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Antibacterial activity of *salvia officinalis* L. against periodontopathogens: An in vitro study

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ABSTRACT

Being aware of the remarkable antimicrobial potential of *S. officinalis* L., we aimed to evaluate the antimicrobial activity of the *S. officinalis* dichloromethane crude extract (SOD), dichloromethane-soluble fractions (SODH and SODD), SODD subfractions (SODD1 and SODD2), and pure substances (manool, salvigenin, and viridiflorol) against periodontopathogens. This bioassay-guided study comprises five antimicrobial tests—determination of the Minimum Inhibitory Concentration (MIC), determination of the Minimum Bactericidal Concentration (MBC), determination of the antibiofilm activity, construction of the Time-kill curve (determination of Bactericidal Kinetics), and determination of the Fractional Inhibitory Concentration Index—on six clinical bacterial isolates and three standard bacterial strains involved in periodontal disease. SOD has moderate activity against most of the tested bacteria, whereas SODD1, SODH1, SODH3, and manool afford the lowest results. The *Porphyromonas gingivalis* (ATTC and clinical isolate) biofilm is considerably resistant to all the samples. In association with chlorhexidine gluconate, only SODH1 exerts additive action against *P. gingivalis* (clinical isolate). Therefore, SODH1 and manool are promising antibacterial agents and may provide therapeutic solutions for periodontal infections.

1. Introduction

The advent of new scientific discoveries in the field of drug synthesis and the progressive development of medical science have brought back a traditional way to fight infections: the use of herbal medicines. In 2016, Newman and Craag [1] cataloged 326 anti-infective (antibacterial, antifungal, antiparasitic, and antiviral) drugs of direct natural origin or containing a compound that mimics the action of a natural compound and which have been discovered and introduced as treatment. By definition, the pharmacognosy branch denominated herbal medicine explores the application of medicinal plant-derived compounds (phytochemicals) in the treatment or prevention of various diseases. Plants employed for this purpose have shown efficient and promising action against several infections, including periodontal disease [2,3].

Periodontal disease is defined as an inflammation of the tooth-supporting tissues (cementum, bone, and ligament) that is initiated by an imbalance between bacterial growth and the host immune system. This disease is frequently diagnosed in dental clinics. The diagnosis is based on a bleeding sign after any mechanical injury, periodontal pocket formation, hyperplasia and gingival attachment loss, commitment of the ligament and bone that support the tooth, excessive tooth mobility, or even tooth loss. During the development of this infection, metabolic products from potential periodontopathogenic bacteria trigger leucodiapedesis and release

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inflammation mediators that arrive at the damaged tissue to combat infection and to conduct subsequent repair [4–6].

The presence of periodontopathogens undeniably characterizes the periodontal disease [7,8]. The bacteria that are usually isolated from patients with periodontal disease include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Treponema denticola* [7,9–13]. The most efficient prevention for this disease is to eliminate the pathogens, which has proven to be challenging. In fact, the high incidence of periodontal disease is a major problem that needs to be overcome.

Salvia officinalis is a member of the family Lamiaceae. It is a traditional medicinal herb that is characterized as a perennial low shrub originating in the Mediterranean region. Its family is known to comprise over 900 species [14,15]. In Brazil, the genus *Salvia* contains 68 species, which are distributed in the Southern, Southeastern, Midwestern, and Northeastern regions of the country and have been used to treat digestive and circulation disturbances, bronchitis, cough, asthma, angina, mouth and throat inflammations, depression, excessive sweating, and skin diseases [15]. More specifically, monoterpenes, diterpenes, triterpenes, and phenolic components occurring in *S. officinalis* are known to have multiple functions, like toning, emmenagogue, antiperspirant, choleretic, hypoglycemic, stimulant, astringent, antimicrobial, and antihypertensive actions, not to mention their role in the treatment of stomach pain, dental abscess, and other buccopharyngeal disorders [15–17]. The *S. officinalis* essential oil contains major terpenes such as manool, viridiflorol, eucalyptol, borneol, and thujone. In turn, carnosol, carnosic acid, rosmarinic acid, flavonoids, polysaccharides, tannic acid, oleic acid, ursonic acid, ursolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic substances have already been identified in *S. officinalis* leaves [18,19].

The plant *S. officinalis* is widely applied as condiment, and its many medical uses have paved the way for a new and promising research line. Throughout the years, studies have proven the *S. officinalis* antibacterial effect against numerous strains, including bacteria that cause pharyngitis [20], food and air-borne pathogens [21,22], and cariogenic bacteria [23–25].

In addition, Oliveira et al. [26] have tested the antimicrobial and non-cytotoxic profile of effective *S. officinalis* glycolic extract concentrations against clinical samples of *Streptococcus mutans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida* species. At 50 mg/mL, this extract eliminates 100% of the strains without toxicity. Another study against cariogenic bacteria has shown that this extract in the powder form associated with glass-ionomer cement (GIC) inhibits *S. mutans* and *Lactobacillus casei* [27].

Although *S. officinalis* has been used as an antimicrobial along the years, either in the most rudimentary way or in modern research, its action against bacteria isolated from periodontal disease is not fully understood. Being aware of this gap in the knowledge concerning this research line and of the remarkable *S. officinalis* potential, we aim to analyze the microbiological activity of the *S. officinalis* crude extract, partitions, fractions, and pure substances by means of five antimicrobial tests against six clinical isolates and three standard strains of periodontopathogens.

2. Material and methods

2.1. Crude extract, fractions, metabolites, and bioguided assay

Bioguided assay fractionation with *S. officinalis* was performed because its dichloromethane crude extract (SOD) has already proven to be effective against oral bacterial infections [28]. All the tested samples were provided by the Research Group on Natural Products from the University of Franca. SOD and its derivatives (fractions and compounds) were obtained as described previously [28–30]. Briefly, *S. officinalis* L aerial parts were extracted with dichloromethane, to give SOD after solvent evaporation. This extract was successively partitioned with *n*-hexane and dichloromethane, to afford the *n*-hexane-soluble and dichloromethane-soluble fractions (SODH and SODD, respectively). SODH and SODD were then submitted to vacuum liquid chromatography with silica gel 60H (Merck, art. 7736) as stationary phase and a gradient elution system of increasing polarity (*n*-hexane and ethyl acetate) as mobile phase, to yield seven SODH subfractions (SODH1 to SODH7) and two SODD subfractions (SODD1 and SODD2). SODH2 and SODH5 were subjected to liquid chromatography with silica gel 60 (Merck, art. 7734) and *n*-hexane/ethyl acetate (9:1) as eluent, to give fifty fractions each. On the basis of thin layer chromatography, the fractions were then combined to afford the pure substances manool, salvigenin, and viridiflorol. The three purified compounds were identified by ^{1}H and ^{13}C NMR [28–30].

All the samples were used to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). The samples that yielded the best results were employed to construct the Time-kill curve (TKC) and to determine the antibiofilm activity and the Fractional Inhibitory Concentration Index (FICI).

2.2. Bacterial strains

To test *S. officinalis* against periodontal pathogens, six standard strains from the American Type Culture Collection (ATCC, Manassas, VA, USA) and three clinical isolates (CI) were used, namely *Porphyromonas gingivalis* (ATCC 33277 and CI), *Aggregatibacter actinomycetemcomitans* (ATCC 43717 and CI), *Prevotella intermedia* (ATCC 49046 and CI), *Prevotella nigrescens* (ATCC 33563), *Fusobacterium nucleatum* (ATCC 33277/CI), and *Prevotella melaninogenica* (ATCC 700524). The evaluated clinical isolates were isolated from clinical trials and kept in the Laboratory of Research on Antimicrobial Trials library under cryopreservation.

2.3. Determination of the Minimum Inhibitory Concentration (MIC) and of the Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) was determined in triplicate by using the microdilution broth method in 96-well microplates [31]. To this end, the tested samples were prepared at concentrations ranging from 0.195 to 400.0 μg/mL. Aerobic and microaerophilic bacteria were incubated for 24 h; anaerobes were incubated at 37 °C for 72 h in an anaerobic workstation (Don Whitley Scientific, Bradford, UK) with 5 or 10% H_2, 10% CO_2, 85 or 80% N_2 atmosphere. After that, resazurin (30 μL) in aqueous solution (0.02%) was added to the microplates, to indicate microorganism viability [32]. Before resazurin was added and to determine the MBC, an aliquot of the inoculum (10 μL) was aseptically removed from each well and plated onto BHI agar supplemented with 5% sheep blood for aerobic and microaerophilic bacteria and onto Brucella agar supplemented with 5% sheep blood and with hemin (5 mg/mL, Sigma, St. Louis, MO, USA) and menadione (1 mg/mL, Sigma for anaerobic bacteria). The plates were incubated as described previously. The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of the sample where no bacterial growth occurred.

2.4. Biofilm formation inhibition as assessed by the Minimum Inhibitory Concentration of Biofilm ($MICB_{50}$)

To evaluate the antibiofilm activity standardization, data on biofilm formation (data not shown) obtained in a previous work by

our research group were used. The bacterial inoculum and the procedure were the same as those described for the MIC determination. However, 200 μL of Brucella (Difco) broth supplemented with hemin (5 mg/mL, Sigma) and menadione (1 mg/mL, Sigma) for the anaerobic bacteria and 100 μL of Brain Heart Infusion (BHI) broth for *A. actinomycetemcomitans*, were added to the wells to give final concentrations ranging from 0.195 to 400 μg/mL. A sterility control and a growth control (wells containing biofilm but no drug or samples) were included. To investigate the antibiofilm activity, two methods were employed: optical density (OD) and counting of microorganisms in Colony Forming Units per milliliter (CFU/mL) [33]. Many protocols have been proposed for the analysis of relative biofilm formation. However, to develop and to screen substances for their antibiofilm activity rapidly, a fast, easy, reproducible, and inexpensive method like the one described here is necessary. $MICB_{50}$ determination is a static biofilm assay that typically allows for 96-well plate formats and is more amenable to high-throughput screening approaches [34]. Contemporary MIC tests, which measure only planktonic susceptibility, may account for treatment failures and development of resistance among bacterial biofilms [35].

2.5. Construction of the time-kill curve (bactericidal kinetics)

The Time-kill assays were performed in triplicate on the basis of the method described by D'Arrigo et al. [36]. Tubes containing Brucella broth (Difco) supplemented with hemin (5 mg/mL, Sigma) and menadione (1 mg/mL, Sigma) for the anaerobic bacteria and with BHI for *A. actinomycetemcomitans* at final concentrations of 12.5, 50, and 100 mg/mL were inoculated with the tested microorganisms. Aliquots (50 μL) were removed to count viable colonies at 0 and 30 min and at 6, 12, 18, and 24 h for the microaerobic bacteria and at 0 and 30 min and at 6, 12, 18, 24, 48, and 72 h for the anaerobic bacteria. Incubation was followed by serial dilution to 10^4 in sterile broth. The diluted samples (50 μL) were spread onto Brucella and BHI agar, and viable colonies were counted after incubation for 24 and 72 h for the microaerobic and the anaerobic bacteria, respectively. The data were analyzed, and the Time-kill curves were constructed by plotting $\log_{10}$ CFU/mL versus time with the aid of the Prism software (version 7.0; GraphPad, Inc.).

2.6. Determination of the Fractional Inhibitory Concentration Index

The Fractional Inhibitory Concentration Index (FICI) was determined by microdilution in the wells of 96-well microplates, which resembled a “checkerboard” [37] and contained serial dilutions of the samples and chlorhexidine being tested alone or in combination. The culture broth consisted of Brucella (Difco) added with hemin (5 mg/mL) and menadione (1 mg/mL) for the anaerobic bacteria and BHI for *A. actinomycetemcomitans*. The microplates containing the anaerobic bacteria were incubated in an anaerobic chamber at 36 °C for 72 h, and the microplate containing *A. actinomycetemcomitans* was placed in a microaerophilic jar at 37 °C for 24 h. After incubation, 30 μL of 0.02% aqueous resazurin (Sigma) was added to each well, to reveal microbial growth. The developed color was blue (no bacterial growth) or pink (bacterial growth). The interpretation was based on the criteria described by Lewis [37], which define action as synergistic when FICI is ≤ 0.5, additive when FICI ranges from >0.5 to 1.0, indifferent when FICI ranges from 1.0 to 4.0 ˃, and antagonistic when FICI ˃ 4.0.

3. Results

Here, MIC values higher than 400 μg/mL corresponded to absence of antibacterial action since this was the highest concentration that we employed in the assays. SOD provided the lowest MIC value, 50 μg/mL, against *A. actinomycetemcomitans* (ATCC). As for the other tested bacteria, SOD gave MIC values that ranged from 100 to 400 μg/mL. SODD1 afforded noteworthy results: this sample was active against a higher number of bacteria and MIC values were equal to 50 μg/mL (Table 1). Therefore, SODD1 was satisfactorily active against *P. gingivalis*, *P. nigrescens*, *F. nucleatum*, *P. melaninogenica*, and *A. actinomycetemcomitans*. SODH1 and SODH2 displayed pronounced antibacterial effect against one bacterial strain only: *A. actinomycetemcomitans* (ATCC), for which the MIC value was 25 μg/mL. On the other hand, SODH3 exhibited activity against *P. gingivalis* (ATCC and CI) and *A. actinomycetemcomitans* (ATCC), with MIC equal to 50 μg/mL. We achieved moderate antibacterial activities with SODH4, SODH5, SODH6, and SODH7, for which MIC values varied from 100 to 400 μg/mL (Table 1). As for the pure compounds, the diterpene manool provided the lowest MIC values, in contrast to salvigenin and viridiflorol, which did not give relevant MIC values; that is, MIC was higher than 400 μg/mL (Table 1). In particular, manool afforded satisfactory antibacterial action against *P. gingivalis* (ATCC) and *F. nucleatum* (ATCC and CI), for which MIC values were equal to 50 μg/mL. Manool is also worthy of mention for its expressive effect against *A. actinomycetemcomitans* (ATCC), which provided the lowest MIC: 3.12 μg/mL. Overall, these values were the same as or lower than values that are considered satisfactory [38].

Fig. 1 shows the results concerning biofilm formation expressed as $MICB_{50}$ values. The $MICB_{50}$ values ranged from 3.668 to 200 μg/mL. The *P. gingivalis* (ATCC and CI) biofilm was the most resistant to all the tested samples: $MICB_{50}$ was 200 μg/mL. In contrast, the *F. nucleatum* (ATCC) biofilm was the most sensitive to all the assayed samples, with $MICB_{50}$ values varying from 12 to 100 μg/mL. SODD1 and SODH3 afforded $MICB_{50}$ of 25 μg/mL, which indicated satisfactory action against the *P. melaninogenica* (ATCC) biofilm. Again, the diterpene diterpeno manool was the most active purê compound against the most sensitive strain: $MICB_{50}$ was 12.5 μg/mL against *A. actinomycetemcomitans* (ATCC).

We conducted the Time-kill curve assay (curves illustrated in Fig. 2) on the basis of the promising MBC values (Table 1). Manool and SODH1 gave the most promising bactericidal kinetics against *P. gingivalis* (ATCC), which was totally eliminated at manool or SODH1 concentrations of 50 and 200 μg/mL within 18 and 12 h, respectively. Other important results were *P. nigrescens* (ATCC) elimination by 400 μg/mL SODH3 within 12 h, *P. melaninogenica* elimination by 100 μg/mL SODD1 within 24 h, and *P. nigrescens* elimination by 50 μg/mL SODD1 within 48 h. The FICI determined for *P. gingivalis* was 0.75, which corresponded to an additive effect [37]. FICI results pointed to an antagonistic effect for CDH combined with any of the samples against *A. actinomycetemcomitans* (ATCC). The FICI values varied from 7.9 to 23.9, and synergism was the most frequent result (Table 2).

4. Discussion

Studies of medicinal plants displaying antibacterial activities have already produced great results for the human health. The need to make new discoveries is clear when bacterial resistance, new and more aggressive diseases, or the requirement for individualized treatment is concerned. This study aimed to evaluate the antimicrobial action of the *S. officinalis* dichloromethane crude extract (SOD), dichloromethane-soluble fractions (SODH and SODD), SODD subfractions (SODD1 and SODD2), and pure substances (manool, salvigenin, and viridiflorol) against periodontopathogens.

After a review of the literature, we did not find studies on the *S. officinalis* antimicrobial activity against periodontopathogens even though the potential of this species has been proven in

Table 1
In vitro antibacterial activity (MIC and MBC) of the *S. officinalis* crude extract, fractions, partitions, and pure substance (manool) against periodontopathogens - Results >400 (μg/mL).

Samples	Minimum Inibitory Concentration (μg/mL)/Mininum Bacterial Concentration (μg/mL)								
	Bacteria								
	P. gingivalis ATCC 33277	*P. gingivalis* Clinical Isolate	*P. intermedia* ATCC 49046	*P. intermedia* Clinical Isolate	*P. nigrescens* ATCC 33563	*F. nucleatum* ATCC 49046	*F. nucleatum* Clinical Isolate	*P. melaninogenica* ATCC 700524	*A. actinomycetemcomitans* ATCC 43717
SOD	200/200	200/200	–	–	200/200	400/-	400/400	200/400	50/100
SODD1	50/50	50/200	–	200/400	50/200	200/-	50/50	50/100	50/50
SODD2	–	–	–	–	–	–	–	–	–
SODH1	100/200	100/200	–	400/400	100/200	200/-	100/100	100/200	25/25
SODH2	400/400	400/400	–	–	400/400	400/-	400/400	400/-	25/25
SODH3	50/200	50/100	–	200/200	100/400	200/-	100/200	100/100	50/50
SODH4	200/400	200/400	–	–	400/400	–	200/-	200/-	100/100
SODH5	200/400	400/-	–	–	400/400	400/-	400/-	400/-	100/100
SODH6	400/400	–	–	–	–	–	400/-	–	400/400
SODH7	–	–	–	–	–	–	–	–	100/-
Manool	50/50	100/-	–	–	400/-	50/-	50/-	400/-	3.12/6.25
Salvigenin	–	–	–	–	–	–	–	–	–
Viridiflorol	–	–	–	–	–	–	–	–	–
Clorhexidine	7.375/7.375	7.375/14.75	14.75/14.75	7.375/14.75	7.375/14.75	3.688/3.6888	0.922/14.75	3.688/29.5	7.375/7.375

previous investigations. MIC assays have been extensively applied to test the antibacterial action of medicinal plants [39,40]. Alves et al. [41] suggested that these assays are the best strategy to assess the antimicrobial activity of natural products as compared to the efficacy of four other screening techniques. Holetz et al. [38] reported standards for the MIC results of compounds from crude plant extracts and established that values less than 100 μg/mL correspond to good antimicrobial activity; between 100 and 500 μg/mL refer to moderate antimicrobial activity; from 500 to 1000 μg/mL mean weak antimicrobial activity, and exceeding 1000 μg/mL mean inactivity. Here, we have considered values above 400 μg/mL as inactivity.

The results from this research confirm the reports on the *S. officinalis* antimicrobial action and highlight that manool is the component with a crucial role in the *S. officinalis* antimicrobial activity, as previously observed with *S. officinalis* leaves [42,43]. Along with the MIC, we also assessed the MBC. Our data show that most of the lowest results correspond to inhibitory action, which is followed by bactericidal activity for the very next concentration, as in the case of manool against *A. actinomycetemcomitans* (ATCC): we observed inhibitory action and bactericidal effect at 3.12 and 6.25 μg/mL, respectively (Table 1).

Individual planktonic cells are important to understand aspects of bacteria correctly. On the other hand, biofilm formation is advantageous to microorganisms: it increases pathogenic factors as well as cohesion and adhesion to surfaces, offers metabolic protection against competing microorganisms, and provides a barrier against antimicrobials and environmental stress [44,45]. Therefore, using bacteria in the planktonic mode to select antimicrobial agents may be inadequate in some circumstances. The antibiofilm activity as assessed by the Minimum Inhibitory Concentration of Biofilm ($MICB_{50}$) is defined by the lowest antibacterial agent concentration leading to 50% or more biofilm formation inhibition [33]. Here, we determined the optical density (O.D.) and the number of microorganisms (log_{10} CFU/mL) in the antibiofilm activity assay of *S. officinalis* samples with the lowest MIC results and a standard dehydroabietic acid and chlorhexidine dichlorohydrate sample.

Although no previous studies about *S. officinalis* against the selected bacteria exist, Marangoni et al. [46] tested three diterpenes of the pimarane type against endodontic bacteria. Their results suggested that all the diterpenes have good action against *P. nigrescens*, *P. intermedia*, *P. gingivalis*, and *A. actinomycetemcomitans* clinical isolates and standard strains: the compounds inhibit at least 50% of the assayed bacteria, with $MICB_{50}$ values ranging from 6.25 to 25 μg/mL. In 2017, Moreti et al. [47] determined the antibiofilm activity of a diterpene isolated from *Mikania glomerata* extract (*ent*-kaurenoic acid) against *A. actinomycetemcomitans* (ATCC 43717), *P. nigrescens* (ATCC 33563), and *P. gingivalis* (clinical isolate), to obtain 3.12, 6.25, and 200 μg/mL, respectively. In the present investigation, analysis of the results in Fig. 1 revealed that the bacterial count, expressed as Log_{10} CFU/mL, progressively decreases while the absorbance increases. When total elimination of the bacteria is close, the biofilm matrix production augments. This effect could be due to an attempt of the strain to become less susceptible to the samples.

A previous study had already proven the antibacterial potential of *S. officinalis* metabolites: Moreira et al. [28] tested manool and SODH1 samples against cariogenic bacteria. According to their results, manool at 12.48 μg/mL (twice its MBC value) requires 6 h to kill the bacteria completely; SODH2 at twice its MBC (31.36 μg/mL) needs 12 h. Souza et al. [48] investigated the bactericidal kinetics of copalic acid at 3.1 μg/mL against *P. gingivalis* (ATCC 33277). They found that log_{10} CFU decreases significantly, which culminates in total bacterium elimination within 24 h. In the present study, we detected SODH1 action for the same strain within in 12 h, but at a higher SODH1 concentration (200 μg/mL). Marangoni et al. [46] also plotted the Time-kill curve of three diterpenes; they considered that the antibacterial action corresponds to a decrease of over 3 Log_{10} in the number of microorganisms. They found that the three diterpenes cause a reduction of over 3 Log_{10} for *P. gingivalis* (CI) and *P. nigrescens* (CI) after incubation for 6 h.

We determined the FICI by following the standards defined by CLSI [31]. We combined SODH1, SODH3, or manool with chlorhexidine (CDH). The combination of standard antimicrobials with substances derived from plants like *S. officinalis* could represent a strategy to prevent antimicrobial resistance. Previous studies have shown encouraging results with essential oils and fluconazole against *Candida* spp [49,50]. Marangoni et al. [47] concluded that CDH combined with two of the three tested diterpenes acts synergistically against *P. gingivalis* (ATCC 33277), and any of the three diterpenes in combination with CDH exerts synergistic or additive effect against its clinical isolate (Table 2).

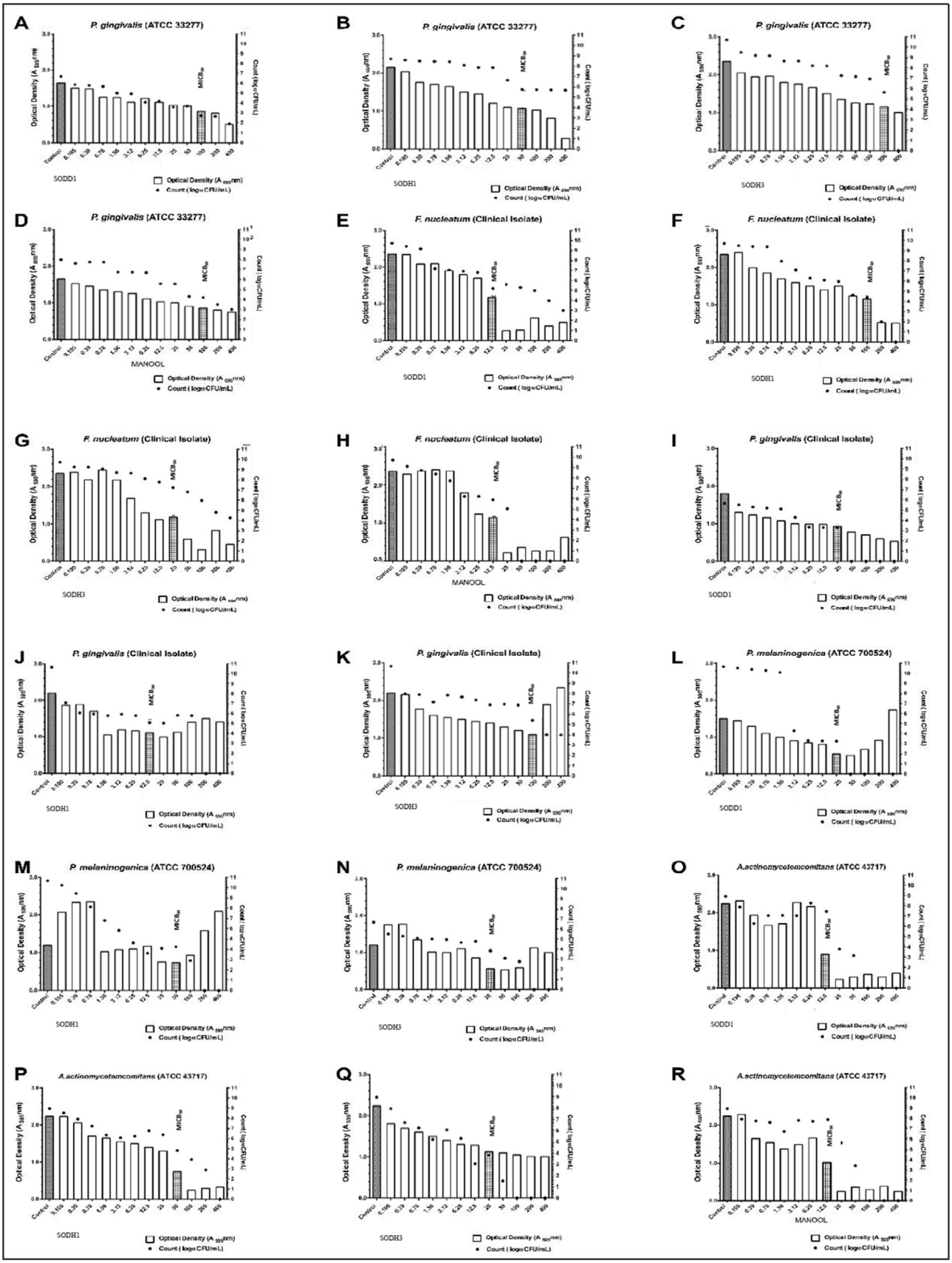

Fig. 1. Biofilm formation inhibition as assessed by the Minimum Inhibitory Concentration of Biofilm ($MICB_{50}$) for the *S. officinalis* fractions, partitions, and pure substance (manool) against periodontopathogens.

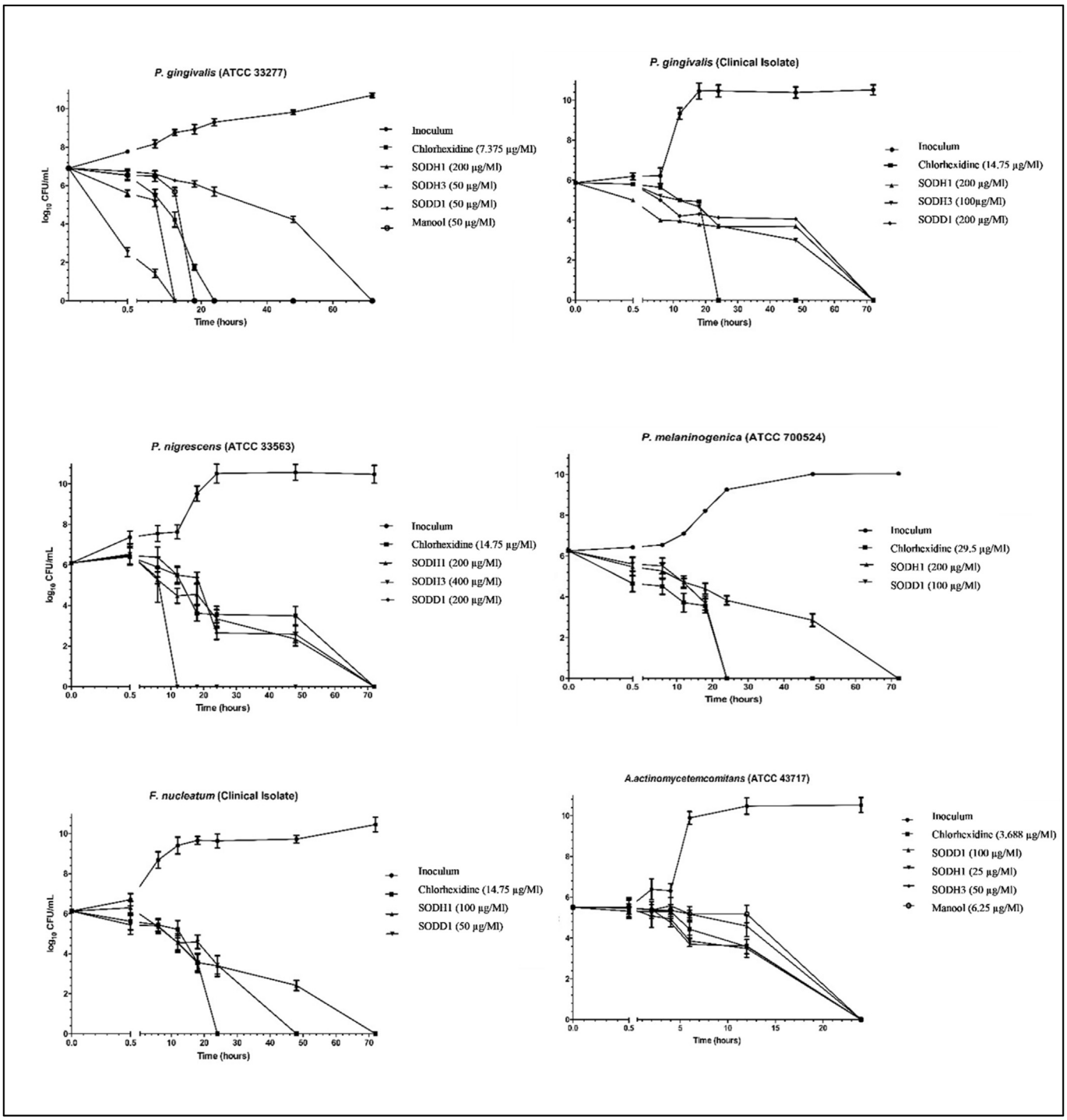

Fig. 2. Time-kill curve for *S. officinalis* fractions, partitions, and pure substance (manool) against periodontopathogens.

The use of manool, or any other sample analyzed in this study for that matter, as antibacterial in products for oral hygiene or clinical treatment depends on cytotoxicity, as well. Manool has already been demonstrated to have highly selective cytotoxicity against tumor cell lines as compared to the normal cell line V79 [30]. Nicolella et al. [29] previously suggested that manool exhibits a protective effect against chromosome damage induced by methyl methanesulfonate in HepG2 cells, but not in V79 cells. These data indicate that high manool concentration is cytotoxic, and that one of its metabolites may underlie the antigenotoxic effect, which justifies its further investigation.

We verified that the *S. officinalis* crude extract (SOD) displays moderate action against all the periodontal bacteria tested herein. SODD1, SODH1, SODH3, and manool are promising antimicrobial agents with low MIC and MBC values. Among the microorganisms evaluated here, *A. actinomycetemcomitans* is the most sensitive to the tested samples: the lowest MIC values are detected against this microorganism. The biofilm of *P. gingivalis*, an important pathogen in periodontal disease, is resistant to all the tested samples. SODH1 is the only subfraction that exerts additive action when associated with chlorhexidine gluconate. Therefore, SODH1 and manool are

Table 2
Fractional Inhibitory Concentration Index results for the *S. officinalis* fractions, partitions, and pure substance (Manool) against periodontopathogens according to the criteria of LEWIS et al. [37].

Bacteria	Samples		
	SODH1	SODH3	Manool
P. gingivalis (CI)	Additive (0.7)	Indifferent (2.2)	–
P. gingivalis (ATCC)	Indifferent (1.5)	Indifferent (2.0)	Indifferent (1.0)
F. nucleatum (CI)	Indifferent (1.5)	Indifferent (3.0)	Antagonistic (4.2)
P. melaninogenica (ATCC)	Indifferent (1.5)	Indifferent (2.0)	–
A.actinomycetemcomitans (ATCC)	Antagonistic (23.9)	Antagonistic (11.9)	Antagonistic (7.9)

(): Fractional Inhibitory Concentration Index. -: Not evaluated.

the most promising among the samples assayed herein, which indicates the need to study them further for use as antimicrobial agents against periodontal disease pathogens.

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Declarations of competing interest

None.

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