# Journal Pre-proof

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PII: S0003-9969(19)30622-3

DOI: https://doi.org/10.1016/j.archoralbio.2019.104585

Reference: AOB 104585

To appear in: *Archives of Oral Biology*

Received Date: 19 June 2019

Revised Date: 27 September 2019

Accepted Date: 5 October 2019

Please cite this article as: Veloso DJ, Abrão F, Martins CHG, Bronzato JD, Gomes BPFA, Higino JS, Sampaio FC, Potential antibacterial and anti-halitosis activity of medicinal plants against oral bacteria, *Archives of Oral Biology* (2019), doi: https://doi.org/10.1016/j.archoralbio.2019.104585

ORIGINAL RESEARCH

# POTENTIAL ANTIBACTERIAL AND ANTI-HALITOSIS ACTIVITY OF MEDICINAL PLANTS AGAINST ORAL BACTERIA

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**Highlights**

Medicinal plants; Jucá, Cinnamon, Mallow, Pomegranate, Rosemary, Macassá, Clove, and Tamarind, have antimicrobial activity on oral PLFURRUJDQLVP¶V SURGXFHUV RI YRODWLOH VXO compounds; such as Fusobacterium nucleatum, Parvimonas micra, Porphyromonas gingivalis, and Prevotella intermedia. They have the potential to reduce halitosis of oral origin

**Abstract**

This study aimed to evaluate the *in vitro* activity of the crude extracts obtained from *Caesalpinia ferrea* Mart. (Jucá), *Cinnamomum cassia* B. (Cinnamon), *Mallow sylvestris* L. (Mallow), *Punica granatum* L. (Pomegranate), *Rosmarinus officinalis* L. (Rosemary), *Aeolanthus suaveolens* (Als.) Spreng. (Macassá), *Sysygium aromaticum* L. (Clove), and *Tamarindus indica* L. (Tamarind) against oral microorganisms (e.g., *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Parvimonas micra*) that produce volatile sulfur compounds (VSC). The pure extracts were placed in culture medium for the diffusion test in agar. The Minimum Inhibitory Concentration (MIC) was determined by the microdilution method, and microbial growth was assayed with resazurin. Total polyphenols in the extracts were measured by using the Prussian Blue Method. For the salivary sediment test, the sediments were exposed to the Jucá and Pomegranate extracts, which was followed by incubation and organoleptic measurements with a monitor (Halimeter®) at 1-, 2-, 4-, and 24-hour intervals. The diffusion test revealed mixed results for the extracts. When the zone of inhibition was present, it ranged from 1.6 to 10.3 mm. The Pomegranate extract was the only extract that inhibited all the evaluated microorganisms; the 0,& YDOXHV UDQJHG IURP WR JP/ . The Pomegranate and Jucá extracts presented higher levels of polyphenols, 7.3% and 3.9%, respectively, and less VSC formation as compared to the negative control. In conclusion, the extracts display antimicrobial activity against the tested microorganisms. The investigated plants have the potential to reduce the main substances related to halitosis of oral origin.

**Keywords:** Dentistry; Phytotherapy; Medicinal plants; Halitosis; Anaerobic bacteria

## INTRODUCTION

Halitosis is the generic term that is used to describe an unpleasant and disgusting odor emanating from the oral cavity. It is one of the dental care problems that lead people to approach dentists for treatment (Kandalam, Ledra, Laubach, & Venkatachalam, 2018; Zalewska et al., 2012). According to Bicak (2018), halitosis can be divided into two groups: delusional (pseudohalitosis, halitophobia) and genuine halitosis. Genuine halitosis is divided into two subgroups: physiological and pathological halitosis. Pathological halitosis can be oral or extraoral. Extraoral halitosis may originate from the respiratory system or other systems.

In 85% of the patients with malodor, the problem is particularly caused by Gram-negative bacteria present in the oral cavity (Danser, Gomez, & Van der Weijden, 2003), which produce volatile sulfur compounds (VSC). Odor components cannot emerge in the absence of microorganisms. The main reasons why microorganisms underlying bad breath exist in the oral cavity are the presence of tongue biofilm, bad oral hygiene, food impacts, soft diet, use of orthodontic appliances, gingival and periodontal diseases (gingivitis, periodontitis, acute necrotizing ulcerative gingivitis, and pericoronitis), dental abscess, and bone diseases (alveolitis, osteomyelitis, and osteonecrosis), among others (Bicak, 2018; Scully & Greenman, 2012).

Chromatography-based studies have been able to identify and to quantify VSC in air samples from the oral cavity, which has helped to establish the importance of these substances for bad oral odor formation (Sopapornamorn, Ueno, Shinada, Yanagishita, & Kawaguchi, 2007; Tonzetich, 1971). Sulfide ($H_2S$) and methylmercaptan ($CH_3SH$), together with lower participation of dimethyl sulfide ($[CH_3]_2S$), are the main VSC (Sopapornamorn et al., 2007).

Application of oral hygiene methods is among the strategies that are used to reduce the number of sulfur compounds in the mouth. Mouthwashes often contain antibacterial agents such as cetylpyridinium chloride, chlorhexidine, zinc gluconate, essential oils, and

chlorine dioxide (Kandalam et al., 2018). Chlorhexidine, which has generally been effective, may disrupt homeostasis in the oral cavity along time, which is a significant negative side effect (Yoo et al., 2017). Nevertheless, the existing mouthwashes cannot cure chronic halitosis, and new solutions to this dental care problem are required (Kandalam et al., 2018).

Another method to treat halitosis is to use medicinal plants (Akkaoui & Ennibi, 2017), which are the resources that are available to some communities and ethnic groups (Brandao et al., 2008). In Brazil, medicinal plants are applied in the poorest regions of the country, as well as in large cities. Several plants are marketed in free markets and popular markets or even grow in home backyards (Brandao et al., 2008).

*C. ferrea* Mart. (Jucá), *C. cassia* B. (Cinnamon), *M. sylvestris* L. (Mallow), *P. granatum* L. (Pomegranate), *R. officinalis* L. (Rosemary), *A. suaveolens* (Als.) Spreng. (Macassá), *S. aromaticum* L. (Clove), and *T. indica* L. (Tamarind) are Brazilian medicinal plants that the population has employed to treat various diseases. These plants have been studied against numerous pathogens (Bernardes et al., 2010; Huang, Xub, Liua, Zhanga, & Hua, 2014; Martins et al., 2016; Nwodo, Obiiyeke, Chigor, & Okoh, 2011; Razavi, Zarrini, Molavi, & Ghasemi, 2011; Sampaio et al., 2009; Vahid Dastjerdi et al., 2014; Zhang et al., 2017).

Our research group has also tested the antibacterial activity of some crude extracts obtained from Brazilian plants against anaerobic oral bacteria (Bardaji et al., 2016; Carvalho et al., 2011; Leandro et al., 2016; Moraes et al., 2016; Moreti et al., 2017; Pimenta et al., 2019; Souza et al., 2011). However, no literature study has evaluated the action of Jucá, Cinnamon, Mallow, Pomegranate, Rosemary, Macassá, Clove, or Tamarind against VSC producers like *F. nucleatum*, *P. gingivalis, P. intermedia*, and *P. micra*. Therefore, it would be interesting to verify whether medicinal plants could be used against malodor instead of drugs, like chlorhexidine.

This research aimed to verify the antibacterial activity of Brazilian plant extracts against microorganisms that produce VSC and to determine the inhibitory effect of the plant extracts on VSC formation.

## MATERIAL AND METHODS

### Plant material

*C. cassia* (Cinnamon), *M. sylvestris* L. (Mallow), *P. granatum* (Pomegranate), *R. officinalis* (Rosemary), *A. suaveolens* (Als.) Spreng. (Macassa), *S. aromaticum* L. (Clove), and *T. indica* L. (Tamarind) extracts were prepared at the Department of Pharmaceutical Sciences of the Federal University of Pernambuco in Recife (PE), Brazil. All the evaluated species were identified at the Toxicology Laboratory of the same institution. *C. ferrea* Mart. extract was prepared at the Federal University of Manaus under the same conditions as the other extracts. **Table 1** summarizes the extraction of the studied plants.

### Chemical constituents extraction

After washing and drying in an oven at 33 °C for one week to eliminate moisture and to stabilize the enzymatic content, the material was pulverized in an electric mill, and the active substances were extracted. The continuous flow leaching or percolation method was used at room temperature. In this process, the extractive solution was constantly renewed for 24 h. After this time, the markers or active substances had been totally extracted. For the extraction to be complete, the process required eight liters of hydroalcoholic solution for each kilogram of pulverized raw material. Approximately 6,600 mL of each extract was recovered. After filtration to remove impurities, the extracts were stored in amber bottles in a cold room.

In the specific case of Tamarind (*Tamarindus indica* L.), the plant sugar and pectin content demanded that extraction be accomplished by fractionated maceration, which prevented an excessively turbid extract from being produced. At the first maceration, the fruit was washed and cut into small pieces, placed in a flat bottom flask, and covered with the extractive solution (70% hydroalcoholic solution). After one week, the mixture was

filtered, and the first filtrate, designated extract A, was obtained and refrigerated. The material collected in the filter apparatus was placed in a flat bottom flask and covered with a new extractive solution (70% hydroalcoholic solution). After one week, this other mixture was filtered, to give extract B, which was refrigerated. The process was repeated for the new material collected in the filter apparatus after extract B was achieved, which provided extract C. After that, the frame was totally depleted. In the final step, extracts A, B, and C were mixed and concentrated in a rotary evaporator.

After the chemical constituents were extracted, they were concentrated at the fluid extract level (1:1, w/v). This process was carried out in a Rotavapor apparatus (Model Ika ± Werke, Staufen, Germany) at a constant temperature of 40 °C. Table 1 shows how the employed part of the plant was stained, the extractive substance, and the final concentration of the obtained extract.

**Antibacterial activity determination**

The antibacterial assays, diffusion tests in agar, and determinations of the minimum inhibitory concentration and minimum bactericidal concentration were conducted at the Microbiology Laboratory coordinated by the Division of Endodontics of the Piracicaba Dental School ± UNICAMP, Piracicaba, SP, Brazil. The antibacterial activity of the extracts was determined with previously cultivated wild type bacteria, namely *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Parvimonas micra*. The evaluated anaerobic bacteria were isolated from clinical trials; identified by means of 16S rRNA genetic sequencing according to Nóbrega, Montagner, Ribeiro, Mayer, and Gomes (2016); and kept in the Endodontic Microbiology library under cryopreservation. Chlorhexidine gluconate 2% (Sigma-Aldrich, St Louis, MO, USA) in the liquid form was employed as positive control. Double triplicates were carried out for each bacterium and experiment.

***Diffusion test in agar***

The solid medium diffusion method has already been described (Koo et al., 2000). First,

/RI)$%PHGLXP FAB = Fastidious Anaerobe Broth, LAB M, Bury, UK) containing inoculum corresponding to standard 1 of the McFarland scale adjusted with a spectrophotometer (Femto, São Paulo, SP, Brazil- wavelength 800 nm) was plated on FAA-containing plates (FAA = Fastidious Anaerobe Agar, LAB M, Bury, UK) enriched with defibrillated sheep blood (Ebefarma, Araras, SP, Brazil). The choice of McFarland standard 1 for the inoculum agrees with the growth characteristics of the studied bacteria. Steel cylinders (8.0 X 10.0 mm ± with an inner diameter of 6.0 mm) were placed on the previously seeded FAA medium. Then, 50 µL of the tested extracts and chlorhexidine gluconate were placed in the cylinders, and the plates were maintained at room temperature for 2 h to allow the tested bacteria to diffuse in the agar (preserved in anaerobic jars at room temperature). The plates containing strictly anaerobic cultures were manipulated under continuous nitrogen flow to reduce the time during which such bacteria were exposed to the ambient atmosphere. The plates were then incubated in an anaerobic chamber (Don Whitley Scientific, Bingley, UK) under atmosphere containing 5± 10% $H_2$, 10% $CO_2$, and 80±85% $N_2$ for 72 h.

Zones of inhibition were measured after the incubation period. The zone of inhibition was the shortest distance (mm) measured from the outer margin of the cylinder to the initial point of microbial growth. Double triplicates were performed for each substance that was tested on the different microorganisms. After the incubation period and after the zones of inhibition were read, the cylinders were removed, and material was collected from the solid medium that was in direct contact with the test substance. This material was seeded on FAA plates and incubated under the same conditions described previously. After the incubation period, reading was conducted to verify the inhibition by direct contact, represented by the absence of bacterial growth.

***Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Determination***

The minimum inhibitory concentration and the minimum bactericidal concentration

methods have already been described (Bardaji et al., 2016). The minimum inhibitory concentration (MIC) of the plant extracts was determined in double triplicates. The microdilution broth method in 96-well microplates was employed. Dilutions of plant extracts were used: WKHILQDOFRQFHQWUDWLRQVUDQJHGIURPWRJP/LQDWRWDORI different concentrations. For chlorhexidine, the concentrations ranged from 0.0115 to 5.9 JP/LQDWRWDORI GLIIHUHQWFRQFHQWUDWLRQV7KHLQRFXOXPZDVSUHSDUHGDQGDGMXVW to standard 1 of the Mc Farland scale with a spectrophotometer (800 nm). The controls were dispensed on the microplate containing the medium liquid only (FAB), the liquid medium (FAB) with the extract, or the liquid medium (FAB) with inoculum.

After incubation in an anaerobic chamber for 72 h, the liquid media were stained with an aqueous resazurin solution (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 0.02%. To this end, /RI WKH UHVD]XULQ VROXWLRQ ZDV DGGHG WR HDFK PLFURWLWHU wells containing the inoculum and the test substance as well as to the wells containing the controls. The lowest concentration corresponding to the test well that maintained the blue resazurin staining was interpreted as the MIC. A change in color to pink-purple or pink indicated that resazurin was reduced and implied the presence of bacterial growth. The reading was carried out 20 min after the dye was applied. The different concentrations were distributed in duplicate microplates so that after the MIC was read, a 20-/DOLTXRW was removed and seeded in plates with FAA medium. Aliquots were taken from the well corresponding to the MIC reading and from three consecutive wells with a higher concentration of the extracts. After incubation, bacterial growth was read.

**Determination of the ability of the extracts to inhibit the formation of sulfurated volatile compounds (VSC) and other odors**

The VSC concentration was measured in tubes containing total salivary culture incubated after exposure to the two extracts that had the highest polyphenols concentration. Each extract was used separately. A total culture of saliva not exposed to any of the extracts was used as negative control. As positive control, 0.12% chlorhexidine gluconate (Periogard®, Colgate, São Paulo, SP, Brazil) was employed (this is the usual

chlorhexidine concentration that is present in mouthwashes). The study was developed with double triplicates.

***Sediment preparation***

The method that was used to prepare the salivary sediment system (SSS) had been described previously (Kleinberg & Codipilly, 1999). Briefly, total saliva was collected from two individuals who had not conducted oral hygiene for 24 h and who had fasted for 12 h prior to collection. Saliva was placed in Falcon-type test tubes (15 mL); the stimulated method that applied Hiperboloide® (SaudBucal, São Paulo, SP, Brazil) was employed. After collection, the saliva samples were centrifuged at 3500 rpm (centrifuge QUIMIS, Diadema, SP, Brazil) for 15 min. The supernatant was removed and stored in a freezer at 4 °C7KHQ/ of the pellet was placed in 10 Falcon tubes. The pellet present in each tube was washed with 1 mL of deionized water for 30 s, three times. Each washing cycle entailed addition of 1 mL of deionized water to the sediment present in each tube, which was agitated. After 30 s, the tube was centrifuged for 1 min. Excess water was removed, leaving the sediment in each tube. Three different concentrations of the extracts were tested, namely the concentrations corresponding to the dilutions 1: 1 (D1, 50%), 1: 2 (D2, 25%), and 1: 4 (D3, 12.5%). The test samples were exposed to 1 mL of the extract for 30 s, which was followed by centrifugation for another minute. After exposure, another wash session was performed for 30 s. The tubes containing the positive control were exposed to 1 mL of 0.1% chlorhexidine solution, and the tubes containing the negative control (only sediment) were exposed to deionized water for 1 min. Then, to simulate clearance by saliva, all the sediment samples were centrifuged, washed with 1 mL of deionized water, and centrifuged again. Water was removed so that each tube remained with the sediment.

In each of the Falcon tubes containing the sediment, SSS was prepared by XVLQJ/ of salivary sediment at 16.7% (v/v), 250 /RIWKHVXSHUQDWDQWDWY v)DQG/ of 6 mM L-cysteine (Sigma-Aldrich, St Louis, MO, USA). The control and the test samples were incubated at 37 °C for 24 h, and Halimeter® was performed at the following times:

after incubation for 1, 2, 4, and 24 h.

***Data collection***

An organoleptic test was carried out to check for odor. A scale of 0 to 4, measured via the sterilized glass rod method, was employed, where 0 represents the absence of odor and 4 corresponds to the most intense odor. Immediately after opening, a glass rod with diameter of 0.5 cm and length of 30 cm was introduced into the falcon tube containing the salivary culture so that the glass rod had its end layered in the sediment system. Then, circular movements were made to stir the tube contents. The rod was then horizontally placed 10 cm away from the H[DPLQHU¶V nostrils, and the corresponding scale value was established according to the odor intensity. pH measurements were performed with an electrode (Orion-ThermoFisher Scientific, Waltham, MA, USA) coupled to a potentiometer 710A (Orion- ThermoFisher Scientific, Waltham, MA, USA). Calibrations were carried out with pH 4 and 7 standards and 95% slope. Readings were taken after the last VSC reading.

**Statistical analysis**

The data obtained in the different stages of this study were tabulated in Excel program (version 2003, Microsoft, Redmond, WA, USA) and SPSS (version 11.0, IBM, Armonk, NY, USA). In these programs, mean values, standard deviations, and percentage values were calculated when appropriate. The results are presented in the tables.

**RESULTS**

Table 2 describes the results of the diffusion test in agar for the different extracts. The Pomegranate extract afforded the largest mean zone of inhibition on *P. micra*. Interestingly, the Pomegranate extract was the only extract that produced a zone of inhibition on all the tested microorganisms. This extract gave a higher average value than chlorhexidine on *P. micra*.

Table 3 lists the Minimum Inhibitory Concentration and the Minimum Bactericidal

Concentration results. The extracts displayed MIC ranging from 50 to 400 µg/mL. At the tested concentrations, most of the extracts were not effective against *F. nucleatum*² MIC results were higher than JP/7KH3 omegranate extract was the only extract that did not have an inhibitory effect on *P. micra*. However, it was the only extract that inhibited *P. intermedia* (MIC = JP/

Microorganism growth in the subculture in solid medium indicates a bactericidal action, while the absence of growth indicates a bactericidal action. The Rosemary, Macassá, and Mallow extracts displayed bactericidal results against *P. micra*. The Jucá extract displayed bactericidal effect on *F. nucleatum* (XQR I P/ The Rosemary, Cinnamon, Clove, Macassá, and Mallow extracts exhibited bactericidal action against *P. gingivalis*. In turn, chlorhexidine showed a bactericidal effect on all the tested bacteria (Table 3).

Table 4 summarizes the polyphenols concentration in the extracts used in this work. The polyphenols concentrations varied between 0.1 and 7.3%. The Tamarind, Mallow, and Macassá extracts displayed the lowest polyphenols concentration, while the Jucá and Pomegranate extracts displayed the highest concentrations, 3.9 and 7.3%, respectively.

Table 5 depicts the results achieved with the Jucá and Pomegranate extracts against the formation of VCS and other odors in the salivary sediment system.

Table 6 shows the measurement of the salivary sediment pH system under the action of the Jucá and Pomegranate extracts after incubation for 24 h.

**DISCUSSION**

The discovery that bacteria participate in oral odor formation has greatly contributed to the study of halitosis (Bicak, 2018). The oral cavity is the primary source of breath odors in most clinical situations (Gokdogan, Catli, & Ileri, 2015). Here, we used bacteria such as the Gram-negative *F. nucleatum*, *P. gingivalis,* and *P. intermedia (*Krespi, Shrime, &

Kacker, 2006) and the Gram-positive *P. micra*, which are related to VSC formation.

Although *P. micra* is Gram-positive, it has proven ability to produce VSC from amino acids (L-glutathione and L-cysteine) (Carlsson, Larsen, & Edlund, 1993). This finding has demonstrated that several bacterial species of the oral cavity participate in VSC formation. In addition, this finding has pointed out the importance of investigating the action of antimicrobials on both Gram-negative and Gram-positive bacteria (Krespi et al., 2006). It is noteworthy that the total number of species involved in VSC production, and consequently in halitosis development, remains unknown. Some previously unclassified oral bacteria can also produce VSC (Persson, Claesson, & Carlsson, 1989).

The diffusion test in agar has been used to screen the antimicrobial potential of medicinal plants to eliminate bacteria related to halitosis. However, this test does not time their action. In turn, the microdilution test in broth is advantageous because it requires small amounts of reagents and can evaluate the effect of various agents on different microorganisms. The microdilution test in broth is the best option to determine the antibacterial activity of medicinal plants (Valgas, Souza, Smânia, & Smânia Jr., 2007). The microdilution results obtained in this study showed the dynamics of the interaction between the antimicrobial agent and the tested bacteria. For example, the Cinnamon extract did not produce a zone of inhibition on *P. micra* in the diffusion test in agar. Nevertheless, the microdilution test revealed 0,& EHORZ JP/ IRU WKLV VDPH bacterium. We should consider the possibility that the liquid test allowed the extract constituents and the bacterium to interact differently as compared to their interaction in a test conducted in solid medium. Therefore, the absence of antimicrobial effect in the diffusion test in agar should be interpreted with caution because the same extract may present antimicrobial activity in the microdilution test.

According to the microdilution method in broth, the antibacterial activity of *R. officinalis* L. ranged from 50 to >400 µg/mL. Our results agree with literature data (Bernardes et al.,

2010). However, the latter article employed microorganisms that underlie dental caries; for example, *Streptococcus mutans*, *S. salivarius*, *S. sobrinus*, *S. mitis*, *S. sanguinis*, and *Enterococcus faecalis* (Bernardes et al., 2010).

The *S. aromaticum* L. extract exerted antibacterial effect on all the tested microorganisms. Zhang et al. (2017) evaluated the effect and the mechanism of eugenol from *Syzygium aromaticum* against *P. gingivalis* and demonstrated that clove displays bactericidal activity, which is in agreement with our study. The former authors suggested that eugenol and essential oils from *S. aromaticum* leaf might be potential additives in food and personal healthcare products, serving as a prophylactic approach to periodontitis.

*A. suaveolens* exhibited bactericidal action against *P. micra* and *P. gingivalis*. MIC ranged from 50 to 400 mg/mL regarding all the tested microorganisms. Our present results were better than the ones reported for different microorganisms (Martins et al., 2016), including two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella* sp. ATCC14028) and a Gram-positive bacterium (*Staphylococcus aureus* ATCC6538). In the latter study, *Escherichia coli* and *Salmonella* were more susceptible to the *A*. *suaveolens* essential oil, with MIC of 50 mg/mL, as compared to *Staphylococcus aureus,* with MIC of 100 mg/mL.

*C. cassia* exerted a bactericidal effect on *P. gingivalis*, and its MIC ranged from 50 to 400 mg/mL on all the tested microorganisms. No literature study contains data that can be compared to our results. However, a study on *C. cassia* with respect to food-related bacteria showed a better result against *Staphylococcus aureus* ATCC 25923, with MIC of 2.5 mg/mL (Huang et al., 2014).

In the present study, we showed the antibacterial activity of methanol extracts from *M. sylvestris* leaves against four VSC producers. Our results are supported by the literature, which describes that methanol extracts from *M. sylvestris* flowers and leaves exhibit high bactericidal activity against a range of microorganisms, such as *Escherichia coli* (PTCC

1047) (Persian Type Culture Collection), *Staphylococcus aureus* (PTCC 1112), *Enterococcus faecalis* (PTCC 1190), *Streptococcus agalactiae* (PTCC 1321), *Erwinia carotovora* (PTCC 1675), and *Staphylococcus aureus* (Razavi et al., 2011).

The *T. indica* extract had MIC values ranging from 190 to 400 mg/mL against all the tested microorganisms. *T. indica* has already been evaluated against 13 Gram-negative and five Gram-positive bacterial strains. MIC ranged from 7.81 mg/mL against *Bacillus subtilis* ATCC 6051 to 31.25 mg/mL against *Escherichia coli* ATCC 11775 (Nwodo et al., 2011).

The *P. granatum* extract was the only extract that produced a zone of inhibition against all the tested microorganisms. We observed the highest mean inhibition against *P. micra*. The Pomegranate extract did not present MIC against *P. micra*. WJP/LW was not effective on *P. intermedia*. The antibacterial property of *P. granatum* L. water extract has been evaluated against *S. mutans* ATCC 35608, *S. sanguinis* ATCC 10556, *S. sobrinus* ATCC 27607, *S. salivarius* ATCC 9222, and *Enterococcus faecalis* CIP 55142 (Vahid Dastjerdi et al., 2014). The water extract demonstrated maximum antibacterial effect on *Streptococcus sanguinis* ATCC 10556 with minimum inhibitory concentration of 6.25 mg/mL and maximum bactericidal effect on *S. sanguinis* ATCC 10556 and *S. sobrinus* ATCC 27607 with minimum bactericidal concentration of 25 mg/mL.

The quantification of polyphenols in products of plant origin is widely used because these components are important for their antioxidant and antimicrobial properties. Different methods have been employed to quantify them; most of the methods refer to the equivalence with the amount of gallic acid present in the studied sample (Romani et al., 2006). We decided to apply the Blue Prussian modified method (Graham, 1992) because it provides color stability after the reagents are added. In our study, the Jucá and Pomegranate extracts provided the highest concentrations of polyphenols, 3.9 and 7.3%, which are related to their antimicrobial effect.

Due to their higher concentration of polyphenols, we used the Pomegranate and Jucá extracts to test the salivary sediment. Nevertheless, their consistency proved unfit for this test, so we had to use dilutions of 50% (D1), 25% (D2), and 12.5% (D3). Exposure of the salivary sediment to the Pomegranate and Jucá extracts led to lower VSC formation, which could be a consequence of their antibacterial action.

Another important point is the role that pH plays in the sediment system. Acidic pH inhibits VSC formation (Codipilly & Kleinberg, 2008). The pH results in Table 6 shows values close to 4.0 for the Jucá extract dilutions D2 and D3, which indicate a possible influence on VSC formation and on the reading of the organoleptic test. However, the D1 pH value did not reduce VSC, which could be attributed to the inhibitory effect of this extract on the bacteria.

The organoleptic readings also show that the Pomegranate and Jucá extracts inhibited odor formation in the exposed salivary sediment system as compared to the control.

pH reduction can be a direct result of extract composition in the environment or their action on different bacteria. The fermentation process is related to the presence of glucose in the salivary sediment system, which increases the number of saccharolytic bacteria, thereby reducing the pH.

The reduction of odor from other substances (indole, escatol) measured by the organoleptic test may be due to increased cysteine degradation and lower degradation of other amino acids, and vice-versa (Codipilly & Kleinberg, 2008). Table 6 shows the effect of the Pomegranate extract on the salivary sediment system. For the dilution D2, the organoleptic test values increased, while the VSC values decreased in the readings at 1 and 2 h. As discussed previously, the organoleptic test simultaneously perceives odors of other substances together with the VSC.

The VSC data obtained by Halimeter® reading on the salivary sediment represented by

the negative control (Tables 4 and 6) showed high values and represented clinically extreme cases of VSC concentration in the oral cavity. Likewise, these data also indicated that the inhibition resulting from exposure of the salivary sediment system to the extract occurs in a medium that offers the ideal conditions for VSC production due to cysteine addition to the system. In an *in vivo* study, measurement above 75 ppb was used to include the individuals in the halitosis carrier group (Liu et al., 2006).

This research showed the potential use of herbal medicines in the fight against halitosis-related microorganisms. The studied extracts inhibited oral microorganisms that produce VSC. The extracts with the highest content of polyphenols; i.e., the Jucá and Pomegranate extracts, inhibited VSC formation in a salivary sediment model, pointing to their potential to reduce the main substances related to halitosis from oral origin. Further studies should investigate, from a clinical standpoint, whether the tested medicinal plants could be used in the composition of mouthwashes. Given that medicinal plants are usually inexpensive and even homegrown items, they are a fascinating option to enhance the SRSXODWLRQ\DFFHVVprevention of oral diseases.

## CONCLUSION

The tested extracts have antimicrobial activity against the assayed microorganisms. These plants have the potential to reduce the main substances related to halitosis from oral origin.

The authors declare no conflict of interest.

## Acknowledgements

This work was supported by the Brazilian grant agencies Sao Paulo Research Foundation (FAPESP), Brazilian Coordination for the Improvement of Higher Education and Graduate Training Personnel (CAPES, finance code 001), and National Council for Scientific and Technological Development (CNPq, process no. 308162/2014-5).

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Table 1. Description of the plants and materials used in the preparation of the extracts.

| Plant | | | Extraction concentration | |
|---|---|---|---|---|
| Popular name part | Scientific name | Used plant | Extractive substance | Final extract concentration |
| Rosemary | Rosmarinus officinalis L. | Leaf | Ethanol 70% | 1000 mg/mL |
| Cinnamon | Cinnamomum cassia Blareyn. | Bark | Ethanol 70% | 321 mg/mL |
| Clove | Syzygium aromaticum L. | Floral peduncle | Ethanol 70% | 396 mg/mL |
| Jucá | Caesalpinia ferrea Mart | Full pod | Methanol 80% | 500 mg/mL |
| Mallow | Mallow sylvestris L. | Leaf | Ethanol 80% | 1000 mg/mL |
| Macassá | Aeolanthus suaveolens | Floral leaf, stem, and vanity | Ethanol 80% | 125 mg/mL |
| Tamarind | Tamarindus indica L. | Fruit without seed | Ethanol 70% | 411 mg/mL |
| Pomegranate | Punica granatum L. | Fruit bee | Ethanol 80% | 540 mg/mL |

Table 2. Mean and standard deviation of the zone of inhibition in mm (n = 6) and action by direct contact (+ or -) of the hydroalcoholic extracts and chlorhexidine on the tested oral cavity microorganisms

| Extract | Microorganisms | | | |
|---|---|---|---|---|
| | P. micra | F. nucleatum | P. gingivalis | P. intermedia |
| Rosemary | 1.6 ± 0.6 (-) | 0.0±0.0 (-) | 3.0 ± 0.0 (-) | 0.0±0.0 (-) |
| Cinnamon | 0.0±0.0 (-) | 0.0±0.0 (-) | 0.0±0.0 (+) | 0.0±0.0 (-) |
| Clove | 3.0 ± 0.0 (-) | 0.0±0.0 (+) | 0.0±0.0 (+) | 0.0±0.0 (-) |
| Macassá | 0.0±0.0 (-) | 0.0±0.0 (+) | 0.0±0.0 (-) | 0.0±0.0 (-) |
| Mallow | 0.0±0.0 (+) | 0.0±0.0 (+) | 0.0±0.0 (+) | 0.0±0.0 (+) |
| Jucá | 6.3 ± 1.2 (-) | 0.0±0.0 (+) | 0.0±0.0 (+) | 3.0±0.0 (-) |
| Pomegranate | 10.3 ± 1.5 (-) | 5.0 ± 0.0 (+) | 3.0 ± 0.0 (-) | 3.6 ± 0.6 (-) |
| Tamarind | 2.6 ± 0.6 (-) | 2.0 ± 0.0 (+) | 0.0±0.0 (-) | 0.0±0.0 (-) |
| Chlorhexidine | 9.3 ± 0.6 (-) | 8.6 ± 0.6 (-) | 8.0 ± 0.6 (-) | 9.6 ± 0.6 (-) |

(-) Absence of bacterial growth after inoculation in solid medium of the material removed from the direct contact area of the microorganism chlorhexidine / extract. (+) Presence of bacterial growth after inoculation in solid medium of the material removed from the direct contact area of the microorganism chlorhexidine / extract. ± standard deviation.

Table 3. Values of Minimum Inhibitory Concentration by microdilution of the hydroalcoholic extracts on the tested oral cavity microorganisms.

| Extract | Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC) - µg/mL | | | |
|---|---|---|---|---|
| | Microorganisms | | | |
| | P. micra | F. nucleatum | P. gingivalis | P. intermedia |
| Rosemary | 50/ 50 | >400/ >400 | 70/ 70 | >400/ >400 |
| Cinnamon | 50/ >50 | >400/ >400 | 190/ 190 | >400/ >400 |
| Clove | 50/ >50 | 400/ >400 | 400/ 400 | >400/ >400 |
| Macassá | 50/ 50 | >400/ >400 | 50/ 50 | >400/ >400 |
| Mallow | 400/ 400 | >400/ >400 | 50/ 50 | >400/ >400 |
| Jucá | 50/ >50 | 400/ 400 | 120/ 130 | >400/ >400 |
| Pomegranate | >400/ >400 | 400/ 400 | 170/ >170 | 160/ >160 |
| Tamarind | 190/ >190 | >400/ >400 | 250/ >250 | >400/ >400 |
| Chlorhexidine | 0.18/ 0.18 | 0.36/ 0.36 | 0.36/ 0.36 | 0.18/ 0.18 |

Table 4. Polyphenols concentration (obtained by spectrophotometry) of the hydroalcoholic extracts

| Plant | Dilution | Reading average | Gallic acid µg/mL | mg of phenols in gallic acid equivalent per mL of extract | Working solution concentration (mg/mL) | Polyphenols from the extract (%) |
|---|---|---|---|---|---|---|
| ES 80% | | 1.17 | 190.0 | | | |
| Jucá | 01:32 | 2.59 | 568.73 | 36.39 | 500 | 7.3 |
| Pomegranate | 01:64 | 1.83 | 329.73 | 21.10 | 540 | 3.9 |
| Cinnamon | 01:08 | 1.62 | 290.92 | 04.65 | 321 | 1.5 |
| Mallow | 01:04 | 0.37 | 67.65 | 0.54 | 100.0 | 0.1 |
| Macassá | PE | 0.59 | 143.07 | 0.14 | 125 | 0.1 |
| ES 80% | | 1.17 | | | | |
| Rosemary | 01:04 | 1.39 | 225.28 | 1.80 | 1000 | 1.3 |
| Clove | PE | 3.34 | 599.80 | 4.80 | 1000 | 0.5 |
| Tamarind | 01:04 | 0.44 | 71.21 | 0.56 | 411 | 0.1 |

ES: Ethanol Standard; PE: pure extract

Table 5. Effect of the Jucá and Pomegranate extract on the formation of volatile sulfur compounds and other odors measured in the salivary sediment system.

| Hour | | D1 | | D2 | | D3 | | PC | | NC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ORG | HAL | ORG | HAL | ORG | HAL | ORG | HAL | ORG | HAL |
| Jucá extract | 1h | 0 | 29.5±0.7 | 0 | 31.5±2.1 | 0 | 43.0±14.1 | 0 | 172.0±2.8 | 1 | 895.0±7.0 |
| | 2h | 0 | 36.5±0.7 | 0 | 35.5±2.1 | 0 | 34.0±4.2 | 0 | 146.0±4.9 | 2 | 735.0±2.4 |
| | 4h | 0 | 38.5±0.7 | 0 | 37.5±2.1 | 0 | 45.0±1.4 | 0 | 50.5±0.7 | 3 | 1275.0±35.3 |
| | 24h | 0 | 37.0±6.5 | 0 | 36.0±5.6 | 0 | 72.5±3.5 | 0 | 30.5±3.5 | 4 | 1500.0±0 |
| Pomegranate extract | 1h | 0 | 30.0±0 | 0 | 1200.0±[illegible] | 0 | 1210.0±14.0 | - | - | - | - |
| | 2h | 0 | 30.0±0 | 0 | 1010.0±14.1 | 0 | 1050.0±70.7 | - | - | - | - |
| | 4h | 0 | 44.0±1.4 | 0 | 925.0±35.3 | 0 | 100.0±141.4 | - | - | - | - |
| | 24h | 0 | 37.0±3.5 | 0 | 1375.0±7.0 | 0 | 1400.0±0 | - | - | - | - |

ORG: Organoleptic test; HAL: Reading with Halimeter®: mean and standard deviation. D1: 50% dilution; D2: 25% dilution; D3: 12.5% dilution. Cont. Posit: Positive control with Periogard®; Cont. Neg .: negative control, sediment not exposed to the test substance or positive control.

Table 6. pH measurement of the salivary sediment system under the action of the Jucá extract after incubation for 24 h.

| Jucá extract | pH | Pomegranate extract | pH |
|---|---|---|---|
| D1 ±A | 6.0 | D1 ±A | 5.7 |
| D1 ±B | 6.0 | D1 - B | 7.1 |
| D2 ±A | 4.6 | D2 - A | 6.3 |
| D2 ±B | 5.0 | D2 - B | 6.7 |
| D3 ±A | 3.0 | D3 ±A | 5.6 |
| D3 ±B | 4.4 | D3 ±B | 5.2 |
| PC ±A | 7.3 | PC ±A | 6.6 |
| PC ±B | 7.0 | PC ±B | 6.6 |
| NC ±A | 6.0 | NC ±A | 6.0 |
| NC ±B | 6.0 | NC ±B | 6.0 |

D1: 50% dilution; D2: 25% dilution; D3: 12.5% dilution. Cont. Posit: positive control with Periogard®; Cont. Neg .: negative control, sediment not exposed to the est substance or positive control. The sample; B: duplicate