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Novel biosubstances, α-mangostin and gartanin, from mangosteen (*Garcinia mangostana* L.) candidate for anti-saprolegniasis agent

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Introduction

Saprolegniasis is known as one of the most important fungal diseases on varieties of fishes along with high mortality. It is caused by aquatic fungi belonging to the family Saprolegniaceae particularly spreading by three genera and examples are *Aphanomyces* spp., *Achlya* spp. and *Saprolegnia* spp. *Aphanomyces invadans*, *Saprolegnia diclina* and *Achlya bisexualis* are the most damaging in hatchery both freshwater and estuarine fishes in Thailand. At present the aquaculture system, treatment of infected fish or eggs has been used malachite green, formalin, and antibiotics that could affect the residues in products and environment [1]. Currently, plant extracts have been known for medicinal as well as antimicrobial activities; and several Thai herb extracts were revealed that antifungal activity against zoospores of *Achlya bisexualis* and *Aphanomyces invadans* [2].

Garcinia mangostana L., (mangosteen) belonging to family Guttiferaceae, is cultivated throughout Southeast Asia especially in eastern and southern regions of Thailand [3,4]. The extract of pericarp has been used in traditional medicine for treatment of pain, diarrhea, dysentery, skin infection and antimicrobe [5]. The most phytochemi in pericarps are α-mangostin and gartanin which have been tested pharmaceutical properties such as antioxidant [6], anticancer [7], anti-inflammatory [8], anti-allergy [9] and antimicrobial activities [10,11]. Therefore, this study was aimed to investigate efficiency of α-mangostin and gartanin against mycelium and zoospores of *Aphanomyces invadans*, *Achlya bisexualis* and *Saprolegnia diclina* with in vitro conditions.

Materials and methods

Aphanomyces invadans NJM 9701, *Saprolegnia diclina* H3 and *Achlya bisexualis* NJM 0611 were obtained from the Laboratory of Fish Diseases, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Japan. The fungi were cultured on GY agar medium that used in this experiment [12].

Mangosteen was purchased from the local market in Chiangmai, Thailand. The pericarp was extracted and subsequently purified. Preparation of α-mangostin and gartanin analyses were performed using a system consisting of an Agilent technologies HPLC system (model 1100 series) which was equipped with a DAD detector and a Hypersil® BDS C-18 column (4.6 × 250 mm, 5 µm). The solution was carried out by isocratic solvent system with a flow rate 1 ml min^{-1} at room temperature. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) with ratio of 20:80 v/v, and mobile phase was prepared daily, filtered through a 0.45 µm that wavelength of UV-vis detector was at 320 nm. The α-mangostin and gartanin fractions were collected and the concentrations were measured by freezing dried weight.

Fungistatic and fungicidal effects of α-mangostin and gartanin on mycelium and zoospores were determined by agar disc diffusion and immersion respectively, at concentrations of 125, 250, 500 and 1,000 ppm, also observing for the fungal growth for 7 days. Zoospores growth was observed by agar plate culture and morphological changes were detected with SEM technique. There was no growth of fungal indicated that in the hyphae and zoospores were inhibited by the biosubstances.

Results and discussion

The α-mangostin and gartanin in pericarp of mangosteen extracts were determined by isocratic solution with HPLC method (Fig. 1). The wavelength at 320 nm was used to detect α-mangostin and gartanin because it is tricyclic aromatic structure enables good absorption at this wavelength [3,4]. The fractions of

α-mangostin and gartanin from mangosteen pericarps were purified and used for demonstration of antifungal activity at concentration 125, 250, 500 and 1,000 ppm.

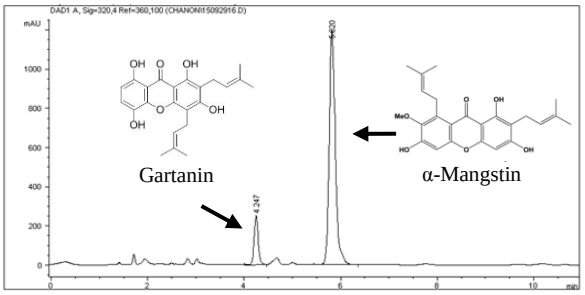

Fig. 1. HPLC fingerprint of *Garcinia mangostana* L. pericarp extract.

Fungistatic effects of α-mangostin and gartanin on mycelium growth was presented in Table 1. The results revealed that the values of 3 fungi were *A. invadans* NJM 9701 at 125 and 250 ppm respectively, *S. diclina* H3 at 125 ppm, and *A. bisexualis* NJM 0611 at 250 ppm. It also verified that inhibition efficacy gradually increased upon higher concentration. The abnormal hypha growth, with short branch and dense, was observed at the mycelium edge of inhibition zone.

Table 2 was uttered the fungicidal effects of α-mangostin and gartanin on zoospore growth, and the values on zoospores growth of 3 fungi were *A. invadans* NJM 9701 and *S. diclina* H3 at 500 and 1000 ppm respectively, and *A. bisexualis* NJM 0611 at 1000 ppm.

Morphological effect was investigated by SEM technique. It confirmed that our both biosubstances were obviously toxic on zoospores surface and inhibited their growth.

Table 1. Fungistatic effects of α-mangostin and gartanin on mycelium

Fungal strain	α-Mangostin (ppm)				Gartanin (ppm)			
	125	250	500	1000	125	250	500	1000
Aphanomyces invadans NJM 9701	-	-	-	-	+	-	-	-
Saprolegnia diclina H3	-	-	-	-	-	-	-	-
Achlya bisexualis NJM 0611	+	-	-	-	+	-	-	-

-, No growth; +, Growth.

Table 2. Fungicidal effects of α-mangostin and gartanin on zoospores

Fungal strain	α-Mangostin (ppm)				Gartanin (ppm)			
	125	250	500	1000	125	250	500	1000
Aphanomyces invadans NJM 9701	+	+	-	-	+	+	+	-
Saprolegnia diclina H3	+	+	-	-	+	+	-	-
Achlya bisexualis NJM 0611	+	+	+	-	+	+	+	-

-, No growth; +, Growth.

Conclusions

The study results suggested that α-mangostin and gartanin were important phytochemicals agents which against aquatic fungal pathogens. These biosubstances have been reported to exhibit antifungal activity against dermatophytes in human [13]. This research was the first report of α-mangostin and gartanin were effective against mycelium and zoospores growth of saprolegniais pathogens; *Aphanomyces invadans*, *Saprolegnia diclina* and *Achlya bisexualis*. Furethermore, we will develop these biosubstances to be anti-saprolegniais product in further.

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