ANTIMICROBIAL ACTIVITY OF *CRATOXYLUM FORMOSUM* ON *STREPTOCOCCUS MUTANS*

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Abstract. The gum of *Cratoxylum formosum*, commonly known as mempat, is a natural agent that has been used extensively for caries prevention by hill tribe people residing in Thailand. The objective of this study was to investigate the antimicrobial activity of *Cratoxylum formosum* gum on *Streptococcus mutans (S. mutans) in vitro*. The gum extracted from stem bark of *Cratoxylum formosum* was investigated for antimicrobial activity against different strains of *S. mutans,* including *S. mutans* KPSK2 and 2 clinical isolates. Inhibition of growth was primarily tested by agar diffusion method. A two-fold broth dilution method was then used to determine the minimum inhibitory concentration (MIC) of the extract. The extract of *Cratoxylum formosum* was effective against *S. mutans* with the inhibition zones ranging from 9.5 to 11.5 mm and MIC values between 48 µg/ml and 97 µg/ml. The gum of *Cratoxylum formosum* has high antimicrobial activity against *S. mutans* and may become a promising herbal varnish against caries.

INTRODUCTION

Natural products have been used for thousands of years as folk-medicine, and are promising sources for novel therapeutic agents (Cragg *et al*, 1997). They have been used or investigated as promising agents to prevent oral diseases, especially plaque-related diseases, such as dental caries (Koo *et al*, 2002; Hwang *et al*, 2003). Among various medicinal plants used in folk-medicine in Thailand, *Cratoxylum formosum*, a shrub 10-20 meters tall known as Tew, stands out because of its multiple pharmacological properties.

Cratoxylum is a small group belonging to the Guttiferae family, distributed in several Southeast Asian countries (Iinuma *et al*, 1996). Species of this genus have been used for their diuretic, gastric and tonic effects, as well as for diarrhea, flatulence, food poisoning and internal bleeding (Grosvenor *et al*, 1995). *C. formosum* is widely distributed in northeastern Thailand. A decoction of the bark is used to cure colic. The gum has been used extensively by hill tribe people of Thailand for painting on tooth surfaces as varnish to prevent dental caries.

Dental caries are one of the most common infectious diseases in the Thai population. The prevalence of dental caries in Thai children age 5-6 years in 2000 has been reported to be 87.4% with a dmft (decayed missing and filled teeth) value of 5.97 (Ministry of Public Health, 2002). *Streptococcus mutans* is closely associated with the pathogenesis of dental caries because of its ability to synthesize water-insoluble glucans that mediate adhesion to and colonization of the teeth (Freedman *et al*, 1978). *S. mutans* also produces large amounts of acid, particularly

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lactic acid, which are potent in driving tooth demineralization (Johnson *et al*, 1980). Therefore, the use of antimicrobial agents to control these cariogenic bacteria is one of the strategies for caries prevention and treatment. The objective of this study was to investigate the antimicrobial activity of *C. formosum* gum on *S. mutans in vitro*.

MATERIALS AND METHODS

Plant material and extraction

The trunk parts of *Cratoxylum formosum* were collected from mountainous areas of northern Thailand, in January-Febuary, 2006 and authenticated by the herbarium staff of the Faculty of Agriculture, Kasetsart University, Thailand. They were cut and burned. Black gum was collected immediately and transferred directly to a sterilized Petri dish.

Microorganisms and antimicrobial activity assay

The microorganisms used in this study were *S. mutans* $KPSK_2$, and two recent clinical isolates. The antimicrobial activity was determined by disk diffusion method and then further investigated for the minimum inhibitory concentration (MIC). In the disk diffusion susceptibility method, Muller-Hinton agar was used and prepared according to the instructions of the manufacturer (Oxford, UK). All agar plates were prepared in 90 mm Petri dishes with 20 ml of agar, giving a final depth of 4 mm.

Overnight broth cultures were prepared in Brain heart infusion broth (Difco), buffered with PBS (phosphate buffer saline, pH 7.4) in order to yield approximately 1.5 x 10^8 CFU/ml. Whatman paper disks (Whatman International, UK) 6 mm diameter were placed on the inoculated agar surfaces and were impregnated with 20 µl of the extract. For MIC determination, the gum extract of *C. formosum* was dissolved in DMSO (dimethylsulfoxide, 50% v/v) in order to overcome the limited solubility of the gum in aqueous media. Two-fold dilution series of the extract were tested against the starting inoculum of 1 x 10^8 CFU/ml. The vehicle (50% DMSO, v/v) was used as a negative control and chlorhexidine gluconate was used as a positive control for growth. The final concentration of DMSO was 0.24% (v/v). The MIC was defined as the lowest concentration of extract that had restricted growth to a level lower than 0.05 at 600 nm (no visible growth). Each experiment was repeated four times.

RESULTS

The antimicrobial activity of *Cratoxylum formosum* gum extract against 3 strains of *S. mutans* was quantitatively assessed by the presence of inhibition zone diameters and the MIC values (Tables 1 and 2).

Table 1
Antimicrobial activity of *Cratoxylum formosum* gum extract against *S. mutans* strains tested based on the disk diffusion method.

S. mutans strain	Inhibition zone diameter (mm)	
	Extract	Chlorhexidine
KPSK2	11.5 ± 0.4	20.5 ± 0.5
Clinical isolate I	11.0 ± 0.9	19.3 ± 0.6
Clinical isolate II	9.5 ± 0.4	19.5 ± 0.5

Table 2
The MIC value of *Cratoxylum formosum* gum extract against *S. mutans* strains on the tube dilution assay.

S. mutans strain	MIC (µg/ml)	
	Extract in 50%DMSO	Chlorhexidine
KPSK2	48	<1.15
Clinical isolate I	97	<1.15
Clinical isolate II	97	<1.15

DISCUSSION

Applying a black varnish to the teeth has been observed as a habit in hill tribe people residing in northern Thailand. This habit is a wide-spread Asian tradition, not only in Thailand but also in Lao PDR, Vietnam, Myanmar, Cambodia and China (Nguyen, 1990). It has been maintained for many countries, recognized as a symbol of elegance and good taste. The techniques and materials used vary by country and population.

Cratoxylum formosum or Tew is a Thai medicinal plant used in folk-medicine as an agent to prevent oral diseases, especially dental caries. Hill tribe people use black gum from the burned stem bark to stain their teeth by smearing it on the buccal and occlusal surfaces. It is believed to have dental preservative effect against decay and pain.

In this investigation, gum extracted from *C. formosum* showed high antimicrobial activity against *S. mutans* with an MIC value between 48 µg/ml and 97 µg/ml. According to Rios *et al* (1988), natural crude extracts that exhibit activity at concentrations lower than 100 µg/l may have great antimicrobial potential, since the active compounds can be isolated and used at lower concentrations. The MIC values for the clinical isolates were higher than those of the culture collection strain. This observation is important because the laboratory strains commonly used to determine susceptibility to antimicrobials, may not express the same virulence or resistance patterns compared to the strains recently isolated from the oral cavity (Duarte *et al*, 2003). It is noteworthy that the extract was effective against recently isolated bacteria.

The antimicrobial activity of an agent may be indicative of the presence of metabolic toxins or antimicrobial substances. It has been reported that xanthones and anthraquinones are the main components of *C. formosum* (Chantrapromma *et al*, 2006). Xanthones have antimicrobial activity against both gram-positive and gram-negative bacteria, such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis* and *Samonella typhi* while anthraquinones have little activity. Not much is known about the antimicrobial activity of *C. formosum* against oral microorganisms. Tayanin and Bratthall (2006) found little inhibitory effect of *C. formosum* on the growth of salivary Mutans streptococci (MS). In their study, plastic strips of the Strip Mutans Test kit were partly covered with the extract and stimulated saliva from persons with high MS levels was added. After incubation, a few colonies were observed. It should be noted that the strip method used in their study is not the standard method to investigate antimicrobial activity. In addition, the amount of extract and the concentration of MS in saliva were not indicated in their study.

Natural products have recently been studied as an alternative to synthetic chemical substances for dental caries prevention. Black gum from the burned bark of *C. formosum* is considered as a promising candidate. Further studies are needed to clarify the bioactive compound in this plant extract.

In conclusion, the remarkable inhibitory effects of *C. formosum* gum extract suggest that this plant may be a useful source for the development of a novel herbal varnish against dental caries. Further studies should be conducted to examine whether this extract has any influence on the viability of *S. mutans* or the development of dental biofilms.

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