PLANT EXTRACTS OF POPULAR USE AGAINST ORAL INFECTIONS

Extratos de plantas de uso popular contra infecções orais

Extractos de plantas de uso popular contra infecciones orales

Original Article

ABSTRACT

Objetive: To assess the antibacterial effect of plant extracts of popular use on bacteria related to the development of caries and endodontic infections. **Methods:** Experimental study conducted at the University of the Extreme South of Santa Catarina in 2015 in which dyes of Artemisia absinthium, Laurus nobilis, Bidens pilosa, Achillea millefolium L and Foeniculum vulgare at hydro-alcoholic concentrations of 5%, 10%, 15% and 20% in Mueller Hinton agar growth-medium had their antibacterial activity against strains of S. mutans and E. faecalis measured. The solid medium diffusion method was used and the inhibitory activity was analyzed according to the presence and size of inhibition halos – control and chlorhexidine 0.12%. Significance level was set at $\alpha=0.05$. **Results:** There was no inhibition on the strains of E. faecalis in any of the dyes; however, it was observed an inhibitory effect of Artemisia absinthium, Laurus nobilis and Bidens pilosa dyes on the strains of S. mutans at the concentrations tested. **Conclusion:** The dyes of plants of popular use assessed showed no antimicrobial effects on E. faecalis, which causes endodontic infections; however, three of them presented inhibitory effects against S. mutans, which is mainly responsible for caries.

Descriptors: *Enterococcus faecalis; Streptococcus mutans*; Plant Extracts.

RESUMO

Objetivo: *Avaliar o efeito antibacteriano de extratos de plantas de uso popular sobre bactérias relacionadas ao desenvolvimento da cárie e infecções endodônticas.* **Métodos:** *Estudo experimental realizado na Universidade do Extremo Sul Catarinense, em 2015, no qual tinturas de Artemisia absinthium, Laurus nobilis, Bidens pilosa, Achillea millefolium L e Foeniculum vulgare, nas concentrações hidroalcoólicas de 5%, 10%, 15%, 20% em meio de cultura ágar Mueller Hinton, tiveram sua ação antibacteriana mensurada contra cepas de S. mutans e E. faecalis. Usou-se o método de difusão em meio sólido e a sensibilidade inibitória foi analisada pela presença e tamanho de halos de inibição, sendo grupo controle a clorexidina 0,12%. Utilizou-se nível de significância p<0,05.* **Resultados:** *Não houve inibição sobre a cepa de E. faecalis em nenhuma das tinturas, entretanto, observou-se efeito inibitório das tinturas de Artemisia absinthium, Laurus nobilis e Bidens pilosa sobre as cepas de S. mutans nas concentrações testadas.* **Conclusão:** *As tinturas de plantas de uso popular investigadas não apresentaram efeitos antimicrobianos sobre E. faecalis, causadora de infecções endodônticas, porém, três delas demonstraram efeitos inibitórios contra S. mutans, principal responsável pela cárie dentária.*

Descritores: *Enterococcus faecalis; Streptococcus mutans; Extratos Vegetais.*

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RESUMEN

Objetivo: *Evaluar el efecto antibacteriano de extractos de plantas de uso popular sobre bacterias relacionadas al desarrollo de caries e infecciones endodónticas.* **Métodos:** *Estudio experimental realizado en la Universidad del Extremo Sur de Santa Catarina en 2015 en el cual tinturas de Artemisia absinthium, Laurus nobilis, Bidens pilosa, Achillea millefolium L y Foeniculum vulgare, en las concentraciones hidroalcohólicas del 5%, 10%, 15%, 20% en medio de cultivo agar Mueller Hinton, tuvieron su acción antibacteriana medida contra cepas de S. mutans e E. faecalis. Se utilizó el método de difusión en medio sólido y la sensibilidad inhibitoria fue analizada por la presencia y tamaño de halos de inhibición, teniendo como el grupo control la clorhexidina 0,12%. Se utilizó el nivel de significación de p<0,05.* **Resultados:** *No hubo inhibición sobre la cepa de E. faecalis en ninguna de las tinturas, sin embargo, se observó el efecto inhibitorio de las tinturas de Artemisia absinthium, Laurus nobilis y Bidens pilosa sobre las cepas de S. mutans en las concentraciones testadas.* **Conclusión:** *Las tinturas de plantas de uso popular investigadas no presentaron efectos antimicrobianos sobre el E. faecalis, causadora de infecciones endodónticas, sin embargo, tres de ellas han demostrado efectos inhibitorios contra el S. mutans, el principal responsable de la caries dentales.*

Descriptores: *Enterococcus faecalis; Streptococcus mutans; Extractos Vegetales.*

INTRODUCTION

Dental caries is still one of the most prevalent diseases of the oral cavity, affecting the great majority of adults in industrialized countries and 60-90% of schoolchildren(1), which represents a high cost in developed countries where oral health accounts for 5-10% of all public health expenditure(2) and a cost that exceeds the financial capacity of most developing countries(3).

Among the major etiologic agents involved in the pathophysiology of human dental caries is the *Streptococcus mutans* due to its acidogenic and acidic characteristics; this Gram-positive bacterium may also be associated with bacteremias and endocarditis(4-6).

Endodontic infections are responsible for pulpal and periapical alterations, and have an etiology that is directly related to microorganisms such as *Enterococcus faecalis*, a facultative bacterium related to the appearance of refractory edodontic lesions due to its ability to survive chemical and mechanical instrumentation in the root canals(7-9).

The species *Streptococcus mutans* and *Enterococcus faecalis* are opportunistic pathogens and are inserted in the group of resistant microorganisms of the oral cavity and the increase in the prevalence of strains resistant to multiple synthetic antibiotics triggers the search for new strategies to combat infection(10). Thus, medicinal plants stand out as a rich source of antimicrobial agents in view of the increase of bacterial resistance due to the excessive use of antibiotics(11).

Most of the drugs have their origin related to the plant world. Both developed and developing countries use medicinal plants for primary health care(12). With regard to the use of phytotherapy, Brazil stands out as a privileged country for possessing 25% of the world flora. However, less than 1% of the species have had their medicinal properties analyzed(13). In a review carried out on the subject, phytotherapy was cited in Primary Health Care in three hundred and fifty Brazilian locations; however, only 24 studies on its use were available in the scientific literature(14).

The excess of scientific requirements regarding the quality, efficacy and safety of herbal medicines, as well as the lack of knowledge and experience by health professionals and the difficulty in standardizing the municipal lists of herbal medicines, are pointed out as factors that hinder their insertion in health services. In this perspective, the insertion of phytotherapy demands educational approaches on the use of medicinal plants as a therapeutic alternative, enabling the promotion of health through the recovery of cultural values and intersectoral actions, such as greater team-community bonding, proximity between professionals and users, local development, among others(14).

The assessment of plant extracts for use in Dentistry has been encouraged by the worldwide growth of phytotherapy in preventive and curative programs given they are more affordable to the population and public health services(13) and present lower toxicity and higher pharmacological activity and biocompatibility(12). Many plant species have demonstrated pharmacological activity in the treatment of oral conditions, including antimicrobial effects, and are alternatives to reduce diseases such as caries through their introduction into dentifrices or other forms of use(13,15).

Given the benefits of phytotherapy, both related to the antimicrobial effects on bacteria that compose the biofilm and the lower cost and greater access, it is evident that there is a need to find alternative and economically viable means to combat oral diseases that can be incorporated into preventive and curative programs.

In this context, the present study aimed to assess the antibacterial effect of plant extracts of popular use on bacteria related to the development of caries and endodontic infections.

METHODS

This is an in vitro experimental study carried out at the University of the Extreme South of Santa Catarina (*Universidade do Extremo Sul Catarinense - UNESC*) in the period from March to September 2015. Initially (March and April), a survey of plants of popular use with antimicrobial indication was carrid out in a booklet of the Health Care Pastoral (*Pastoral da Saúde*) of the municipality of Criciúma, Santa Catarina, Brazil. After the survey, the plants that indicated antimicrobial effect were searched in the literature to identify the microorganisms for which they showed effectiveness. Plants that had already been tested through similar methodology for the bacterial strains of interest (*S. mutans and E. faecalis*) were excluded and only those for which conclusive studies reporting in vitro antibacterial effect had not been published yet were tested.

The following plant dyes were obtained in a private compounding pharmacy: *Artemisia absinthium* (absinthe), *Laurus nobilis* (bay laurel), *Bidens pilosa* (black-jack), *Achillea millefolium L.* (yarrow) and *Foeniculum vulgare* (fennel) – at concentrations of 5%, 10%, 15% and 20%, being prepared by macerating the dry plant and using 70% ethanol as extracting fluid.

Microbiological tests were carried out in the laboratory of clinical analyses (LENAC-1) of UNESC by a single researcher with a wide experience in microbiology to avoid variations due to the individual factor. All procedures were performed in a laminar flow hood, with the researcher wearng a lab coat and disposable gloves.

Lyophilized bacteria strains of *Streptococcus mutans* (ATCC 25175) and *Enterococcus faecalis* (NEWP 012) were reactivated in BHI (Brain Heart Infusion - DIFCO) broth, obtaining a suspension of the bacteria. Suspensions of *S. mutans* and *E. faecalis* were respectively incubated in microaerophilia anaerobically for 48 hours at 37°C, and the cultures obtained were blotted onto plates containing BHI agar.

After growth, the bacterial colonies were transferred into a 0.9% saline solution (w/v) to obtain turbidity equivalent to a 0.5 McFarland standard, which corresponds to approximately 1.5 x 108 CFU/mL. After that, the bacterial suspension was seeded in Petri dishes containing 15 mL of Mueller-Hinton agar medium (BD-Becton Dickinson), with a thickness of approximately 4 mm, with the aid of swab[16].

The assessment of the antimicrobial activity of the dyes was performed by the solid medium diffusion technique. After sowing the bacterial suspension, five holes were opened in the culture medium using a sterile 5 ml syringe with luer slip, forming wells with of 6mm which were filled with 50µl of each concentration of the different dyes with the aid of an automatic pipette. A plate of each bacterium was prepared for control, with the wells filled with 50µl of 0.12% chlorhexidine and 50µl of 70% ethanol with the aid of an automatic pipette.

The plates were then incubated in bacteriological oven at 35 ± 1°C in microaerophilia (*S. mutans*) or anaerobiosis (*E. faecalis*). All assays occurred in quadruplicate. After the incubation time, the diameters of the halos of inhibition were measured[17].

After data collection, SPSS version 22 was used for statistical analysis. Normality of the size of the halo of inhibition was checked by the Shapiro-Wilk test, which revealed a non-Gaussian distribution; therefore, the median and the interquartile range were calculated.

The Kruskal-Wallis test was used to compare the size of the inhibition halo in relation to the dyes of *Artemisia absinthium* (absinthe), *Laurus nobilis* (bay laurel), *Bidens pilosa* (black-jack), *Achillea millefolium L.* (yarrow) and *Foeniculum vulgare* (fennel) at each concentration (5%, 10%, 15% and 20%), which was followed by Dunn's post hoc test in the case of statistical significance. The correlation between the concentration (5%, 10%, 15% and 20%) and the size of the inhibition halo was verified by means of the Spearman's test and was considered weak if $<= 0.3$; moderate if $>= 0.40$ and $<= 0.6$; and strong if $>= 0.7$. A significance level of $\alpha = 0.05$ and a 95% confidence interval were used for all the tests.

RESULTS

None of the dyes produced halo of inhibition against the strain of *E. faecalis strain*. With regard to controls, inhibition halo was produced only at 0.12 % chlorhexidine.

Halo of inhibition against *S. mutans* strain was produced by the dyes of the following plants at all concentrations: *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa*. Therefore, no halo of inhibition was produced by *Achillea millefolium L.* and *Foeniculum vulgare*. In the control group, inhibition halo was produced at 0.12% chlorhexidine.

Although significantly higher in the control group with chlorhexidine ($p<0.05$ at all concentrations) when compared to the values presented in the different concentrations of the analyzed plants (Table I), the median of the size of the halo of inhibition was considered similar between all dyes (*Artemisia absinthium, Laurus nobilis and Bidens pilosa*) when compared individually by concentration (5%, 10%, 15% and 20%).

TableI - Size of the halos (mm) of inhibition produced by *Artemisia absinthium*, *Laurus nobilis* and *Bidens pilosa* dyes at concentrations 5%, 10%, 15%, 20% and 0.12% chlorhexidine. Criciúma, Santa Catarina, 2015.

Dye/ Concentration	*Artemisia absinthium*	*Laurus nobilis*	*Bidens pilosa*	Chlorhexidine	p-value
	Median (interquartile range)				
5%	10.00 (10.00-10.75)	10.50 (10.00-11.00)	10.00 (9.00-11.75)	20.50 (19.25-21.00)	0.029**
10%	11.00 (10.63-11.00)	11.00 (10.63-11.75)	10.50 (10.00-11.75)	20.50 (19.25-21.00)	0.025**
15%	11.75 (11.13-12.00)	12.00 (11.25-12.38)	11.75 (10.13-13.00)	20.50 (19.25-21.00)	0.032**
20%	12.00 (12.00-12.00)	13.50 (12.63-14.00)	12.00 (11.00-13.75)	20.50 (19.25-21.00)	0.011**

**p<0.01, statistically significant.

At 5% concentration, the halos of inhibition of the dyes did not present a statistically significant difference between them (Figure 1), even when compared to 0.12% chlorhexidine (control), which demonstrates that the dyes produced inhibitory effects on *S. mutans* that are statistically similar to those produced by 0.12% chlorhexidine.

In general, the halos of inhibition of the dyes did not present a statistically significant difference between them (Figure 2) at 10% concentration. However, there was a statistically significant difference between *Bidens pilosa* dye and 0.12% chlorhexidine (p= 0.035).

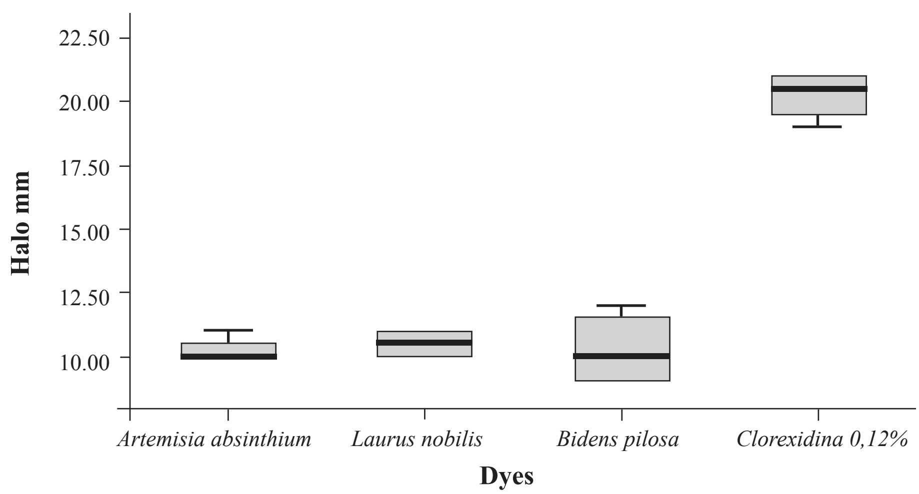

Figure 1 - Distribution of halos (mm) of inhibition of 0.12% chlorhexidine and *Artemisia absinthium*, *Laurus nobilis* and *Bidens pilosa* dyes at 5% concentration. Criciúma, Santa Catarina, 2015.

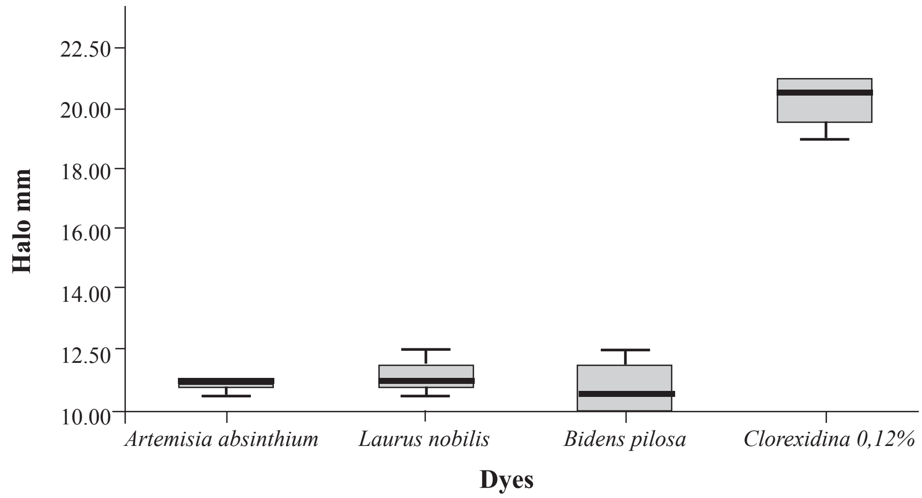

Figure 2 - Distribution of halos (mm) of inhibition of 0.12% chlorhexidine and *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* dyes at 10% concentration. Criciúma, Santa Catarina, 2015.

There was no statistically significant difference (Figure 3) between the halos of inhibition of *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa*, even when compared to 0.12% chlorhexidine (control), at 15% concentration, which deonstrates that the dyes produced inhibitory effects on *S. mutans* that are similar to those presented by 0.12% chlorhexidine.

The halos of inhibition of *Artemisia absinthium* and *Bidens pilosa* dyes presented a statistically significant difference when compared to 0.12% chlorhexidine 0.12% (p=0.016 and p=0.046, respectively) at 20% concentration, which shows that at this concentration the *Artemisia absinthium* and *Bidens pilosa* dyes produced significantly smaller halos of inhibition compared to 0.12% chlorhexidine.

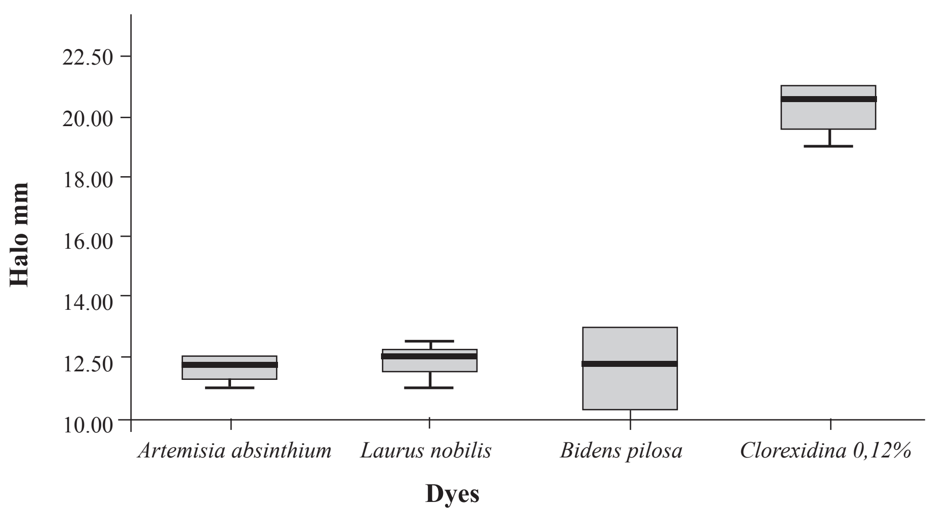

Figure 3 - Distribution of the halos (mm) of inhibition of 0.12% chlorhexidine and *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* dyes at 15% concentration. Criciúma, Santa Catarina, 2015.

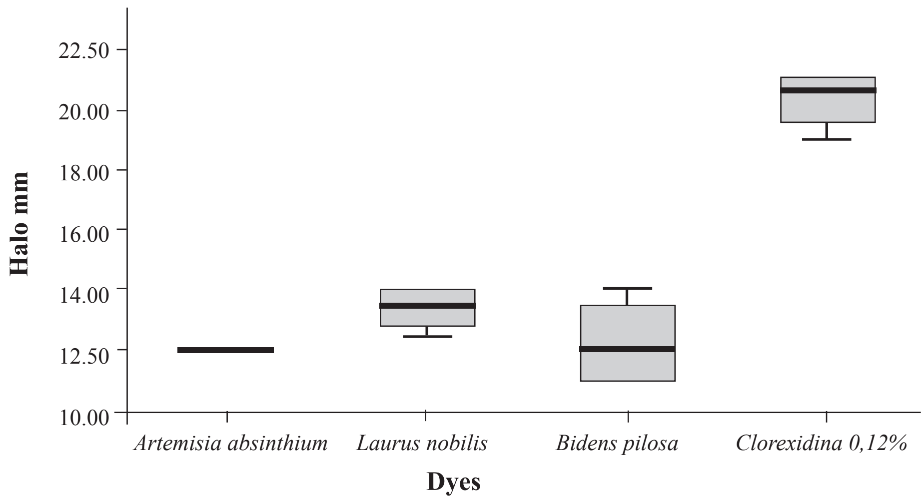

Figure 4 - Distribution of halos (mm) of inhibition of 0.12% chlorhexidine and *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* dyes at 20% concentration. Criciúma, Santa Catarina, 2015.

It should be noted that *Laurus nobilis* dye did not present statistically significant difference when compared to 0.12% chlorhexidine, demonstrating similar inhibitory effect.

A strong positive correlation (table II) was found between the concentration (%) and the size of the halo (mm) of *Artemisia absinthium* (r_s=0.901) and *Laurus nobilis* (r_s=0.872) dyes – both presented statistically significant correlation ($p<0.001$), i.e., the size of the halos of inhibition increases as the concentration increases. However, *Bidens pilosa* (r_s=0.505) presented a moderate positive correlation, but which was also considered statistically significant ($p=0.046$).

Table II - Correllation between the concentration (%) and the size of the halo (mm) of inhibition of *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* dyes. Criciúma, Santa Catarina, 2015.

Dye	r_s	p-value
Artemisia absinthium	0.901	<0.001
Laurus nobilis	0.872	<0.001
Bidens pilosa	0.505	0.046

DISCUSSION

Oral infections, such as dental caries and gingivitis, are common problems in the Brazilian population, especially in preschool children. The dental biofilm (bacterial plaque) has been implicated in the etiology of these infections[18]. Therefore, agents capable of reducing the bacterial population of the biofilm, such as plant extracts, can be allies in preventing these infections, which can even progress into more severe conditions.

The recognition of the microbial etiology of endodontic infections has triggered the search for new therapeutic approaches. The selection of the strain to be used in the present study was based on the failure of most endodontic treatments to combat *E. faecalis*[9]. Odontogenic infections are complications that are difficult to treat in dentistry, particularly in countries with high rates of dental caries and periodontal disease[19].

The use of phytotherapy as an alternative to treat such infections is based on the search for products with good antimicrobial activity, with less harmful effects and with a lower cost, facilitating the access by both the population and health services[12,13,15].

In the present study, the dyes analyzed showed no antibacterial effect on the strain of *E. faecalis*; however, at 5%, 10%, 15% and 20% concentrations, *Artemisia absinthium* (absinthe), *Laurus nobilis* (bay laurel) and *Bidens pilosa* (black-jack) presented effects on strains of *S. mutans*. It was also found a correlation between the increase in the concentrations of *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* dyes and the increase in the size of the halos of inhibition, demonstrating a relationship between the concentration of the active ingredient and the antimicrobial action. This finding is similar to that observed in a study carried out with a glycolic extract of *Punica granatum*, whose halo of inhibition of the growth of *S. mutans* ranged from 11 mm at 1% concentration to 30 mm at 10% concentration[20].

In the Dentistry field, research on natural products has been conducted in recent years in search of new products with greater pharmacological activity, lower toxicity and greater biocompatibility, aiming to provide the population with access to these drugs[21].

None of the dyes used in the present study presented inhibitory effects on the growth of *E. faecalis* at the concentrations tested. This bacterium is characterized by being resistant and able to survive in extreme conditions even after endodontic treatment performed in ideal conditions and long periods of intracanal medication[7]. Several substances are used in the treatment of endodontic infections; even natural extracts have been investigated as irrigating solutions as they help in the chemical and mechanical preparation for the cleaning and disinfection of the root canal system[9]. In this context, it has been proven that propolis exerts therapeutic effects against *E. faecalis* and can be used as an effective alternative for the control of this bacterium[22].

According to the results of the present research, extracts of *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* have been shown to be efficient for the inhibition of bacterial growth of *S. mutans*. This bacterium is associated with pathological processes ranging from dental caries, in patients with poor oral hygiene and a diet rich in fermentable sugars, to bacteremia – and the bacterium may also be associated with periodontal disease[23].

The results of the present research showed antimicrobial effect of *Bidens pilosa* dye on *S. mutans* with the production of halos of inhibition in vitro; however, at 10% and 20% concentrations, the inhibitory effect was considered statistically lower when compared to 0.12% chlorhexidine. In an experimental study conducted in Thailand, researchers assessed extracts of *Bidens pilosa* and found antibacterial action against Gram-positive and Gram-negative bacteria[24]. Likewise, a French study demonstrated that *Bidens pilosa* extract has a moderate antibacterial effect on *S. mutans*[25]. *Bidens pilosa* presents in its essential oil a substance called β-caryophyllene, which has already been cited in the literature as having an important antibacterial effect[26].

According to the results of the present study, *Laurus nobilis* showed antibacterial effect against *S. mutans* with the production of halo of inhibition at all concentrations. Unlike the other dyes tested (*Artemisia absinthium, Bidens pilosa, Achillea millefolium* and *Foeniculum vulgare*), when it was analyzed at 20% concentration it presented an effect similar to that of 0.12% chlorhexidine. By using the distillation method, the authors obtained the essential oil of *Laurus nobilis*. The oil, when inoculated in Petri dishes, led to the production of halos of inhibition against bacteria *Salmonella typhi, Salmonella typhimurium, Salmonella enteritidis, Staphylococcus aureus* and *Listeria monocytogenes*. The size of the halos of inhibition of bacterial growth decreased according to the reduction of the concentration of the essential oil[27]. These results may occur because *S. mutans* is a Gram-positive bacterium; therefore, it is more sensitive to the action of antibiotics due to lack of an external membrane, which facilitates the action of dyes[28].

Inhibition of bacterial growth of *S. mutans* through *Artemisia absinthium* was observed at all concentrations tested in the present study. However, at 20% concentration, the halo of inhibition was considered statistically smaller when compared to 0.12% chlorhexidine. No studies have been found in the literature to report the antibacterial effect of *Artemisia absinthium* on *S. mutans*, but a study reported the effects of this plant on *S. aureus, Bacillus cereus, Listeria, Salmonella* and *Escherichia coli bacteria* – the essential oil presented inhibitory effects on these microorganisms[29]. Differently, other authors observed that the dry extracts of *Artemisia absinthium* up to the concentration of 200 mg/mL presented little or no antimicrobial effect against 15 different microorganisms[30]. These differences may occur because the studies used different methodologies regarding the species of the microorganism studied and the way of obtaining the extracts.

Although medicinal plants are already part of popular culture, interest in phytotherapy has increased considerably among users, health services and researchers in recent decades. Even the WHO has pointed out the need to value the use of medicinal plants in the health field and primary health care[31].

The results of the present study may serve as a basis for the development of new protocols to assess the antimicrobial activity of plant extracts and the implementation of policies on the use of medicinal plants in the public health system. The institutionalization of the use of phytotherapics in primary health care services has faced drawbacks, mainly due to the reluctance and lack of knowledge on the subject by health professionals; however, the promotion of health through medicinal plants can and should be experienced within the health service, providing, among other advantages, the approximation of the user with the system and a lower cost[31].

The main limitations of the present study were the use of an in vitro method, which differs from oral cavity conditions, and the use of concentrations limited to 20%; therefore, further studies should be carried out using more concentrated dyes for plants that showed antibacterial effects; in addition, the antimycobial effect of plant extracts should be assessed "in vivo".

CONCLUSION

The results of the present study showed that plant extracts of *Artemisia absinthium, Laurus nobilis* and *Bidens pilosa* – as hydroalcoholic solutions – at concentrations of 5%, 10%, 15% and 20% showed potential in vitro inhibitory effects against *S. mutans* (ATCC 25175), the main microorganism involved in the genesis of caries. Therefore, they consitute possible alternatives for the use of medicinal plants in clinical dentistry. The extracts did not show in vitro antimicrobial effect on *E. faecalis* (NEWP 012) at any of the concentrations tested.

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