motile by monotrichous flagellum, strictly aerobe, hydrolyse starch and gelatin very well. The bacteria

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overseason in leaf, twig, and fruit canker lesions. During warm (27-30°C), rainy weather the bacterial ooze exuding from lesions and splashed onto young tissues, enter through stomata or wounds. Free moisture and strong winds seem to greatly favor the widespread of the disease. Control of citrus canker obtained by using disease-free stocks, pruning, burning all infected, and applying copper formulations sprays. (Agrios, 1977; Schaad,1988; Fawcett, 1936)

For the last 10 years, the herbal extract has been used in the human and animal diseases therapy. There are many reports on using herbal extract in plant disease control. However, only few of them are on phytopathogenic bacteria. Wongkaew *et al*. (1997) reported that crude extract of Koon (Golden shower : *Cassia fistula*) leaf , Chum-hed-ted (Ringworm brush : *Cassia allota*) leaf and tumeric (*Curcuma longa*) rhizome could inhibit the growth of *Erwinia carotovora, Pseudomonas solanacearum,* and *Xanthomonas campestris* pv. *vesicatoria.* Molina *et al*.(1999) reported that Amides affinin from *Heliopsis longipes* root at high concentration level could inhibit the growth of *Escherichia coli, Saccharomyces cerevisiae* and *Pseudomonas solanacearum.* Capsaicin from chille fruit at high concentration could retard the growth of *P. solanacearum* and inhibit *Bacillus subtilis* but could not inhibit *S. cerevisiae*. Therefore studying on efficacy of Thai herbal extract for growth inhibition of phytopathogenic bacteria is very interesting topic for researchers.

MATERIALS AND METHODS

Twenty-three kinds of Thai herbs which previously recorded on antimicrobial effects were selected for this experiment (Table 1).

Two hundred g of each Thai herb was cut or grinded to small pieces. Each of them was soaked into 400 ml of the 95 % ethyl alcohol. After soaking for 7 days, the plant tissue was removed by filtration. The solution was centrifuged at 8000 rpm for 10 minutes to remove plant debris. The supernatant was transferred and concentrated in the rotary vacuum evaporater. The 0.1 g of dried herbal extract was resuspended in 95% ethyl alcohol to make the concentration of 100,000 ppm and diluted to 50,000, 10,000, 5,000 and 1,000 ppm respectively.

Xanthomonas axonopodis pv. *citri* was isolated from diseased citrus by tissue transplanting method on SX agar. After incubated for 48 hours at room temperature (30°C), the light yellow colony exuted from plant tissue with clear zone surround them. A single colony was picked up and suspended in steriled water and cross streak on nutrient glucose agar (NGA) or yeast dextrose calcium carbonate agar (YDC) to make pure culture. Virulent colonies of small round, yellow, convex were picked up and maintained in sterile distilled water and kept in a cooler at 13°C. The bacterial suspension was prepared by transfering the virulent colony to nutrient glucose broth (NGB), incubated in rotary shaker at 30°C for 24 hours. The optical density (OD) was measured by spectrophotometer to reach 0.1 at 590 nanometer wavelength. Some of the bacterial suspention was inoculated into disease-free lime leaves by Detach leaf technique (Boonyawattana,1991) for pathogenicity test.

Double layer nutrient glucose agar was prepared by pouring 10 ml at 55°C nutrient agar into steriled petridish and allowing it cool for basal layer. The top layer prepared by mixing 0.5 ml of bacterial suspension with 10 ml of nutrient agar at 55°C. These media were immediately used for efficacy test.

Ten microlitre of each herbal extract at the concentration of 100,000 ppm was drop three times on 0.6 cm diameter steried paper disc by micropipette, air dry in steriled chamber and placed on double layer NGA. The 95% ethyl alcohal was used as the control treatment. The diameter of inhibition zone was measured after 24 hours incubation at 30°C. The extract of herbs that could inhibit the growth of bacteria were diluted to 5

Table 1 Twenty-three kinds of Thai herbs that used in this experiment.

No.	Common name / Thai name	Scientifics name	Family name	Part of use
1	Kam-phaeng-chet-chun	*Litosanthes biflora*	Rubiaceae	Leaf
2	Kae-lae	*Broussonetia kurzii*	Moraceae	All part
3	Tumeric	*Curcuma longa*	Zingiberaceae	Rhizome
4	Annatto Tree	*Bixa orellana*	Bixaceae	Flower
5	Cha-plu	*Piper aurantiacum*	Piperaceae	Leaf
6	Toy-ting	*Hygrophila erecta*	Acanthaceae	Seed
7	Flame of forest	*Butea monosperma*	Papilionaceae	Leaf
8	Thong-phun-chung	*Rhinacanthus nasutus*	Acanthaceae	Leaf
9	Pomegranate	*Punica granatum*	Punicaceae	Fruit peel
10	Egyptian rivet	*Lowsonia inermis*	Lythraceae	All part
11	Copper pod , Non-tri	*Peltophorum pterocarpum*	Caesalpiniaceae	Bark
12	Nut Gall	*Polyscias scutellaria*	Araliaceae	Fruit
13	Sappan Tree	*Caesalpinia sappan*	Caesalpiniaceae	All part
14	Guava	*Psidium guajava*	Myrtaceae	Leaf
15	Phlai	*Zingiber cassumunar*	Zingiberaceae	Rhizome
16	Otaheite Apple , Olive	*Spondias cytherea*	Anacardiaceae	Fruit
17	Mango	*Mangifera indica*	Anacardiaceae	Seed
18	Ra-cha-dud	*Brucea javanica*	Simaroubaceae	Seed
19	Lep-yeil	*Zizyphus oenoplia*	Rhamnaceae	All part
20	Wan-chug-mod-loog	*Curcuma xanthorrhiza*	Zingiberaceae	Rhizome
21	Sea myrobalan	*Sapium nidicum*	Euphorbiaceae	Fruit
22	Myrobalan Wood	*Terminalia chebula*	Combretaceae	Fruit
23	Beleric myrobalan	*Terminalia bellerica*	Combretaceae	Fruit

concentration levels of 100,000, 50,000, 10,000, 5,000 and 1,000 ppm and tested by the same method. There were 13 replications (plates) for each herbal extract.

RESULTS AND DISCUSSION

The efficacy test of Thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. *citri*, the causal agent of bacterial canker of citrus, was conducted by paper disc diffusion method. From 23 kinds of Thai herbal extracted by 95% ethyl alcohol at the concentration of 100,000 ppm. Five kinds of them could inhibit the growth of bacteria on double layer NGA. Those were the extract of guava leaf, beleric myrobalan fruit, pomegranate fruit peel, nut gall fruit, and myrobalan wood fruit . The extract of nut gall fruit showed the largest average inhibition zone of 1.68 cm. The others were the extract of pomegranate fruit peel (1.66 cm), myrobalan wood fruit (1.42 cm), beleric myrobalan fruit (1.21 cm), and guava leaf (1.02 cm). But the other 18 kinds of the herbal extract showed no inhibition zone. When the extract of those high efficacy in inhibit the growth of bacteria were diluted to the 5 concentration levels of 100,000, 50,000, 10,000, 5,000 and 1,000 ppm and were tested by paper disc diffusion method

again. It was founded that the guava leaf extract could inhibit the growth of bacteria at 50,000 ppm, the myrobalan wood fruit extract could inhibit the growth of bacteria at 10,000 ppm, the extract of beleric myrobalan fruit , nut gall fruit and pomegranate fruit peel could inhibit the growth of bacteria at all concentration levels started at 1,000 ppm. The diameter of inhibition zone varied according to the concentration levels of the herbal extract. (Table 2, 3 and Figure 2)

The experimental result showed that the inhibition zone diameters were different according to the kinds and concentration levels of herbal extract. However, the active ingredients in Thai herbal extract depended on the kinds of herbs, kinds and concentration of the solvent and methods of extraction. Eighteen kinds of the herbal extract that showed no inhibition zone did not mean no active ingredient. It might release at a small amount in 95% ethyl alcohol. Vudhivanich and Supanuntorn (2002) reported that each kind of herbal active ingredient was dissolved by different percentages of ethyl alcohol. Crude extract of the same herb could inhibit the growth of some

Table 2 Efficacy test of 23 Thai herbal extracts for growth inhibition of *Xanthomonas axonopodis* pv. *citri*, the bacterial canker of citrus.

No.	Common name / Thai name	Part of use	Reaction
1	Kam-phaeng-chet-chun	Leaf	-
2	Kae-lae	All part	-
3	Tumeric	Rhizome	-
4	Annatto tree	Flower	-
5	Cha-plu	Leaf	-
6	Toy-ting	Seed	-
7	Flame of forest	Leaf	-
8	Thong-phun-chung	Leaf	-
9	Pomegranate	Fruit peel	+
10	Egyptian rivet	All part	-
11	Copper pod , Non-tri	Bark	-
12	Nut gall	Fruit	+
13	Sappan tree	All part	-
14	Guava	Leaf	+
15	Phlai	Rhizome	-
16	Otaheite apple , Olive	Fruit	-
17	Mango	Seed	-
18	Ra-cha-dud	Seed	-
19	Lep-yeil	All part	-
20	Wan-chug-mod-loog	Rhizome	-
21	Sea myrobalan	Fruit	-
22	Myrobalan wood	Fruit	+
23	Beleric myrobalan	Fruit	+

+ showed inhibition zone - no inhibition zone

phytopathogenic bacteria but could not inhibit other species as the reported by Wongkaew *et al.* (1997) that crude extract of tumeric rhizome could inhibit the growth of *E. carotovora, P. solanacearum,* and *X. campestris* pv. *vesicatoria.* Leksomboon *et al.* (1998) and Vudhivanich and Supanuntorn (2002) reported that crude extract of guava leaf could inhibit the growth of *R. solanacearum.* In this experiment, crude extract of tumeric rhizome and guava leaf could not inhibit *X. axonopodis pv. citri.* However, the difference of extraction methods would cause the different experimental results and conclusions. Therefore the study on the suitable method of extraction for each Thai herb need further investigation.

CONCLUSION

From twenty-three kinds of Thai herbal extract which were tested, five kinds of them could inhibit the growth of *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus canker, and showed visible inhibition zone. Those were the extract of guava leaf, beleric myrobalan fruit, pomegranate fruit peel, nut gall fruit, and myrobalan wood fruit. The guava leaf extract could inhibit the

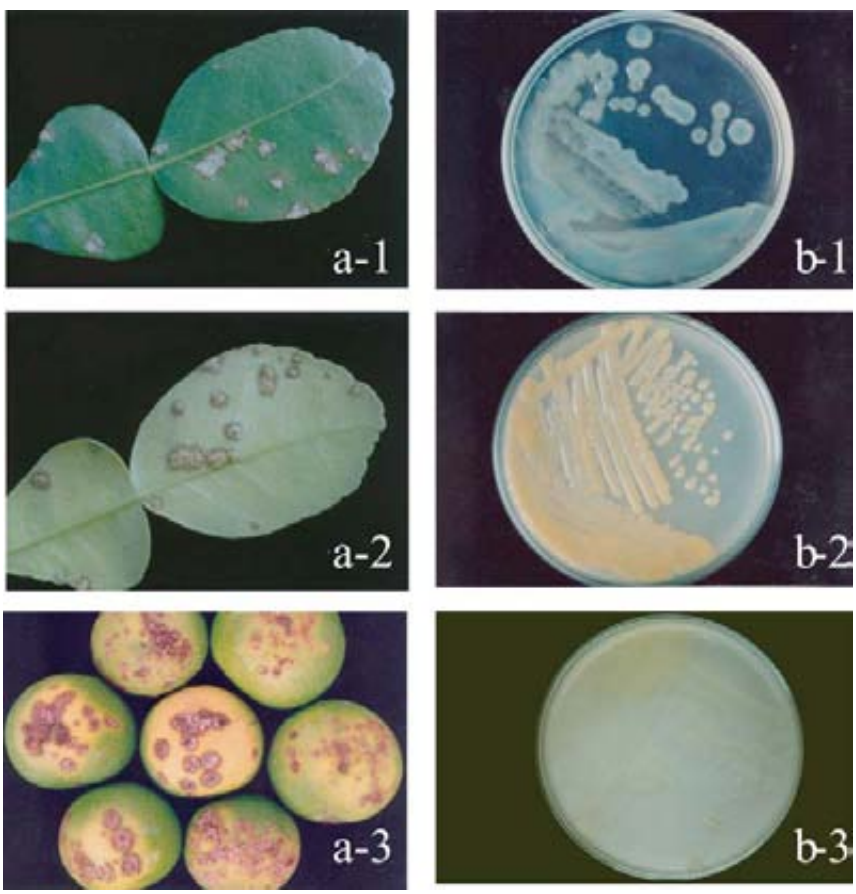

Figure 1 Canker disease symptoms and cultural characteristic of *Xanthomonas axonopodis* pv. *citri.*
a. The symptom shows brown corky raise lesions surrounded by water soak area and yellowish halo. (a-1 upper leaf, a-2 lower leaf, a-3 fruit)
b. Colony of bacteria on culture media. (b-1 SX agar , b-2 YDC , b-3 NGA)

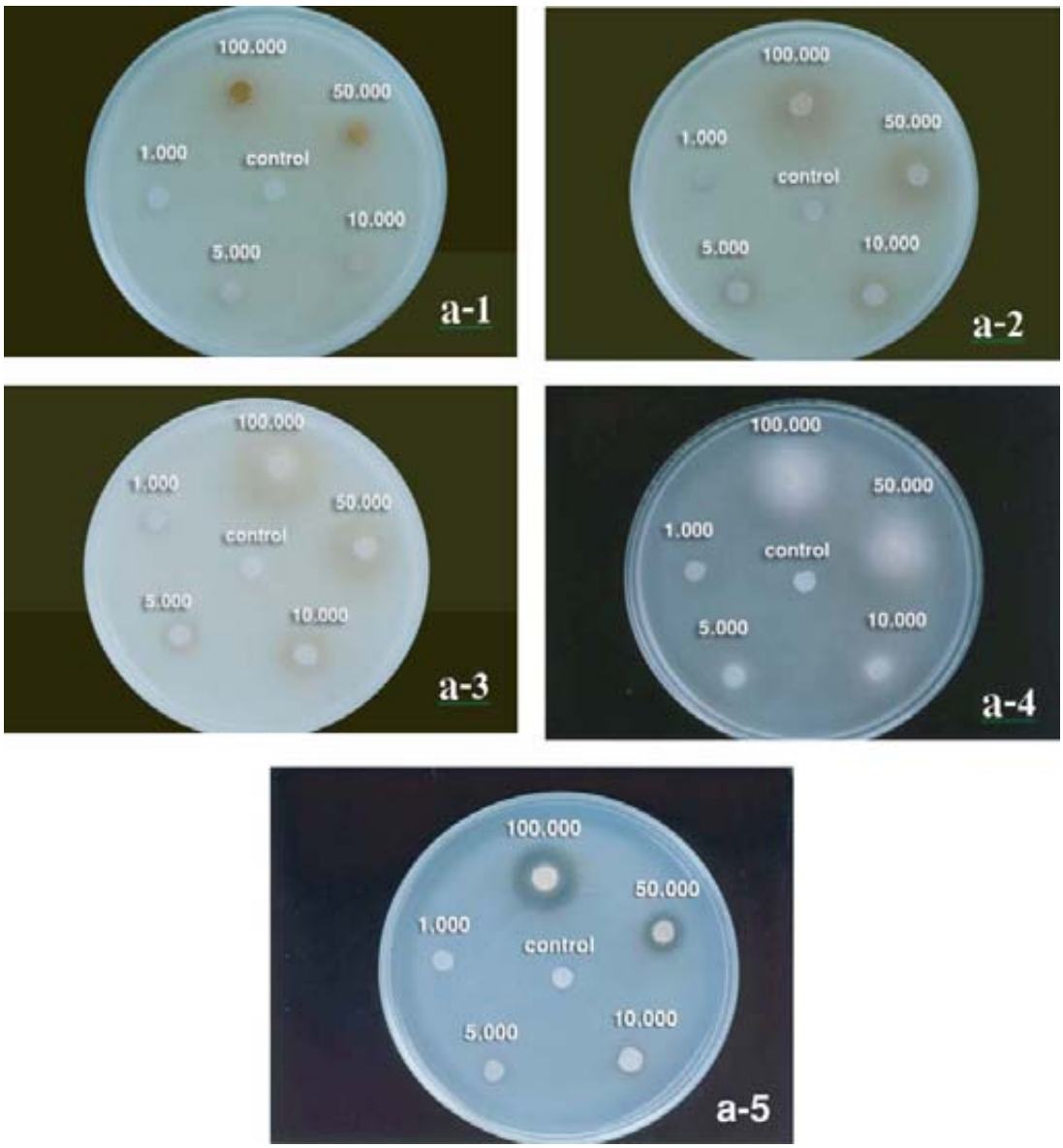

Figure 2 Visible inhibition zone on double layer NGA by paper disc diffusion method.

a-1 The extract of guava leaf

a-2 The extract of beleric myrobalan fruit

a-3 The extract of pomegranate fruit peel

a-4 The extract of nut gall fruit

a-5 The extract of myrobalan wood fruit

Table 3 Efficacy test of Thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. *citri*.

Plant extract	Conc.	Diameter of inhibition zone (cm) 13 rep.													
	ppm	1	2	3	4	5	6	7	8	9	10	11	12	13	Aver.
1 Guava	100,000	0.9	0.9	1	0.9	1.2	1.2	1.1	1.1	1	1	1	1	1	**1.02**
(leaf)	50,000	0.9	1	1	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.9	**0.88**
	10,000	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
	5,000	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
	1,000	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
2 Pomegranate	100,000	1.7	1.8	1.8	1.5	1.7	1.6	1.7	1.8	1.4	1.7	1.6	1.5	1.8	**1.66**
(fruit peel)	50,000	1.3	1.2	1.2	1.2	1.2	1.2	1.3	1.1	1.2	1.3	1.1	1.2	1.3	**1.22**
	10,000	0.9	0.9	0.8	1	0.8	0.8	0.9	0.8	0.9	0.8	0.9	0.9	0.8	**0.88**
	5,000	0.8	0.7	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.8	0.8	0.7	**0.76**
	1,000	0.7	0.6	0.6	0.7	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.6	**0.65**
3 Beleric myrobalan	100,000	1.2	1.2	1.1	1.3	1.2	1.3	1.2	1.1	1.2	1.1	1.3	1.3	1.2	**1.21**
(fruit)	50,000	1	1.1	1	1.2	1.1	1	1.1	1	1	1	1.1	1.1	1	**1.05**
	10,000	0.9	0.8	0.9	0.8	0.9	0.9	1	0.9	0.8	0.9	0.9	0.8	0.8	**0.87**
	5,000	0.8	0.7	0.8	0.7	0.7	0.8	0.9	0.8	0.7	0.8	0.8	0.7	0.8	**0.77**
	1,000	0.7	0.6	0.7	0.6	0.6	0.7	0.8	0.7	0.6	0.6	0.7	0.6	0.6	**0.65**
4 Nut gall	100,000	1.8	1.6	1.8	1.7	1.5	1.6	1.7	1.6	1.7	1.7	1.8	1.5	1.5	**1.68**
(fruit)	50,000	1.4	1.5	1.3	1.4	1.2	1.1	1.2	1.1	1.4	1.5	1.3	1.2	1.2	**1.31**
	10,000	1.1	1	0.9	1	0.9	1	1	0.9	1.1	0.9	1.1	1	1	**1**
	5,000	0.9	0.8	0.8	0.9	0.8	0.8	0.9	0.8	0.9	0.8	1	0.9	0.9	**0.87**
	1,000	0.8	0.7	0.7	0.8	0.7	0.7	0.8	0.6	0.8	0.7	0.8	0.8	0.8	**0.75**
5 myrobalan wood	100,000	1.4	1.5	1.4	1.5	1.3	1.5	1.4	1.5	1.5	1.3	1.4	1.4	1.4	**1.42**
(fruit)	50,000	1	1	0.9	1.1	0.9	1.1	1	1	1	0.9	1	1	0.9	**0.98**
	10,000	0.7	0.7	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.8	0.8	0.7	**0.73**
	5,000	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
	1,000	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**

growth of bacteria at 50,000 ppm, the myrobalan wood fruit extract could inhibit the growth of bacteria at 10,000 ppm, the extract of beleric myrobalan fruit , nut gall fruit and pomegranate fruit peel could inhibit the growth of bacteria at all concentration levels started at 1,000 ppm.

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