

## Comparative In Vitro Activity of Sanguinarine Against Oral Microbial Isolates

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**MICs of sanguinarine were determined for 52 oral reference strains and 129 fresh isolates from human dental plaque. Sanguinarine was found to completely inhibit the growth of 98% of the isolates at a concentration of 16 µg/ml.**

Caries and periodontal diseases are the most common human dental diseases. It has been demonstrated that plaque microorganisms are the primary etiological agents of these diseases. Therefore, reduction or elimination of plaque is essential in preventing or reducing the incidence of these diseases. Various dentifrices and oral rinses containing agents such as chlorhexidine and quaternary ammonium compounds to combat dental plaque have been developed with varying degrees of success. Sanguinarine, an agent in one oral rinse, is an alkaloid extract from the rhizome of the plant *Sanguinaria canadensis*. It has been shown to possess a broad spectrum of in vitro activity against a wide variety of microorganisms including fungi, yeasts, and phages (1, 5, 7, 14, 15). An extract of *Sanguinaria* has been incorporated in cough syrups and cold remedies as an expectorant for decades, and a structurally similar compound called fagaronine, in the form of the "Nigerian chewing stick", has been beneficial to the oral hygiene of African natives (10). It was recently shown that sanguinarine is retained in high concentrations for several hours in the oral cavity in both plaque and saliva after oral rinsing and has the ability to reduce the formation of plaque on tooth surfaces (11). The purpose of the present investigation was to determine the MICs of sanguinarine for fresh oral isolates and oral reference strains.

A total of 129 fresh isolates obtained from human dental plaque and 52 oral reference strains were tested to determine the MICs of sanguinarine. Since sanguinarine is incorporated in oral dentifrices and rinses, the microbial strains selected for the present investigation were representative of those commonly found in supragingival plaque. Fresh isolates from plaque samples were obtained from periodontal pockets by using an oxygen-free gas-flushed sampling device, as described by Newman and Socransky (9). The plaque samples were dispersed in prerduced, anaerobically sterilized 1/4-strength Ringer solution by sonic oscillation under a constant flow of oxygen-free gas (80% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>) for 10 s. The samples were then subjected to 10-fold serial dilutions in prerduced, full-strength Ringer solution, and 0.1-ml portions were plated in duplicate onto Trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.), as described by Manganiello et al. (8) and Tanner et al. (12). After 5 to 7 days of anaerobic incubation at 35 to 37°C, colonies were chosen from high-dilution plates and subcultured onto Trypticase soy agar-blood plates. Cultures were transferred until pure, characterized (2, 12), and identified

(3, 12; J. L. Dzink, A. C. R. Tanner, A. D. Haffajee, and S. S. Socransky, *J. Clin. Periodontol.*, in press). Isolates were identified by a probabilistic identification program with the algorithm of Lapage et al. (6). Gram-positive rods which failed to fit existing species descriptions were grouped by cluster analysis (4).

The bacterial isolates were maintained on Trypticase soy agar-blood plates in an anaerobic atmosphere at 35 to 37°C. The isolates were placed into 4 ml of sterile Mycoplasma broth (BBL) supplemented with 5 µg of hemin per ml and 0.1% glucose and incubated for 24 h. The broth cultures were adjusted with sterile broth to approximate a 0.5 McFarland turbidity standard. Established microtiter methods for susceptibility testing were used (13). Microtiter plates containing various concentrations of sanguinarine were prepared as follows. A chemically defined medium consisting of 14 inorganic salts, 23 amino acids, 24 vitamins and other factors, 7 purines and pyrimidines, and glucose (S. S. Socransky, J. L. Dzink, and C. M. Smith, submitted for publication) was dispensed in 60-ml portions into flasks. Sufficient sanguinarine was added to each flask to form final concentrations of 1, 2, 4, 8, 16, 32, 64, 128, and 512 µg/ml. In addition, the medium without sanguinarine was used as a controls. After the media were filter sterilized through a 0.2-µm (pore size) Nalgene filter, 0.1-ml portions were aseptically dispensed into the wells of sterile microtiter plates with an MIC 2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.). The plates were used immediately after preparation.

Samples (approximately 1.5 µl) of the broth inocula were transferred to the wells of microtiter plates with an MIC 2000 inoculator. The plates were incubated anaerobically for 2 days at 37°C; they were then removed, and the MIC values were recorded. The MIC was defined as the lowest concentration of test agent at which no growth was observed (13).

The MICs of sanguinarine for the oral reference strains and fresh isolates are shown in Table 1; they ranged from 1 to 16 µg/ml for all but four isolates (98%). Two strains each of *Wolinella succinogenes* and *Peptococcus prevotii* showed elevated MICs, of 32 µg/ml.

Preliminary susceptibility studies of sanguinarine against oral microbial strains were performed on blood agar plates using a Steer replicator. When attempts were made to repeat the study with complex broth media, the sanguinarine precipitated, obscuring the MIC values. The precipitation was due to the binding of sanguinarine to protein in the complex media and was averted by the use of a protein-free chemically defined medium. The values obtained with this medium

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TABLE 1. Susceptibility of oral microbial species to sanguinarine

Species	No. of strains	MIC range ( $\mu\text{g/ml}$ )	MIC <sub>50</sub> <sup>a</sup> ( $\mu\text{g/ml}$ )	MIC <sub>90</sub> <sup>a</sup> ( $\mu\text{g/ml}$ )
<i>Actinobacillus actinomycetemcomitans</i>	4	8-16	— <sup>b</sup>	—
<i>Actinomyces</i>	18	1-8	4	8
<i>A. israelii</i> (2) <sup>c</sup> , <i>A. meyerii</i> (2), <i>A. odontolyticus</i> (2), <i>A. naeslundii</i> (2), <i>A. viscosus</i> (3), <i>Actinomyces</i> sp. (7) <sup>d</sup>				
<i>Arachnia propionica</i>	2	4-8	—	—
<i>Anaerovibrio lipolytica</i>	2	8	—	—
<i>Bacteroides</i>	12	1-16	4	8
<i>B. gingivalis</i> (2), <i>B. intermedius</i> (1), <i>B. oralis</i> (3), <i>B. oris</i> (1), <i>B. corporis</i> (1), <i>B. melaninogenicus</i> (1), <i>Bacteroides</i> sp. (3)				
<i>Bacterionema matruchotii</i>	1	4	—	—
<i>Bifidobacterium</i>	10	2-16	2	4
<i>B. adolescentis</i> (1), <i>B. dentium</i> (2), <i>B. infantis</i> (1), <i>Bifidobacterium</i> sp. (6) <sup>d</sup>				
<i>Capnocytophaga</i>	9	1-4	2	4
<i>C. gingivalis</i> (1), <i>C. ochracea</i> (5), <i>C. sputigena</i> (3)				
<i>Campylobacter concisus</i>	1	1	—	—
<i>Campylobacter</i> sp.	1	16	—	—
<i>Eikenella corrodens</i>	3	8-16	—	—
<i>Eubacterium</i>	12	1-16	4	8
<i>E. aerofaciens</i> (2), <i>E. brachy</i> (1), <i>E. combesii</i> (1), <i>E. contortum</i> (1), <i>E. cylindroides</i> (1), <i>E. lentum</i> (2), <i>E. necrogenes</i> (2), <i>E. timidum</i> (1), <i>Eubacterium</i> sp. (1) <sup>d</sup>				
<i>Fusobacterium nucleatum</i>	10	1-4	1	4
<i>Lactobacillus</i>	6	2-8	—	—
<i>L. acidophilus</i> (3), <i>L. casei</i> (2), <i>Lactobacillus</i> sp. (1) <sup>d</sup>				
<i>Leptotrichia buccalis</i>	1	8	—	—
<i>Peptococcus prevotii</i>	4	4-32	—	—
<i>Peptostreptococcus</i>	8	4-8	8	8
<i>P. anaerobius</i> (4), <i>P. micros</i> (3), <i>P. productus</i> (1)				
<i>Propionibacterium</i>	21	1-8	2	4
<i>P. acnes</i> (12), <i>P. avidum</i> (1), <i>P. jensenii</i> (1), <i>P. lymphophilum</i> (1), <i>Propionibacterium</i> sp. (6) <sup>b</sup>				
<i>Selenomonas sputigena</i>	2	2-8	—	—
<i>Staphylococcus</i>	7	4-16	—	—
<i>S. capitus</i> (1), <i>S. hominis</i> (3), <i>S. xylosum</i> (3)				
<i>Streptococcus</i>	41	1-8	4	8
<i>S. acidominimus</i> (2), <i>S. intermedius</i> (8), <i>S. mitis</i> (5), <i>S. morbillorum</i> (12), <i>S. mutans</i> (4), <i>S. sanguis</i> (6), <i>S. uberis</i> (4)				
<i>Wolinella</i>	6	4-32	—	—
<i>W. curva</i> (1), <i>W. recta</i> (3), <i>W. succinogenes</i> (2)				

<sup>a</sup> MIC<sub>50</sub>, MIC for 50% of strains; MIC<sub>90</sub>, MIC for 90% of strains.

<sup>b</sup> —, Number of strains too small for MICs to be determined for 50 or 90% of strains.

<sup>c</sup> Numbers in parentheses indicate numbers of strains of that species.

<sup>d</sup> Strains placed in cluster groups which failed to fit existing species.

may be representative of the activity of free sanguinarine, but the amount of sanguinarine which remains active in saliva or crevicular fluid or on tooth or mucosal surfaces has yet to be determined.

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