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Short report

Bacteriostatic effect of flavonoids isolated from leaves of *Psidium guajava* on fish pathogens

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Abstract

The antimicrobial activity against fish bacterial pathogens of flavonoids (morin, morin-3-*O*-lyxoside, morin-3-*O*-arabinoside, quercetin, and quercetin-3-*O*-arabinoside) isolated from the leaves of *Psidium guajava* was evaluated. The flavonoids were shown to have bacteriostatic effect on all of the tested bacteria.

Keywords: *Psidium guajava*; Flavonoids; Antimicrobial activity

1. Plant

Psidium guajava L. (Myrtaceae), leaves were obtained from Lampang Herb Conservation Assembly, Lampang province, Thailand.

2. Uses in traditional medicine

P. guajava leaves have been used as herbal medicine for the treatment of various human ailments such as wounds, ulcers, cholera, and coughs [1,2]. They also possess antimicrobial, anti-inflammatory, analgesic, spasmolytic, antipyretic antidiarrheal and antimutagenic activities [1–7].

3. Previously isolated constituents

Essential oils [8,9], triterpenoids [1], and flavonoids [4].

4. Tested materials

Five flavonoids, morin, morin-3-*O*-lyxoside, morin-3-*O*-arabinoside, quercetin, quercetin-3-*O*-arabinoside, prepared according to Arima and Danno [4].

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Table 1
Antimicrobial activity of the *P. guajava* leaves flavonoids against fish pathogens

Flavonoids	MIC (μg/ml)					
	A. h	A. s	F. c	L. g	S. a	V. s
Morin	300	150	200	250	300	250
Morin-3-*O*-lyxoside	250	250	200	250	200	250
Morin-3-*O*-arabinoside	300	250	250	300	150	300
Quercetin	200	200	300	250	200	300
Quercetin-3-*O*-arabinoside	250	150	250	250	200	300

A. h=*Aeromonas hydrophila*; A. s=*Aeromonas salmonicida* subsp. *salmonicida* ATCC 14174; F. c=*Flavobacterium columnare* ATCC 23463; L. g=*Lactococcus garvieae* ATCC 49156; S. a=*Streptococcus agalactiae*; V. s=*Vibrio salmonicida* ATCC 43839.

5. Studied activity

Minimum inhibitory concentration (MIC) values of the flavonoids against all fish pathogens used in this study were determined as described previously [4]. To examine mode of action of the flavonoids against the fish pathogens, each flavonoid (at the final concentration equals to the MIC value) was added to 10 ml cultures of fish pathogens (ca. 10^2 CFU/ml). After incubation at 37 °C for 24 h, 100 μl of each mixture was inoculated into 10 ml of fresh Muller Hinton broth. The optical density at 600 nm (OD600 nm) of the culture was determined at the time of inoculation and after incubation at 37 °C for 24 h.

6. Used microorganisms

Fish bacterial pathogens listed in Tables 1 and 2. ATCC bacteria were obtained from American Type Culture Collection, USA. *Aeromonas hydrophila* and *Streptococcus agalactiae* isolated from infected fish were kindly gifted by Dr. Nilubon Kitacharoen, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand.

7. Results

All flavonoids isolated from leaves of *P. guajava* were shown to be able to inhibit all of the fish pathogens used in this study with the different degree of inhibition (Table 1). The recovery of the fish pathogens in fresh medium after inhibited by the flavonoids indicated that they had bacteriostatic mode of action against the tested bacteria (Table 2).

8. Conclusion

The findings showed that the leaves of *P. guajava* are an interesting source for biologically active compounds that may be applied for therapy of bacterial fish diseases.

Table 2
Recovery ability of the flavonoid-inhibited bacteria in fresh medium

Flavonoids	OD600 nm[a]											
	A. h		A. s		F. c		L. g		S. a		V. s	
	0[b]	24[b]	0[a]	24[a]	0[a]	24[a]	0[a]	24[a]	0[a]	24[a]	0[a]	24[a]
Morin	0	0.88	0	0.76	0	0.91	0	0.98	0	0.82	0	0.76
Morin-3-*O*-lyxoside	0	0.92	0	0.77	0	0.99	0	0.13	0	0.79	0	0.81
Morin-3-*O*-arabinoside	0	0.80	0	0.81	0	0.87	0	0.91	0	0.77	0	0.88
Quercetin	0	0.86	0	0.75	0	0.93	0	0.99	0	0.70	0	0.72
Quercetin-3-*O*-arabinoside	0	0.84	0	0.79	0	0.89	0	0.92	0	0.83	0	0.83

A. h=*Aeromonas hydrophila*; A. s=*Aeromonas salmonicida* subsp. *salmonicida* ATCC 14174; F. c=*Flavobacterium columnare* ATCC 23463; L. g=*Lactococcus garvieae* ATCC 49156; S. a=*Streptococcus agalactiae*; V. s=*Vibrio salmonicida* ATCC 43839.

[a] Optical density at 600 nm values are the mean of three replicates.

[b] Time after inoculation of the flavonoid-inhibited bacteria into fresh Muller Hinton broth (h).

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