

Inhibitory Effect of *Cratoxylum formosum* Gum on *Candida glabrata* and Its α -mangostin Content

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Abstract. *Candida glabrata* is the most common fungal species isolated in patients with severe mucosal inflammation. The high resistance to traditional antifungal therapies makes this species a growing concern in clinical settings. *Cratoxylum formosum* is a plant widely distributed in mountainous area of Asian countries. This study aims to examine antifungal activity of *C. formosum* gum against *C. glabrata* and its α -mangostin content. Inhibition of fungal growth was primarily tested by agar diffusion. Broth dilution method was then used to determine the minimum inhibitory concentration (MIC). The α -mangostin content was determined by high performance liquid chromatography (HPLC). Inhibitory effect of the gum was seen against *C. glabrata* (clinical isolate and ATCC22019) with zones of inhibition ranging from 14.3 to 10.2 mm. MIC value against *C. glabrata* ATCC22019 and the clinical isolate was 1.25 mg/mL. By HPLC, the α -mangostin content of *C. formosum* gum was determined as 4.08% (w/w). In conclusion, the anticandidal activity of *C. formosum* gum suggests that this plant may be a useful source for the development of a novel antifungal agent against candidal infection. Further *in vitro/in vivo* studies should be conducted to understand the mechanisms of action and to establish the safe profile of this gum for clinical usage.

1 Introduction

Candida glabrata is normally commensal of humans that can be found especially in the oral cavity and the gastrointestinal tract of most healthy humans. However, it can turn into opportunistic pathogen causing mucosal and blood stream infections in persons with predisposing factors, such as treatment with antibiotics, diabetes, cancer, extreme age, immunosuppression or long-term hospitalization [1]. Apart from being the most common non-*Candida albicans* (NCAC) species isolated in oral cavity of patients with severe mucosal inflammation, *C. glabrata* exhibits the high resistance to traditional antifungal therapies [2].

Cratoxylum formosum is a plant widely distributed in mountainous area of various Asian countries. The gum extracted from the burned bark has been used to stain on tooth surfaces to prevent tooth pain, tooth decay or other oral diseases [3]. Previous studies have shown the antimicrobial properties of *C. formosum* gum against tooth decay associated bacteria and *Candida albicans* [4,5]. However, the effect on *C. glabrata* does not exist. Therefore, the objective of this study was to examine the antifungal activity of *C. formosum* gum against *C. glabrata in vitro*.

2 Materials and Methods

2.1 Plant materials preparation

The trunks of *Cratoxylum formosum* were collected from mountainous regions of northern Thailand. They were cut and burned to obtain a black colored extract, which was immediately collected and used to prepare the gum.

2.2 Candida preparation

Candida glabrata ATCC 22019 and clinical isolate were used in the study, which were obtained from the culture collection of the Department of Oral Microbiology, Faculty of Dentistry, Mahidol University.

2.3 Antifungal activity testing

The antifungal property of *C. formosum* gum was determined by disk diffusion method then further investigated for the minimum inhibitory concentration (MIC).

Overnight broth cultures of the test microorganisms were prepared in Brain Heart Infusion broth (Difco, USA), buffered with phosphate buffer saline (PBS, pH 7.4) to yield a concentration of approximately 1.5×10^8 CFU/mL. Paper disks (Whatmann International, UK) of 6 mm diameter, were placed on the inoculated agar surfaces and impregnated with 20 μ L of the gum. For determination of MIC, the gum was dissolved in dimethyl sulfoxide (DMSO, 50% v/v). Two-fold dilution series of the gum were tested against the starting inoculums of 1×10^8 CFU/mL. The vehicle (50% DMSO, v/v) was used as a negative control and nystatin (100,000 units/mL, InterThai Pharmaceutical, Thailand) as a positive control

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for growth. The MIC was defined as the lowest concentration of gum that restricted growth to a level lower than 0.05 at 600 nm (no visible growth).

2.4 Determination of α -mangostin content

C. formosum gum (50 mg) was diluted in methanol to obtain a final concentration of 1 mg/mL. The solution was filtered through 0.45 μ m membrane filter prior to analysis. For standard solution preparation, a stock solution of α -mangostin reference standard was prepared to obtain concentrations of 200, 100, 50, 25 and 10 μ g/mL.

The validated HPLC method was performed on Water 600 Controller, 2996 Photodiode Array Detector, a rheodyne injector fitted with a 20 μ L loop and 717 Plus Auto-sampler. An XTerra® RP18 (3.9 mm x 150 mm, 5 μ m particle size) with a RP 18 guard column was used. The elution was carried out with a gradient solvent system consisting of 0.1% v/v ortho phosphoric acid (solvent A) and acetonitrile (solvent B) with a flow rate of 1 mL/min at ambient temperature. The gradient program was as follows: 70% B for 0-15 min, 70% B to 75% B in 3 min, 75% B to 80% B in 1 min, constant at 80% B for 6 min, 80% B to 70% B in 1 min with 11 min of post-run for reconditioning. The wavelength of the detector was set at 320 nm. The content of α -mangostin was calculated using its calibration curve regarding the dilution factor and was expressed as g/100 g of the gum. All the tests were performed in triplicate on three separate occasions.

3 Results

The antifungal activity of *C. formosum* gum against 2 strains of *C. glabrata* was quantitatively assessed by the presence of inhibition zones and the MIC values (Table 1). The amount of α -mangostin in the gum determined by HPLC was 4.08% (w/w).

Table 1 Mean inhibition zones(mm) and MIC values (mg/mL) of *C. formosum* gum against *C. glabrata*

<i>C. glabrata</i>	<i>C. formosum</i>		Nystatin	
	zone	MIC	zone	MIC
ATCC 22019	14.3	1.25	12.0	0.20
Clinical isolate	10.2	1.25	11.7	0.25

4 Discussion

Cratoxylum formosum is a small tree, 10-20 m tall, which distributes in several Asian countries. It has been known for its therapeutic use on the treatment of diarrhea, food poisoning and internal bleeding [6,7]. In dentistry, the gum obtained from the burned bark of this plant has been used extensively by hill tribe people of Thailand to paint on their tooth surfaces for the prevention of tooth and gum diseases [4,8,9]. The antimicrobial properties have been reported against many types of oral microorganisms including bacteria related to tooth decay (*Streptococcus mutans*) [4] and periodontal diseases (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*) [10] as well as candida (*Candida albicans*) [5]. In the present study, the gum extracted from this plant showed moderate antifungal activity against *C. glabrata* (MIC was 1.25 mg/mL) which is the second most prevalent human yeast pathogen after *C. albicans*. The effect seems less potent compared to *C. albicans* (MICs of *C. albicans* were 0.5-1.25 mg/mL). This may be due to the differences in their metabolic pathways and resistance mechanisms between these two pathogenic candida species.

In the past, *C. glabrata* was considered a relatively non-pathogenic saprophyte of the normal flora in healthy humans and was not readily associated with serious infection. However, *C. glabrata* can rapidly disseminate throughout the body and infection with this species is associated with a high mortality rate [11]. Nowadays, *C. glabrata* is of added concern because of its inherent resistance to certain antifungal agents. Data from the study of Baddley *et al.* [12] indicated that yeast species with high MICs obtained from patients with candida infection are associated with lower success rates and higher mortality than those with low or susceptible MICs [13]. Furthermore, antifungal resistance has consequences in terms of elevated MICs that are associated with poorer outcomes and breakthrough infections during antifungal treatment and prophylaxis.

Considering the toxicity of *C. formosum* gum, mildly cytotoxicity or grade 2 cytotoxicity has been demonstrated in mouse fibroblast cell line (L929) at the concentration of 1.25-12.5 mg/mL [5]. However, the acceptable biocompatibility of the agent has been set as grade 2 or lower [14].

The active components present in the plant products, xanthenes are the prominent bioactive compounds in the bark or exuded gum of trees belonging to the family Clusiaceae, of which *Garcinia mangostana* L. and *Cratoxylum* spp. were traditionally employed for painting in the oral cavity [8,9,15]. Many studies have shown α -mangostin to exert the most potent antimicrobial activity against both bacteria and fungi, among all the xanthone compounds [16,17]. This study also focused on the quantification of α -mangostin and it was revealed that the amount of this xanthone derivative present in the gum was 4.08% w/w. Compared with that demonstrated by Kaomongkolgit *et al.* [17], the yield of α -mangostin from the dried pericarp of mangosteens (*Garcinia mangostana*)

was approximately 10 times (0.4% w/w) less than that from *C. formosum*.

In conclusion, *C. formosum* gum showed anticandidal property in inhibiting the growth of *C. glabrata* with the MIC in acceptable biocompatibility range. Based on our data, it can be suggested that *C. formosum* gum may be a candidate for antimicrobial action against oral yeast. However, further studies should be conducted to clarify the mechanisms of action followed by clinical studies to establish the safe profile of this gum for clinical use.

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