Effect of *Streblus asper* Leaf Extract on Selected Anaerobic Bacteria

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**Keywords:** antibacterial effect, disc diffusion, endodontic infection, herbal extract, medicinal plant

**Abstract**  
The purpose of this in vitro study was to determine the antibacterial effects of leaf extract of koi (*Streblus asper*) against six anaerobic bacteria: *Porphyromonas gingivalis* W50, *Prevotella intermedia*, *Actinomyces naeslundii* (T14V), *Peptostreptococcus micros*, *Actinobacillus actinomycetemcomitans* ATCC 43717 and ATCC 43718. The dried-pulverized leaves were extracted using 50% (v/v) redistilled ethanol. Employing the disc diffusion method, it was demonstrated that 15 µL of the leaf extract at 250 and 500 mg/mL had inhibitory effects towards all bacterial strains tested except *A. actinomycetemcomitans* ATCC 43717. The minimum inhibitory concentration of *S. asper* leaf extract on *P. gingivalis* W50, *A. actinomycetemcomitans* ATCC 43717 and ATCC 43718, *P. micros*, *P. intermedia*, and *A. naeslundii* (T14V) were 3.9, 31.3, 7.8, 15.6, 31.3, and 125 mg/mL respectively. The minimum bactericidal concentration of the extract on *P. gingivalis* W50, *P. micros*, *A. actinomycetemcomitans* ATCC 43717 and ATCC 43718, were 31.3, 62.5, 62.5 and 15.6 mg/mL respectively. The extract had no bactericidal activity against *P. intermedia* and *A. naeslundii* (T14V).

**INTRODUCTION**  
Bacteria and their by-products play an essential role in the pathogenesis of pulpal and periapical diseases (Yang, 1992). Of particular importance is the high incidence of anaerobic bacteria of which reports range from 80-93% of the cultured bacteria (Abbott, 1990). One of the primary goals of endodontic therapy is to eliminate bacteria from the root canal system. Available evidence indicates that bacteria and other irritants are substantially reduced by chemo-mechanical cleaning and shaping of the root canal system, followed by placement of an intracanal medicament (Abbott, 1990; Bystrom and Sundqvist, 1981). In selecting root canal medicaments or irrigants, one must weigh their therapeutic benefits against potential deleterious effects. The ideal root canal medicament or irrigants should be strongly antimicrobial, but not toxic to the host tissues (Abbott, 1990). To date, the ideal irrigant or intracanal medicament has not been found. Many irrigants or intracanal medicaments are irritant and highly toxic (Chang et al., 1998; Masillamoni et al., 1981). Some irrigant and intracanal medicament are known to produce allergic reactions and to possess a toxic mutagenic and carcinogenic potential (Kaufman and Keila, 1989; Lewis and Chestner, 1981).

Recently, an interest in medicinal plants as new sources of antimicrobial agents has grown. A wide variety of plant extracts have been reported to have anti-inflammatory properties, antibacterial and astringent effects (Graf, 2000; Smirnova and Iadrova, 1968; Taweechaisupapong et al., 2000). Therefore, several herbal extracts have been added to some cosmetics and health-care preparations. Koi (*Streblus asper* Lour; Moraceae), is a medicinal plant used for several pharmaceutical purposes: bark extracts used in fever, dysentery, relief of toothache and antigingivitis (Gaitonde et al., 1964); leaf extract showed insecticidal activity towards mosquito larvae (Kritsaneepaiboon, 1989); branches used as toothbrush for strengthening teeth and gums (Lewis, 1980); roots applied to unhealthy ulcers, sinuses and locally as antidote to snake bite; and the milky juice has been used as antiseptic and astringent applied to chapped hands and sore heels (Mukherjee and Roy, 1983).
Many studies report the antibacterial actions of *Streblus asper* leaf extract (SAE) towards *Streptococcus mutans* (Taweechaisupapong et al., 2000; Wongkham et al., 2001). However, no reports on the antibacterial effects of SAE on anaerobic bacteria are found. The purpose of this study was therefore to test the antibacterial effects of SAE against selected anaerobic bacteria found in infected root canals.

**MATERIALS AND METHODS**

**Bacteria and Growth Condition**

The bacteria tested in this study were *Porphyromonas gingivalis* W50, *Peptostreptococcus micros*, *Prevotella intermedia*, *Actinomyces naeslundii* (T14V), *Actinobacillus actinomycetemcomitans* ATCC 43717 and 43718. Four bacterial strains (*P. gingivalis*, *P. micros*, *P. intermedia* and *A. naeslundii*) were provided by Prof. R.J. Doyle, Louisville, KY, USA. *A. actinomycetemcomitans* ATCC 43717 and 43718 were provided by Prof. Isao Ishikawa, Tokyo Medical and Dental University, Japan. *P. gingivalis*, *P. micros*, *P. intermedia* were grown in thioglycolate broth (Difco Microbiology Systems, Sparks, MD), supplemented with hemin (5 mg/L) and menadione (0.1 mg/L). *A. naeslundii* and *A. actinomycetemcomitans* were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, MD) and brain heart infusion broth (BBL) respectively. All bacterial strains were incubated at 37°C as static cultures in anaerobic jars containing H₂, CO₂ and N₂ (10:10:80) with GasPaks (BBL) for 48 h. Then their density was adjusted to McFarland 0.5. These bacterial suspensions were used as inoculum for subsequent studies.

**Preparation of SAE**

Koi leaves were locally collected in Khon Kaen province, washed, air-dried, pulverized and extracted as previously described (Taweechaisupapong et al., 2000). Approximately 5 g of dark brown sticky material was obtained from 100 g of the dried-pulverized leaves. The material was dissolved in distilled water at 500 mg/mL, centrifuged at 10,000 rpm (9410 x g) at 4°C for 20 min and passed through a 0.2 µm filter (Acrodisc® PF, GelmanSciences, USA). The filtrate was used as the starting material for subsequent studies.

**Determination of Antibacterial Effects of SAE by the Disk Diffusion Method**

Antibacterial effects of SAE was determined by the disk diffusion method (Barry, 1991). Plates containing Brucella agar (BBL) were seeded with the bacterial suspensions. Seeding was done using sterile swabs that were brushed across the agar surfaces. Paper discs (6 mm in diameter) soaked with 15 µL of 250 and 500 mg/mL SAE, 5% NaOCl (positive control), and distilled water (negative control) were placed onto the agar surface. Then the plates were placed into anaerobic jars and sealed. Anaerobic conditions were maintained using the Gas Pack System (Becton Dickinson Microbiology Systems, Cockeysville, MD). After 1 week of incubation at 37°C, the zones of inhibition were measured across the diameter with a transparent ruler and recorded using the following criteria (- = No zone of inhibition; + = Zone of inhibition > 6-8 mm; ++ = Zone of inhibition > 8 mm). The tests were performed in triplicate for each bacterial strain.

**Determination of Bacteriostatic and Bactericidal Activities of SAE**

Bacteriostatic and bactericidal activities of SAE towards the bacteria were determined as described previously (Barry, 1976). 50 µL of SAE was two-fold serially diluted with thioglycolate broth in a microtitre plate. An equal volume of bacterial suspension was added and mixed with the extract. The plates were incubated for 48 h, at 37°C in an anaerobic chamber (Model – Baclight – 2E, Sheldon Manufacturing, Inc., Oregon). The bacterial growth was examined, and the lowest concentration of the SAE, which inhibited the visible growth of bacteria, was recorded as the minimum growth inhibitory concentration (MIC). The positive growth of each microorganism cultured in
the broth without SAE served as a positive control and the negative growth found in the mixture of broth and SAE without microorganism served as a negative control. Aliquots of the mixture of SAE and bacteria, which showed negative-visible growth after the first 48 h of incubation were inoculated onto the surface of Brucella agar. The lowest concentration of the SAE giving negative growth of bacteria was recorded as the minimum bactericidal concentration (MBC).

RESULTS

The results showed that 15 μL of the SAE at concentration 250 and 500 mg/mL possessed antibacterial activity towards all bacterial strains tested except A. actinomycetemcomitans ATCC 43717 (Table 1). The positive control (5% NaOCl) was effective against all test microorganisms whereas the negative control (distilled water) was always ineffective.

P. gingivalis appeared to be the most susceptible to the extract with a MIC of 3.9 mg/mL (Table 2). Higher concentrations of the SAE were needed to inhibit growth of A. actinomycetemcomitans ATCC 43718, P. micros, P. intermedia, A. actinomycetemcomitans ATCC 43717 and A. naeslundii respectively. Although the SAE was able to inhibit growth of P. intermedia and A. naeslundii, it had no bactericidal activity on these two bacterial strains.

DISCUSSION

The bacteria used in this study were commonly found in endodontic infections (Sundqvist, 1992). An intracanal medicament is used to (1) eliminate any remaining bacteria after canal instrumentation; (2) reduce inflammation of periapical tissues and pulp remnants; (3) render canal contents inert and neutralize tissue debris; (4) act as a barrier against leakage from the temporary filling; (5) help to dry persistently wet canals (Chong and Pitt Ford, 1992). During the period between appointments, bacteria surviving instrumentation and irrigation have been shown to increase rapidly in number in empty root canals (Bystrom and Sundqvist, 1981). Controlled asepsis, including effective root canal disinfection, was shown to be important for successful healing of periapical lesions (Bystrom et al., 1987).

The antibacterial properties of intracanal medicaments have been well researched (Spangberg, 1985), but the choice of medicament remains controversial. To justify the use of these medicaments, their antibacterial activity must be significantly greater than their cytotoxic effect (Messer and Feigal, 1985). Antibacterial agents that are toxic and potent enough to eliminate bacteria may damage periapical tissues. With regard to the toxicity of the SAE, the extract showed no notable signs or symptoms of unwanted side effects in humans (Taweechaisupapong et al., 2000). A study on toxicities of the SAE at subacute doses in rats, revealed that there were no apparent toxic effects after the extract was given orally to rats up to 50 mg/kg body weight, daily for 6 weeks (Wongkham et al., 1996). Therefore, the SAE at concentration 3.9-125 mg/mL, which is able to inhibit growth of all microorganisms used in this study is safe if used in humans whose body weight is at least 40-50 kg.

At present, no antimicrobial agent is effective against all microorganisms. Instead, they have a certain spectrum of activity. Similar to those antimicrobial agents, the SAE is not able to kill all bacteria used in this study. Although the SAE possesses bactericidal properties towards A. actinomycetemcomitans, P. gingivalis and P. micros, it has no bactericidal activity against P. intermedia and A. naeslundii. This is probably due to the difference in cell membrane permeability and cell wall, protein, and nucleic acid synthesis in different strains of bacteria, and each may mediate a different effect. Additional studies are necessary to further determine the mechanisms of antimicrobial action of Koi.

Since Koi possesses antiseptic, antibacterial and potential anti-inflammatory properties, it can be considered as alternative material for intracanal medicament or irrigant. Although the antibacterial effect of the SAE was less effective than NaOCl, the
extract was not irritating and less toxic. Moreover, the anti-inflammatory property of the extract will help to reduce inflammation of pulp remnants or periapical tissues, particularly when time does not permit complete removal of pulp contents. The reduction of inflammation may reduce pain and promote repair of periapical tissues.

ACKNOWLEDGEMENTS
This work was supported by a research grant of Faculty of Dentistry, Khon Kaen University.

Literature Cited


### Tables

**Table 1. Antibacterial activity (zone of inhibition) of the *Streblus asper* leaf extract.**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zone</th>
<th>SAE (250 mg/mL)</th>
<th>SAE (500 mg/mL)</th>
<th>NaOCl (5%)</th>
<th>DW^1</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porphyromonas gingivalis</em> W50</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td><em>Prevotella intermedia</em></td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td><em>Peptostreptococcus micros</em></td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> (T14V)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinobacillus actinomycetemcomitans</em> ATCC 43717</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Actinobacillus actinomycetemcomitans</em> ATCC 43718</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

^1DW, distilled water

**Table 2. The MIC and MBC of the *Streblus asper* leaf extract towards the tested microorganisms.**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porphyromonas gingivalis</em> W50</td>
<td>3.9</td>
<td>31.3</td>
</tr>
<tr>
<td><em>Peptostreptococcus micros</em></td>
<td>15.6</td>
<td>62.5</td>
</tr>
<tr>
<td><em>Prevotella intermedia</em></td>
<td>31.3</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> (T14V)</td>
<td>125</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinobacillus actinomycetemcomitans</em> ATCC 43717</td>
<td>31.3</td>
<td>62.5</td>
</tr>
<tr>
<td><em>Actinobacillus actinomycetemcomitans</em> ATCC 43718</td>
<td>7.8</td>
<td>15.6</td>
</tr>
</tbody>
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