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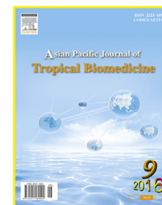

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# Bioprospecting for anti-*Streptococcus mutans*: The activity of 10% *Sesbania grandiflora* flower extract comparable to erythromycin

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ABSTRACT

**Objective:** To search an herbal material, capable of inhibiting plaque producing bacteria *Streptococcus mutans*.

**Methods:** Twenty materials comprising 10 flowers and 10 rhizomes were extracted with 70% ethanol. Their activity was then examined at a concentration of 10% (w/v) against *Streptococcus mutans in vitro* on Mueller–Hinton media. Erythromycin (Oxoid, 20 μg disc) was used as a positive control. Meanwhile, to establish a fingerprint guide for authentication or quality control, the most potent material was further analyzed regarding its chemical constituents by means of reversed phase-high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).

**Results:** Of the tested samples, *Sesbania grandiflora* (*S. grandiflora*) flower and *Costus speciosus* rhizome extracts showed the most potent activity with inhibited zone diameters of 18.5 and 14.8 mm, respectively. On the other hand, other extract plants showed a diameter zone in the range of 0.5–10.6 mm or being inactive (diameter = 0 mm). The activity of *S. grandiflora* was comparable to that of erythromycin (diameter = 18.0 mm). The best separation was achieved on HPLC system with acetonitrile-water with a ratio of 2:8, and a flow rate at 0.5 mL/min. TLC, meanwhile, was featured on chloroform–methanol (8.5:1.5) as a mobile system.

**Conclusions:** *S. grandiflora* flower is a promising material to be developed as the active ingredient of anti-plaque toothpaste as well as mouthwash solution. The developed HPLC and TLC system can be used for a further standard in its material authentication as well as for a fingerprinting of quality control during the manufacturing process.

## 1. Introduction

Tooth decay nowadays is becoming a dental health problem both in several developed and in developing countries [1–3]. As identified, the major cause of this is the plaques, a biofilm material formed by a number of bacteria species in which *Lactobacillus* sp. [4], *Streptococcus viridans* [5], and *Streptococcus mutans* (*S. mutans*) [6,7] are known as the major plaque producers. To prevent the floral producing plaques, community is educated to brush or to use mouthwash routinely. The formulation of antibacterial toothpaste or mouthwash requires a potent active ingredient. However, due to the instant food consumption as well as numerous meals introduced in the communities, more persistent food traces after brushing have been the major complicated aspect of current dental caries [8]. An effective plaque removal and plaque causing bacteria inhibitors, as a result, should be contained within daily toothpaste or mouthwash solution [9–11]. Finding a prospective antibacterial component is becoming an interest. Plant-derived material could be a potential source to discover a potent component of bacteria inhibitor toothpaste. Compared to the synthesized substance, it is more acceptable for the costumers for its good image on "natural" lifestyle in a

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particular safety issue. Hence, to search anti-plaque forming bacteria, we have screened 20 plant materials including the flowers of *Cananga odorata* (*C. odorata*), *Catharanthus roseus* (*C. roseus*), *Euphorbia milii, Illicium verum* (*I. verum*), *Plumeria alba* (*P. alba*), *Plumeria rubra* (*P. rubra*), *Polianthes tuberosa, Saraca indica, Sesbania grandiflora* (*S. grandiflora*), *Tegetes erecta*, and the rhizome of *Acorus calamus* (*A. calamus*), *Alpinia purpurata* (*A. purpurata*), *Costus speciosus, Cyperus rotundus* (*C. rotundus*), *Curcuma heyneana* (*C. heyneana*), *Curcuma soloensis* (*C. soloensis*), *Curcuma zedoaria* (*C. zedoaria*), *Zingiber amaricans* (*Z. amaricans*), *Imperata cylindrica* (*I. cylindrica*), and *Zingiber cassumunar* (*Z. cassumunar*). Since *S. mutans* have been reported as one of the major plaque producers, it was then employed as the targeted model.

## 2. Materials and methods

### 2.1. Plant materials

Twenty plant materials were obtained in the fields in some areas in Solo, Indonesia. They were authenticated by Azis Saifudin and the voucher specimens were preserved at the Laboratory of Pharmacognosy, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia. Meanwhile, *S. mutans* were obtained from Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia.

### 2.2. Sample preparation

About 10 g of each dried plant was macerated twice with 70% ethanol within a 24-h period. The obtained extract was evaporated under a reduced pressure to result in a dried extract. The sample was dissolved at concentration 10% in dimethyl sulfoxide (DMSO) in each disc. Erythromycin (20 μg/disc, Oxoid, USA) was used as the positive control, while a test disc containing only DMSO 10 μL was used as the solvent control.

### 2.3. Antibacterial test

*S. mutans* was grown on the media of brain heart infusion (Oxoid, USA) and shaken at 37 °C for 18–24 h. About 100 μL suspension was added with brain heart infusion media to 1 mL. The suspension was adjusted with saline solution to achieve the concentration of $10^8$ CFU/mL. For bioassay, 200 μL of the culture was spreaded on solid Mueller-Hinton (Oxoid, USA) media in a Petri dish of 37 °C. After 24 h, the disc was removed and placed in a biosafety cabinet. Five discs containing five tested samples including positive control and solvent were laid on the media to be again incubated for 24 h. Here, the inhibition zone was measured in millimeter (mm).

### 2.4. Chromatography analyses

To establish an analytical parameter used as a standard for future quality control during the manufacturing of the most active plant, the chemical analyses through thin-layer chromatography (TLC) method and high performance liquid chromatography (HPLC) system were developed.

#### 2.4.1. TLC analysis

Considering that the report on the chemical constituent of *S. grandiflora* has been limited so far, a chemical analysis was conducted through a silica or C-18 plate (Merck, Germany, 0.25 mm in thickness). The system was optimized using a combination of chloroform–methanol, hexane–ethyl acetate, and hexane–chloroform for a normal phase. Meanwhile, a water–acetonitrile–methanol combination was used for an optimization work for the reversed phase. The visualizations were conducted on UV lights 250/366 nm and vanilin-$H_2SO_4$ reagent followed by heating.

#### 2.4.2. HPLC

Qualitative analysis was carried out to determine an optimum peak number and peak shape. HPLC system was Waters Alliance 2965 with PDA 2998 detector, Empower® software, cosmosil column (150 mm × 4.6 mm, particle size of 5 μm, column temperature of 25 °C). Solvent choice was selected based upon the optimization among methanol, acetonitrile (Merck, Germany), and aquabidest (PT. Ika Pharmindo, Indonesia). For sample preparation, 1.0 mg sample was weighed and dissolved in methanol (1 mg/mL). The sample was filtered on a 20 μm paper (Toyo Roshi, Tokyo Japan) prior to the injection. Injection volume was 2 μL. Each procedure was repeated three times.

## 3. Results

### 3.1. Antibacterial test

Of 20 samples examined, *S. grandiflora* flower and *C. specious* rhizome extracts were found to exhibit the most potent samples. They had the inhibition diameter of 18.0 mm and 13.6 mm, respectively (positive control erythromycin = 18.0 mm) (Figure 1). Meanwhile, the flowers of *C. roseus*, *T. eracta*, *P. alba*, and the rhizomes of *A. calamus, C. heyneana, Z. amaricans, I. cylindrica, Z. cassumunar* exhibited moderate activities with a diameter inhibition ranging from 5.0 mm to 12.3 mm. The remaining samples *C. odorata* flower, *A. purpurata*, *C. rotundus*, *C. soloensis*, and *C. zedoaria* rhizomes, in contrast, showed a weak inhibition with an inhibition diameter less than 5 mm or being inactive (0 mm) (Table 1), while DMSO did not show any inhibition effects.

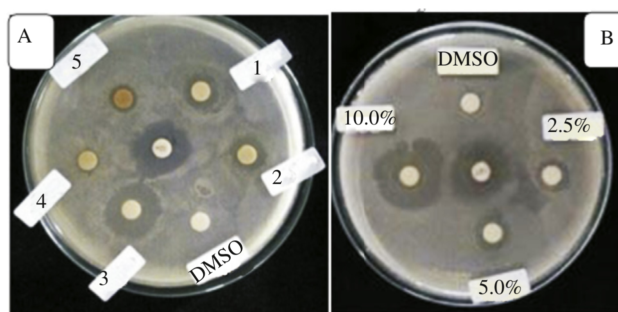


**Figure 1.** Inhibition zones of *C. odorata*, *I. verum*, *S. grandiflora*, *P. alba*, and *P. rubra* against *S. mutans*.

A: The middle is the positive control (erythromycin) while the others showed mild activities; B: The activity of *S. grandiflora* extract at concentrations of 2.5%, 5.0% and 10.0%, respectively. 1: *C. odorata*; 2: *I. verum*; 3: *S. grandiflora*; 4: *P. alba*; 5: *P. rubra*.

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