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Antibacterial Activity of Propolis Supplemented-Chewing Candy Against *Streptococcus mutans*

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Streptococcus mutans is considered to play a major etiological role in development of human dental plaque believed to related to dental caries, the most prevalent disease of the human oral cavity. The objectives of the present study were to formulate and produce propolis supplemented-chewing candy and to investigate its antibacterial activity against *S. mutans*. Propolis is a natural resinous bee-hive product thought to have antimicrobial, anti-inflammatory and immunostimulating activities. Propolis was extracted from hives of bees of *Trigona* spp. using ethanol. The extract was coated with maltodextrine and homogenized to generate propolis microparticles. The particles were introduced into chewing candy preparations for the production of propolis supplemented-chewing candy. The candy was then subjected to *in vitro* antibacterial assays to test its activity against *S. mutans* isolated from human dental plaque. Results showed that the ethanol extracted propolis of *Trigona* spp. bee-hives can be homogenized to form propolis microparticles. The propolis microparticles could be used as a supplement in the formulation of chewing candy preparations. The propolis supplemented-chewing candy showed antibacterial activity against *S. mutans.* The candy, therefore, has the potential to be used as an antiplaque agent for prevention of dental caries.

Key words: antibacterial activity, propolis supplemented-chewing candy, *Streptococcus mutans, Trigona* spp.

Streptococcus mutans diduga memegang peran penting dalam etiologi perkembangan plak gigi pada manusia yang berhubungan dengan karies gigi, penyakit rongga mulut yang paling banyak terjadi. Penelitian ini bertujuan memformulasi dan memroduksi permen kenyal berpropolis serta menentukan aktivitas antibakterinya terhadap *S. mutans*. Propolis bahan alami mengandung resin yang diperoleh dari sarang lebah dan diketahui memiliki aktivitas antibakteri, antiinflamasi, dan imunostimulasi. Propolis diekstraksi dari sarang lebah *Trigona* spp. menggunakan etanol. Ekstrak kemudian dilapisi dan dihomogenisasi untuk membentuk mikropartikel propolis. Partikel ini selanjutnya dicampurkan ke dalam sediaan permen kenyal dalam pembuatan permen kenyal berpropolis. Aktivitas antibakteri permen selanjutnya diuji secara *in vitro* terhadap *S. mutans* yang diisolasi dari plak gigi. Hasil menunjukkan bahwa ekstrak etanol propolis dari sarang lebah *Trigona* spp. dapat dihomogenisasi membentuk mikropartikel propolis. Mikropartikel propolis dapat dijadikan suplemen dalam formulasi sediaan permen kenyal berpropolis. Permen kenyal berpropolis menunjukkan aktivitas antibakteri terhadap *S. mutans*. Oleh karena itu, permen ini berpotensi untuk digunakan sebagai bahan antiplak gigi guna mencegah karies gigi.

Kata kunci: aktivitas antibakteri, permen kenyal berpropolis, *Streptococcus mutans, Trigona* spp.

Dental caries continues to be an important public health problem in some parts of the world and is considered to be the most prevalent disease affecting the human oral cavity (Duailibe *et al*. 2007). The incidence is particularly high during childhood (Gasparini *et al*. 1989; Okada *et al*. 2005). The tooth enamel and dentin are demineralized by acids, such as lactic acid, which are produced as a by-product of carbohydrate metabolism by cariogenic bacteria in dental plaque (Yoo *et al*. 2007). *Streptococcus mutans* is the leading cause of dental caries worldwide, and is considered to be the most cariogenic amongst the oral streptococci. Its etiological role in human dental decay has been extensively discussed by Hamada and Slade (1980). The relationship between dental plaque and the occurrence of dental caries has also been indicated by others (Newman 1986).

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To date, the prevention and control of dental caries, is not restricted to a single procedure. In addition to traditional methods, such as periodic dental follow-ups, brushing with fluoride-containing dentifrices, topical application of fluorides, low-sucrose diets, a more effective control procedure needs to be applied in some cases. Researchers are currently also interested in the prospect that natural substances offer alternatives for the control of dental caries (Duailibe *et al*. 2007).

The use of natural agents against selected oral pathogens has been reported (Li *et al*. 1997). Propolis, a natural product from the common honeybee (*Apis mellifera*) has been shown to exert antibacterial action against a number of oral microorganisms including *S. mutans* (Duailibe *et al*. 2007; Ophori *et al*. 2010). However, the detailed mechanism of propolis antibacterial activity has yet to be elucidated. Propolis has also been reported to inhibit cell adhesion as well as water-insoluble-glucan formation by *S. mutans* (Koo *et*

al. 2000). In addition, it possesses anti-inflammatory, local anesthetic, hepatic-protective, antitumor, and immunostimulating activities (Bankova *et al*. 2000).

The chemical composition of propolis is highly variable and depends on the local flora at the site of pollen collection. Although its biological activity, especially against microorganisms is always present, samples from different geographic and climatic zones have their activities resulting from completely different chemical composistions (Bankova 2005). The chemical composition of propolis produced by *A. mellifera* from different regions in Java, Indonesia, has also been investigated. It was found that the chemical composition of this propolis is strikingly diverse. All of the samples analyzed, however, contain phenolic acids which are thought to play roles in propolis bioactivity (Syamsudin *et al*. 2009).

In addition to *A. mellifera*, propolis is also produced by the stingless bee *Trigona* spp. These bees tend to make less honey and produce more propolis. Previous studies have shown that the propolis of *Trigona* spp. contains flavonoids and shows activity against a number of bacterial strains including *Campylobacter* spp. and *S. mutans* (Fatoni *et al*. 2008). In the present study, propolis of *Trigona* spp. was introduced into chewing candy preparations and the antimicrobial activity of the candy against *S. mutans* was then analyzed in order to elucidate as to whether propolis is still active against *S. mutans* after being blended into a chewing candy preparation.

MATERIALS AND METHODS

Extraction of Propolis. Propolis was extracted using the method of Fatoni *et al*. (2008) with slight modifications. Raw propolis of *Trigona* spp. collected from Pandeglang, Banten Province, Indonesia was cut into small pieces, ground and extracted with 70% ethanol (1:5 w/v) in shaker (EYELA, Japan) at a speed of 130 rpm and at room temperature for 14 days. The clear filtrate was then decanted and followed by solvent evaporation using an evaporator. The solid extract obtained was then solubilized in 70% ethanol (1:1 w/v). The solution was used as the propolis stock.

Generation of Propolis Microparticles. Propolis microparticles were generated using a modified method of Bhaskar *et al*. (2009). Maltodextrine was used as a coating agent to protect propolis bioactive chemicals from conditional factors such as heat and moisture change during the process of nanoparticle formation. A thin coating using maltodextrine was also intended to make propolis water soluble. Magnesium stearate was included as a powdery anti-sticking agent for making the sticky surface of propolis microparticles non-adhesive. Maltodextrine (85 g) and magnesium stearate (5 g) were dissolved in 100 mL water. The mixture was then homogenized at 22 000 rpm for 30 min. To the mixture was added 120 mL of 20% (v/v) propolis stock and the mixture was homogenized at 22 000 rpm for 30 min. The solution was then dried using a vacuum dryer at a temperature of approximately 45 °C. The powder obtained was ground using high energy milling to generate microspheres of less than 500 nm in diameter. The size of microparticles in the resulting powder was then examined under the scanning electron microscope. The moisture of the powder was determined using a moisture balance.

Production of Propolis Supplemented-Chewing Candy. Chewing candy preparations were formulated to contain 11% (w/v) of gelatine, 10% (w/v) of sucrose, 0.05% (w/v) of methylparabene, 0.05% (w/v) citric acid, and a required amount of colouring essence. Propolis was supplemented at three different concentrations ie. 6, 7, and 8% (w/v). Preparations without propolis supplementation were also prepared to act as a control. The propolis concentration in each preparation was determined spectroscopically at a wavelength of 324 nm using a propolis solution of known concentration as the standard. In order to generate elastic chewing candy, gelatine was initially soaked in water for several minutes followed by boiling with stirring until a homogenous gelatine solution was obtained. Separately, a solution containing sucrose, methylparabene, citric acid and essence was prepared and this was added to the gelatine solution. The solution was homogenized by stirring at 6 rpm, at 70 °C. The solution was incubated until the temperature fell to about 50 °C prior to the propolis addition, followed by homogenization. The solution was then poured into a candy template to obtain shaped candy of 15 g pieces. The shaped candy was then cooled at -4 °C in order to obtain an elastic texture.

Antibacterial Activity Assay. Antibacterial activity of propolis supplemented-chewing candy was determined using the disc-diffusion method (Andrew 2009). An aliquot of 50 µL of *S. mutans* culture isolated from human dental plaque was inoculated homogenously into a petri dish containing 20 mL warm PYG agar medium [1% (w/v) peptone, 1% (w/v) yeast extract, 2% (w/v) glucose, 2% (w/v) bacto agar] followed by incubation until the medium solidified. A paper disc of 5 mm in diameter was placed on top of the medium. As much as 10 µL of water solubilized candy (3:4 w/v) was loaded onto the paper disc and the petri disc was incubated at 37 °C for 24 h. The diameter

of the inhibitory zone was measured using micro-callipers. Assays were performed in triplicate.

RESULTS

Generation of Propolis Extract. Macerate of raw propolis, which was dark in colour, resulted in a brown filtrate. Following solvent evaporation, solid propolis extract having a soft consistency, being sticky, and dark brown in colour was produced. The propolis yield obtained was 13% (w/w).

Generation of Propolis Microparticles. Preparation of propolis microparticles resulted in dry propolis microparticle powder. The powder had a water content of 4.4% (w/w). Under the scanning electron microscopy, using 3000 magnification, the average size of the coated particles was about 500 nm in diameter. The size of the propolis microparticles themselves is believed to be smaller. Under these conditions, the smallest particle detected had a diameter of about 180 nm. The particles showed a wrinkled irregular shape with a coarse surface. This might be due to water loss from the particles during the vacuum-drying process (Fig 1).

Production of Propolis Supplemented-Chewing Candy. Propolis could be supplemented into chewing candy preparations. The candy preparation generated was elastic and transparent. The preparation could be shaped by using a candy template.

Antibacterial Activity of Propolis Supplemented-Chewing Candy. The propolis supplemented-chewing candy showed antibacterial activity against *S. Mutans*. This is indicated by the formation of inhibition zones on the test plates. The highest activity was shown by candy supplemented with 8% (w/v) propolis (Table 1).

Fig 1 Propolis microparticles of *Trigona* spp. Propolis microparticles (coated samples) were observed under the scanning electron microscopy at 7 kV in a vacuum.

Table 1 *Streptococcus mutans* growth inhibition zone due to antibacterial activity of propolis supplemented-chewing candy

Propolis concentration of candy (%, w/v)	Diameter of inhibition zone (mm)
0	Not detected (control)
6	6.3 ± 0.6
7	6.3 ± 0.6
8	8.0 ± 1.0
Amoxycillin 10 mg/mL	17.3 ± 2.1

DISCUSSIONS

Dental caries is a disease of the teeth that can lead to pain, infection, tooth loss, and in severe cases, death. Most school children and adults worldwide have experienced dental caries. The disease is most prevalent in Asian and Latin American countries, and has been attributed to a number of factors such as age, culture, dietary habits, race and geographical location (Ophori *et al*. 2010). The goal of the present study was to develop a dental antiplaque-agent in the form of propolis supplemented-chewing candy that potentially can be used in the prevention of dental caries, especially amongst school children. For these purposes the antibacterial activity of the propolis supplemented-chewing candy against *S. mutans* was tested.

Propolis is generally derived from beehives of *A. Mellifera*. In this study propolis was extracted from beehives of *Trigona* spp. collected from Pandeglang District, Banten Province, Indonesia. *Trigona* spp. belongs to stingless bee group the propolis from which has yet to be widely explored. Prior to its use in the candy preparation, the propolis extract was homogenized to microparticles in order to improve its effectiveness as antimicrobial agent by size reduction and hence increased surface area. *In vitro* assays showed that the propolis microparticles have antibacterial activity against *S. mutans* at a propolis concentration of 6, 7, and 8% (w/v) (unpublished data).

From the present study it is clear that the antibacterial activity of the propolis supplemented-chewing candy was due to the bioactive compounds of the propolis. The data also indicated that the bioactivity of the propolis active substances was successfully maintained during propolis nanoparticle preparation and candy production. The inhibitory activity of propolis from other bees on *S. mutans* has also been reported (Duailibe *et al*. 2007; Ophori *et al*. 2010). Ophori *et al*. (2010) showed that an ethanol extract of propolis obtained from beehives of *A. mellifera* has a strong antimicrobial activity against *S. mutans* isolated from dental caries in an *in vitro* studies. Similarly, Duailibe *et al*. (2007) showed that extract prepared with propolis produced by the bee *Melipona compressipes fasciculata* possesses *in vivo* antimicrobial activity against *S. mutans* present in the oral cavity.

Although the detailed chemical composition of the propolis used in the present study has yet to be investigated, previous studies have indicated that propolis of *Trigona* spp. contains flavonoids and tannins thought to be responsible for its antibacterial activity (Fatoni *et al*. 2008). Compounds responsible

for propolis antibacterial activity vary considerably depending on the local flora at the site of collection. European propolis (poplar type) contains flavanones, flavones, phenolic acids and their esters found to be responsible for antibacterial activity. In Brazilian propolis (*Baccharis* type), compounds responsible for antibacterial activity are prenylated-*p*-coumaric acids and labdane diterpenes, while in Cuban propolis they are prenylated benzophenones (Bankova 2005).

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