International Journal of Pharmacology

ISSN 1811-7775

science alert

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.737.742

Research Article
Antimicrobial Effect of the Methanolic Extract *Psacalium decompositum* on Periodontopathogenic Bacteria

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Abstract

Background and Objective: Periodontal disease and dental caries are frequent oral illnesses. Both have an important impact on quality of life are of polymicrobial origin and progress slowly. Plants are a valuable resource in health systems in developing countries and a large part of traditional treatments involve the use of plant extracts or their active substances. The objective of this study is to determine the antimicrobial activity of the methanolic extract of *Psacalium decompositum* using the plate diffusion method *in vitro* and to determine the minimum inhibitory concentration of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Streptococcus mutans*. **Materials and Methods:** The plant was collected and identified, *Psacalium decompositum* and a methanol extract was prepared by mashing. Phytochemical screening was also performed. The methanol extract was evaluted by the plate diffusion method using different microdilutions in broth against the ATCC strains *Porphyromonas gingivalis*, *Prevotella intermedia* and *Streptococcus mutans*. About 0.2% chlorhexidine was used as a positive control and 5% ethanol as a negative control. The nonparametric statistic, the Kruskal-Wallis test was used to compare the three treatments and the Mann-Whitney test for each pair of treatments to identify significant differences between them. **Results:** The minimum inhibitory concentration of the methanolic extract of *P. decompositum* was 500 μg mL^{-1} for *P. gingivalis* and *P. intermedia* and 700 μg mL^{-1} for *S. mutans* ($p<0.002$). The minimum inhibitory concentration for chlorhexidine (positive control) was 900 μg mL^{-1} for the three bacteria studied. **Conclusion:** The methanolic extract was active against cariogenic and periodontopathogenic bacteria and represents a natural alternative for the control of these microorganisms that produce oral diseases. The methanolic extract of *P. decompositum* has antimicrobial activity making it a natural alternative for the treatment of caries and periodontal disease.

Key words: *Psacalium decompositum*, *P. gingivalis*, *P. intermedia*, *S. mutans*, antimicrobial activity

Received: April 22, 2016 Accepted: June 24, 2016 Published: September 15, 2016

Citation: P. García-Palencia, M.A. de la Garza-Ramos, S.A. Galindo-Rodríguez, A. Oranday-Cárdenas, C. Leos-Rivas, M.J. Verde-Star and C. Rivas-Morales, 2016. Antimicrobial effect of the methanolic extract *Psacalium decompositum* on periodontopathogenic bacteria. Int. J. Pharmacol., 12: 737-742.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the World Health Organization (WHO), oral health is defined as the absence of chronic orofacial pain, mouth or throat cancer, oral ulcers, congenital defects such as cleft palate, periodontal disease, dental caries, tooth loss and other diseases. The most frequent oral problems in Mexican population are dental caries and periodontal disease. Both have an impact on quality of life and can be prevented and controlled with simple and economical measures as well as with the help of dental professionals working together with the community[1].

Periodontal disease is characterized by inflammation and loss of supporting tissues, in final stages it causes mobility and loss of teeth[2]. In Mexico, periodontal disease is found in 67.2% of adults between 40 and 49 years of age and in 90% of individuals between 60 and 90 years of age[3].

Chronic periodontitis starts and is sustained by gingival biofilm. This biofilm modifies the pathogenesis of the disease according to age or genetic factor. Periodontitis is polymicrobial and progresses slowly. The microorganisms most frequently related with this disease are *Porphyromonas gingivalis*, *Tannerella fosythensis*, *Prevotella intermedia*, *Aggregatibacter actinoinomycentencomitans*, *Fusobacterium nucleatum*, *Campylobacter* spp., *Eikenella corrodens* and others[4].

Plants are a valuable resource in health systems in developing countries. Although there are no precise data to evaluate the use of medicinal plants, the WHO has estimated that more than 80% of the world population routinely uses traditional medicine to satisfy their primary care health needs and a large part of these traditional treatments involve the use of plant extracts or their active substances[5].

Psacalium decompositum (Asteraceae) grows throughout Mexico from Chihuahua to Chiapas and has several popular names: Matarique, Matari, Materi, Matariki, Maturín, Maturi, Mataril and Pitacawi. The root is traditionally prepared as tea and used for rheumatism, jaundice, colicky pain in infants, diabetes, renal disease, malaria, fever, arthritis, muscle pain, urinary tract diseases as a diuretic, tonic, aseptic and for wound healing. The root is also applied as a cataplasm for wounds. A portion of the root is placed in dental caries to relieve pain[6].

Hypoglycemic sesquiterpenes such as cacalol and a mixture of 3-hydroxycacalolide and epi-3-hydroxycacalolide have been identified; however, their mechanism of action is unknown. The plant also contains alkaloids, essential oils, resins, tannins and glycosides[7].

Different chemical substances are currently used for the treatment of periodontal disease, such as ammonium derivatives, fluorides, phenolic compounds and chlorhexidine gluconate. They are used as oral rinses to eliminate dental plaque but they possess side effects such as pigmenting teeth, irritating the mucosa and altering the sense of taste[8].

The objective of this study was to determine the antimicrobial a ctivity of the methanolic extract of *P. decompositum* using the plate diffusion method on the ATCC bacteria *P. gingivalis*, *P. intermedia* and *S. mutans in vitro* and determine its Minimum Inhibitory Concentration (MIC).

MATERIALS AND METHODS

Plant material: *Psacalium decompositum* was collected in Real de Catorce, Mexico and identified in the Botany Department of the School of Biological Sciences of the UANL. The root was separated, washed with water, cut into slices and dried in the shade for 7 days, afterwards, it was ground and stored in refrigeration until its use.

Preparation of methanolic extract: Two hundred grams sample of pretreated *P. decompositum* was placed in a 500 mL Erlenmeyer flask. Briefly, 120 mL of 99.9% methanol was added and the flask was hermetically sealed and shaken constantly for 7 days using a dual action Lab-line shaker (Thermo Fisher Scientific Inc., Waltham, MA). The solvent was separated from the rest of the plant material and filtered with Whatman No. 1 filter paper; afterwards, the same plant material was extracted two more times. The methanolic extract was concentrated using a Büchi rotavapor at a temperature below 60°C, the extract was then totally dried at room temperature and stored in amber colored vials at 4°C until use.

The yield of the extract was obtained with the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight obtained}}{\text{Initial weight}} \times 100$$

where, weight obtained refers to the weight obtained by extraction and initial weight is the weight of the plant material to be extracted.

Phytochemical screening: The variety of compounds with a different chemical nature, found in the plants was named phytocomplex[8]. This contained secondary metabolites with biologic activity in the oral cavity (antimicrobial, anti-inflammatory and astringent among others)[9].

Phytochemical tests were performed to determine the presence of unsaturated compounds (tetranitromethane), tannins (phenolic oxyhydriles), sterols and triterpenes (Liebermann-Burchard), saponins, carbohydrates, quinines, coumarins, alkaloids (Dragendorff), sesquiterpen lactones (Baljet) and flavonoids (Shinoda)[10].

Evaluation of the antibacterial activity of the methanolic extract of *Psacalium decompositum*: This was determined by the plate diffusion method. Plates with sheep blood agar were inoculated with 100 µL of the bacterial supernatant. Plates were divided into five zones; in three, sterile Whatman No. 1 filter paper was placed with 10 µL of the extract to be tested, in zone 4, the negative control, 99.9% ethanol was placed depending on the extract and in zone 5, 0.12% chlorhexidine was used as a positive control. The procedure was carried out in triplicate. Plates were incubated for 24 h at 37°C in anaerobiosis and inhibition halos were measured to determine activity.

Determination of the minimum inhibitory concentration of the methanolic extract *P. decompositum*: The MIC was determined with the microdilution technique in 96-well plates[11]. The concentrations evaluated were: 1000, 900, 700, 500, 400, 300, 200, 100 and 50 µg mL^{-1}, each concentration was tested 4 times in triplicate. Target samples with the extract in tripticase in soy culture medium were included with the positive control 0.2% chlorhexidine (final concentration in the medium). With this, different concentrations of 1200, 1000, 900, 700, 500 and 400 µg mL^{-1} were evaluated using 5% ethanol as a negative control and microorganisms as a growth control, each of these were performed 4 times in triplicate.

The strains *S. mutans* ATCC® 700610TM UA159 [UAB577], *P. gingivalis* ATCC® BAA-308TM W83 and *P. intermedia* ATCC® 25611TM were activated. An aliquot of 100 µL of each strain was collected and inoculated in 5 mL of trypticase soy broth and incubated at 37°C for 12 h in reduced oxygen conditions necessary for each bacterial strain. An inoculum was prepared adjusting it to 0.5 McFarland (1.5×10^8 CFU). Mother solution was prepared from the extract. About 50 mg portion of the extract was dissolved in 1 mL of solvent (100% ethanol) and a 1/20 dilution was made, 50 µL of the initial extract solution and 950 µL of a fluid medium were mixed to a final volume of 1 mL (2500 µg mL^{-1}). From this, the different concentrations mentioned were prepared. Stock solution of the positive control (chlorhexidine) was prepared at a concentration of 2000 µg mL^{-1}. With this, the previously mentioned concentrations were prepared. A stock solution of 100% ethanol and liquid culture medium were prepared both in the same proportion as the extract as a negative control and the different concentrations to be evaluated were made.

A volume of 200 µL per well was deposited in each microplaque with a different volume of mother solution for each concentration of the extract, plus 100 µL of the inoculum of each bacterial strain, completing the final volume of each well with a fluid medium. This was run in quadruplicate: for the target of the extract a different volume of mother solution, for the chlorhexidine control a different volume of the stock solution was added for each concentration of the positive control, for the negative control a different volume of stock solution for each concentration and for growth control a 100 µL inoculum of each bacterial strain and 100 µL of liquid medium. The plaque was incubated at 37°C for 24 h in appropriate redox conditions for each bacterial strain; after this optical density was measured at 595 nm on a BIO-RAD® iMark Microplate Absorbance Reader (Bio-rad Laboratories, Inc., Hercules, CA). The lowest absorbance between the tested concentrations after subtracting the turbidity of the extract was considered the minimum inhibitory concentration.

Statistical analysis: A statistical analysis Leven test was performed to assess whether there are significant differences in the variances of the treatments. The nonparametric Kruskal-Wallis statistical test was used to compare three treatments and the Mann-Whitney test for comparing each pair of treatments to see if there is a significant difference.

RESULTS

The methanolic extract of *P. decompositum*, presented a significant antimicrobial activity against the three bacteria studied (Table 1). The inhibition halos in the antibiograms of the extract against the microorganisms of the study are shown in Fig. 1 with the against *P. gingivalis* being noteworthy. Minimum Inhibitory Concentration (MIC) of the methanolic root extract of *P. decompositum* was higher for the cariogenic bacterium *S. mutans* at 700 µg mL^{-1} and lower for the periodontal pathogenic bacteria *P. gingivalis* and *P. intermedia* at 500 µg mL^{-1} for both and 900 µg mL^{-1} for chlorhexidine (positive control) for the three bacteria tested (Fig. 2). Levene's test showed variances that differed significantly, $p<0.001$, therefore, the Kruskal-Wallis test was used to compare the three treatments with their absorbances with a significance of $p<0.002$, demonstrating that there was a significant difference between the absorbances of the three treatments. Based on the range table, the Mann-Whitney test was applied for each treatment pair of the methanolic extract

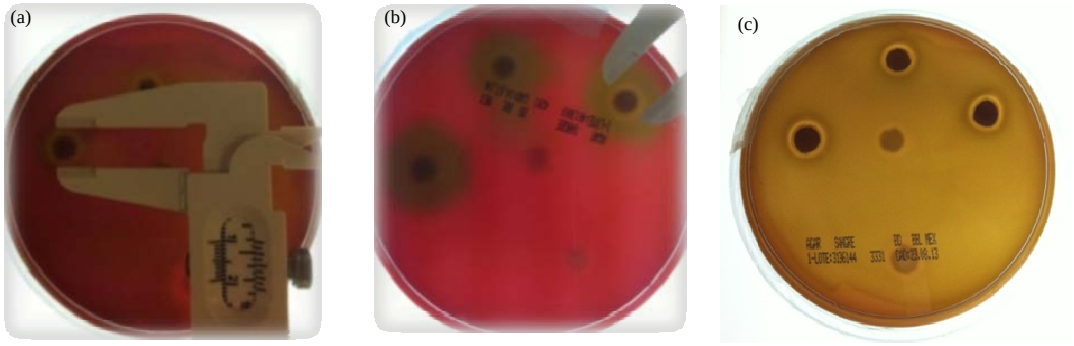

Fig. 1(a-c): Measurement of the inhibition halo of the methanolic extract of *P. decompositum* activity against (a) *P. gingivalis* ATCC® BAA-308TM, (b) *P. intermedia* ATCC® 25611TM and (c) *S. mutans* ATCC® 700610TM

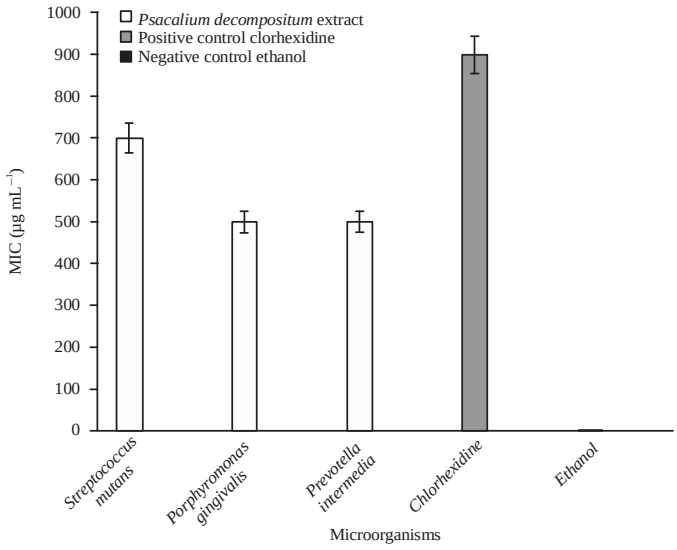

Fig. 2: Minimum inhibitory concentration of the methanolic extract of *P. decompositum* and the positive and negative controls against *S. mutans*, *P. gingivalis* and *P. intermedia*

Table 1: Antimicrobial activity of the methanolic extract of *P. decompositum* on periodontopathogenic and cariogenic bacteria by the plate diffusion method

	Inhibition halo (mm)		
Bacteria	*Psacalium decompositum*	Positive control	Negative control
Porphyromonas gingivalis	10.4	12.0	0
Prevotella intermedia	9.4	8.6	0
Streptococcus mutans	9.0	9.6	0

Positive control: 0.12% clorhexidine and Negative control: 100% ethanol

P. decompositum and the bacteria *P. gingivalis*, *P. intermedia* and *S. mutans*. It was found a significant difference in the first group of $p<0.040$ for the second group the p-value was $p<0.001$ and for the third group there was no significant difference ($p<0.906$).

The yield of the extract was 8.1% using the previously mentioned formula. Phytochemical screening of the methanolic extract demonstrated the following metabolites or functional groups: Unsaturated compounds, carbonyl group, phenolic oxyhydril-tannins and sterols.

DISCUSSION

The methanolic extract of *Psacalium decompositum* was active against the periodontopathogenic bacteria *P. gingivalis* and *P. intermedia* with an MIC of 500 µg mL^{-1} and the cariogenic bacteria *S. mutans* with an MIC of 700 µg mL^{-1}. In dental practice substances currently available for treating periodontal problems are used in higher concentrations. The

main product used as a mouthwash is clorhexidine 0.12 or 0.2% (this corresponds to 1200-2000 μg mL^{-1}).

With these results we can conclude that the *P. decompositum* extract represents a natural alternative for the control of the microorganisms that cause oral diseases[12]. In addition, the concentrations used in this study can be achieved in clinical solutions. These could be at approved concentrations used commercially or at the concentration reported in this study because the extract is active at a lower concentration than commercial products. It is also important to mention that since periodontal disease is chronic and clorhexidine causes teeth discoloration with prolonged use, the use of this natural product could be an advantage.

Marsh and Martin[13], Van Winkelhoff *et al*.[14] and Urena[15] described *P. gingivalis* as the most pathogenic Gram-negative anaerobe bacilli emphasizing its ability to colonize and destroy periodontal tissue and evade host defenses.

Prevotella intermedia are periodontopathogenic microorganisms that play an important role in the establishment and development of polymicrobial periodontal diseases. It is characterized by its ability to adhere and invade gingival epithelial cells, fibroblasts and endothelial cells, although the role that gram-negative enteric bacilli play in the pathogenesis of periodontal disease is unknown. Researchers agree that they have an impact on the progress and treatment of the disease in addition to being invasive and toxigenic bacteria[16]. The *S. mutans* is a lactic acid producing Gram-positive immobile coccus that is capable of changing alkaline pH to acid pH in 24 h. It is a microorganism that is closely related to the most prevalent infectious disease in humans, dental caries, which is characterized by demineralization and remineralization[17].

Caries and periodontitis are caused by an imbalance of bacterial populations that naturally form biofilms. The bacteria in these biofilms are saprophytes that help maintain normal conditions in the oral cavity but when changes occur in host defenses or in the oral environment, the virulence of these bacteria increases.

Almanza-Perez[18] and Alarcon-Aguilar *et al*.[19] reported the presence of alkaloids in rhizomes and roots of *P. peltatum*, in this study of *P. decompositum*, these compounds were not found. In a review performed to study for secondary metabolites present in the family *Asterecea*. De Vivar *et al*.[20] reported the presence of pyrrolizidine alkaloids in all species of the genus *Psacalium*. Secondary metabolites such as coumarins, which have antimicrobial activity[21] and sesquiterpene lactones, that also have antimicrobial activity, were found in the methanolic extract of *P. decompositum*[22,23]. This study with *P. gingivalis*, *P. intermedia* and *S. mutans* coincides with that of Jimenez *et al*.[24], in that some compounds from the organic extracts of roots of *P. decompositum*, such as cacalol and some of its derivatives have antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and certain fungi such as *Candida albicans* and *Cryptococcus neoformans*[25,26].

In this study, it was found that *P. decompositum* has a broader antimicrobial effect since it inhibited the growth of the three bacteria studied in comparison with the results obtained by Almanza-Perez[18] who used hexanic and dichloromethane extracts from a plant of the same genus, *P. pelatum*, which inhibited the growth of only one bacteria, *Bacillus subtilis* of six that were evaluated at a concentration of 10 and 100 μg mL^{-1} for both extracts.

These results show that plants from different species have different properties despite belonging to the same genus. Antimicrobial activity has been reported with diverse plants belonging to the same family *Asteraceae*, such as the study by Portalatino and Medina[27] with hydroalcoholic extracts of *Baccharis genistelloides* (callua), *Jungia paniculata* (matico) and *Perezia multiflora* (escorzonera) which inhibited *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 11774.

Anti-inflammatory and hypoglycemic properties have been reported for *P. decompositum*; however, there are few studies on its antimicrobial activity, making this an area of opportunity for new study.

CONCLUSION

The methanol extract *Psacalium decompositum* was active against the peridontopathogenic bacteria *P. gingivalis* and *P. intermedia* and the cariogenic bacteria, *S. mutans* and *P. decompositum* represent a natural alternative for the control of these microorganisms which produce oral diseases. It is important in future studies to fractionate this methanolic extract to identify the specific molecule that produces this antimicrobial activity. Also, possible toxicity of the extract should be considered. For this reason, toxicity testing and the Ames test are currently being carried out.

ACKNOWLEDGMENT

We thank Sergio Lozano-Rodriguez, M.D. for his help in transalting the manuscript. García Palencia was

supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico) No 557089.

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