Available Online at www.ijcrr.in

Research

International Journal of Contemporary Research and Review
CrossRef DOI: http://dx.doi.org/10.15520/ijcrr/2017/8/11/351

ISSN 0976 – 4852
October, 2017|Volume 08|Issue 10|

# Antimicrobial Effect of Capsaicin against *Aggregatibacter actinomycetemcomitans*

**Karina Sarno Paes Alves Dias[1], Eduardo Saba-Chujfi[2], Silvio Antonio dos Santos-Pereira[3], Ivan Santos Batista Sobrinho[4]**

[1]Faculdade Independente do Nordeste (Fainor). Av. Luis Eduardo Magalhães, 1305, Candeias, Vitória da Conquista – BA, Brazil. Phone: (5577) 3161-1000.

[2]Department of Periodontology, SLMANDIC - São Leopoldo Mandic Institute and Research Center. R. Dr. José Rocha Junqueira, 13 - Pte. Preta, Campinas - SP, Brazil. CEP 13045-755. Phone: (5519) 3211-3600.

[3] Department of Periodontology, SLMANDIC - São Leopoldo Mandic Institute and Research Center. R. Dr. José Rocha Junqueira, 13 - Pte. Preta, Campinas - SP, Brazil. CEP 13045-755. Phone:(5519) 3211-3600.
[4]Universidade Federal da Bahia – UFBA, Campus Anísio Teixeira. Rua Horminio Barros, Qd 17, 58, Candeias, Vitória da Conquista - BA,Brazil.

**Accepted 2017-10-10; Published 2017-11-01**

**Abstract:**

This study aimed to evaluate the in *vitro* antimicrobial activity of synthetic capsaicin and *in natura* capsaicin against *Aggregatibacter actinomycetemcomitans,*and compare them with each other. The *in natura* and synthetic extracts of capsaicin at the concentrations of 0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% were diluted in 1mL of dimethylsulfoxide (DMSO). The positive control was chlorhexidine 0.12% and the negative control was DMSO. The diameter of the zone of bacterial inhibition was measured after 72 hours. The main effect of capsaicin (natural vs. synthetic) origin against *Aggregatibacter actinomycetemcomitans* was not significant; No effect of interaction between capsaicin origin and concentration on bacterial growth was observed. Capsaicin presented *in vitro* antibacterial activity against *Aggregatibacter actinomycetemcomitans*, with no difference between *in natura* and synthetic type, but its effect at the concentrations tested was lower than that of chlorhexidine 0.12%. It was concluded that capsaicin presented antimicrobial activity on *Aggregatibacter actinomycetemcomitans*, irrespective of origin.

**Keywords:** Anti-infectious. Capsaicin. Periodontics. Medicinal plants.

## Introduction:

In the treatment of periodontal disease, it is frequently necessary to associate the use of systemic antimicrobial agents with conventional mechanical scaling and root planning, to help with to eliminate the periodontal pathogens and modulating the host inflammatory response, thereby reducing tissue destruction[1,2]. However, the indiscriminate use of antimicrobial agents

promotes the natural selection of resistant bacterial strains and provides an opportunity for genetic recombination of the resistance and mutation factors. Therefore, the importance is

observed of studies that evaluate the therapeutic efficacy of procedures and medications, especially in relation to the use of antimicrobial agents[3]. Therefore, the research of new antimicrobial substances isolated from vegetable extracts has been a worldwide trend, and from this aspect, the contribution of phytochemistry has been fundamental, because the great structural diversity provided by this field of science, has allowed molecules to be found, which may serve as prototypes for new antimicrobial substances[4]. The phenolic compounds present in plants not only contribute to the flavor, odor and color of diverse foods, but also offer pharmacological properties, such as antifungal, anti-inflammatory and antibacterial activity, being capable of inhibiting the growth of microorganisms according to their concentration [5,6]. The species of the genus *Capsicum* have been studies by researchers all over the world, and studies have attributed the following properties to capsaicin: antihyperlipidemic activity [7]; anti-inflammatory, antioxidant [8,9], and antimicrobial properties [10-12]; in addition to a chemiopreventive effect [13, 14], and effectiveness in the treatment of a number of nerve fiber disorders, including pain associated with arthritis, cystitis and diabetic neuropathy [15]. Capsaicin, the active principle of peppers, has played a significant and traditional role as a medication. According to Molina et al.[16], capsaicin showed strong inhibition of the growth of the species *Bacillus subtilis* at the minimum concentration of 25 μg/mL, whereas at concentrations of 200 to 300 μg/mL it only retarded the growth of the bacteria *Escherichia coli and Pseudomonas solanacearum*. For the species *Saccharomyces cerevisiae*, capsaicin stimulated its growth at high concentrations. Other studies have demonstrated that capsaicin inhibited the growth of the species *Helicobacter pylori* resistant to metronidazole at the concentration of 25 g/mL [17]. Barbosa et al.[18] concluded that the analogs were active against *Penicillium expansum* at higher concentrations than those of capsaicin. Other researchers observed that capsaicin analogs presented efficiency equal to or lower than it for antimicrobial activity against *Streptococcus mutans* [19, 20]. Capsaicin has lipophilic capacity and is capable of interacting with the lipids of the cell membrane, thus explaining its antimicrobial actions [21]. In a study conducted by Zhou et al.[22], capsaicin inhibited the growth of the species *Porphyromonas gingivalis* at minimum inhibition concentration (MIC) and minimum bactericide concentration (MBC) of 1.6% and 6.4%, respectively. In view of the considerations here related and taking into consideration that capsaicin has the potential of being better explored as a source of antimicrobial compounds, the purpose of this study was to evaluate the *in vitro* antimicrobial activity of capsaicin against *Agregatibacter actinomycetemcomitans*.

**Materials and Methods:**

A standard sample of *Agregatibacter actinomycetecomitans* (ATCC 29522) was used, obtained from the "Instituto Oswaldo Cruz" - Rio de Janeiro - Brazil. The microorganism was cultivated in BHI broth (Difco, São Paulo, Brazil) and kept frozen at –20 ºC.The research was developed by means of a quantitative approach by means of microbiological tests, *in vitro*, against *Agregatibacter actinomycetecomitans* (ATCC 29522), using both the *in natura* and synthetic extract of capsaicin, both at the same concentrations, for comparative purposes. The raw material for elaborating the *in natura* capsaicin extract was acquired from the company Galena Química e Farmacêutica LTDA - SP/Brazil. (Attachment B) Whereas, the synthetic extracts were formulated from the product acquired from the company Sigma-Aldrich, Germany (N-VANILILNONANAMIDA) (Table 1). In the process of dilution into the different concentrations, the raw material was ground with a pestle and mortar with added DMSO. Both the *in natura* and the synthetic capsaicin were weighed with an analytical balance. Afterwards, this raw material was dissolved in DMSO (Dimethylsulfoxide), which was bottled in ambar glass. From this dissolution, extracts were obtained in the following concentrations: 0.1%; 0.5%; 1.0%; 1.5%; 2.0%; 2.5% and 3.0%. The media were made in dehydrated Agar BHI, as proposed by the *Clinical and Laboratory Standards Institute* [23]. The positive control used was 0.12% Chlorhexidine, and the negative, DMSO.

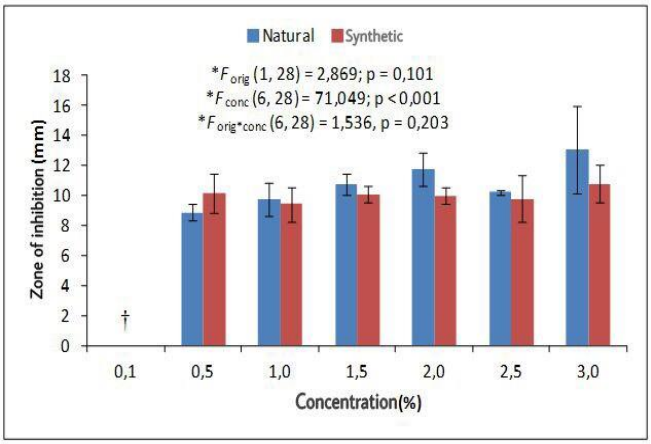
A suspension of bacterial cells was prepared, followed by adjustment with sterile saline, to the MacFarland standard scale of 0.5 [24]. The antimicrobial susceptibility was evaluated by the disc-diffusion method, in accordance with the recommendations of the *Clinical and Laboratory Standards Institute* [25].

A total of 21 plates were prepared with Mueller Hinton agar. The bacterial inoculation was uniformly distributed on the agar surface with the use of a pipette and Drigalski loop. The plates were left to rest at ambient temperature for 3 minutes. With the aid of a sterile paint brush, dry, sterile filter paper discs (Whatman - type 3) 5.0 mm in diameter, were impregnated with 10µl of *in natura* and synthetic capsaicin extract at the above-mentioned concentrations, and the controls, were uniformly distributed on the agar surface. The plates were placed in an Anaerobic Jar and incubated in a BOD (biochemical oxygen demand) type oven at 36±1ºC for 72 hours. Lastly, the formation of or absence of halo formation was observed.The entire experiment was performed in triplicate and measurement was performed with a ruler.

**Results:**

The negative control (dimethylsulfoxide) presented bacterial growth, since no inhibition zone was perceived around the disc. The means and standard deviations of the inhibition zones (Graph 1) are presented according to the origin and concentration of capsaicin. The factorial ANOVA results demonstrated that there was no significant principal effect of the origin of capsaicin (natural *vs.*Synthetic) against *Agregatibacter actinomycetemcomitans;* and no effect was observed of the interaction between the origin and concentration of capsaicin on bacterial growth. However, significant principal effect of capsaicin against *Agregatibacter actinomycetemcomitans* was verified. The multiple comparisons test revealed that there was statistical difference between the lowest concentration (0.1%) and all the others, indicating that the growth of the bacterial strain was inhibited as from the concentration of 0.5%, and was maintained sable up to 3.0%.

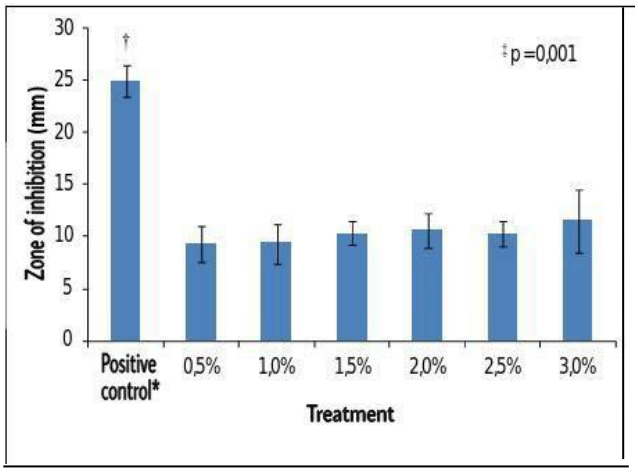
***Graph 1 - Inhibition Zone of Agregatibacter actinomycetemcomitans growth in the presence of the capsaicin extract of different origins (orig) and concentrations (conc).***




**Legend:** Columns represent means and error bars, the standard deviations. * Factorial ANOVA (*two-way*); indicated significant difference in relation to all the other concentrations (p < 001;Games-Howell multiple comparisons test).

In the present study, comparisons between the isolated effects of different concentrations of capsaicin and the positive control 0.12% chlorhexidine (Graph 2) presented inactivity against the effect of origin. It was possible to observe that inhibition of the growth of the bacterial species *Agregatibacter actinomycetemcomitans* was lower at the different concentrations if capsaicin when compared with 0.12% chlorhexidine.

***Graph 2 - Comparison of Agregatibacter actinomycetemcomitans inhibition zone between different concentrations of capsaicin and 0.12% chlorhexidine (positive control).***




**Legend:** Columns represent means and error bars, the interquartile deviations. ‡Kruskal-Wallis test; † indicated significant difference relative to all the concentrations of capsaicin (p = 0.004; Mann-Whitney test). *0.12% Chlorhexidine

**Discussion:**

Other studies have demonstrated the antimicrobial activity of capsaicin against diverse types of organisms of the oral cavity [22, 12]. However, *Agregatibacter actinomycetemcomitans* was not included among the strains analyzed in any of these studies, which makes it impossible to perform comparison of the results found in the present study.

The antibacterial and antiviral properties of capsaicin against *Streptococcus pyogenes*, causes of pharyngotonsillitis were confirmed by Marini et al.[12], probably due to damage to the bacteria cell membrane. The findings are also in agreement with previous studies that documented the *in vitro* bactericide action of capsaicin against Gram-positive and Gram-negative pathogenic agents [11]. In the study of Dima et al.[10] the antimicrobial activity of capsaicin was proved by using disc diffusion sensitivity tests against three bacteria: *Staphylococcus aureus*, *Salmonela*, and *Escherichia coli*. Whereas Zhou et al.[22], as was done in the present study, evaluated the antimicrobial activity of capsaicin against a periodontogenic bacteria, *Porphyromonas gingivalis*. However, they used the microdilution method and also demonstrated the inhibitory effect of a MIH of 1.6% and MBC of 6.4%.

It is very plausible the fact of the inhibition of the growth of the bacterial species *Aggregatibacter actinomycetemcomitans* was lower in the different concentrations of capsaicin when compared with 0.12% of chlorhexidine , since the latter is considered the gold standard oral antiseptic [26] and has antimicrobial power against the bacteria *Agregatibacter actinomycetemcomitans* and various other periodontogenic bacteria [26-29]. Nevertheless, the use of chlorhexidine may lead to undesirably secondary side-effects, such as ulcerative and desquamative oral lesions [30].

**Acknowledgement:**

The results of this research suggested that capsaicin may represent a new option in the arsenal of antimicrobial substances, and it is necessary to develop further researches to prove its clinical efficacy and applicability, especially as an auxiliary substance in the conventional treatment of scaling and planing the roots of periodontally compromised teeth.

**Compliance with Ethical Standards:**

Declaration as regards conflict: The authors declare that there is no conflict of interest.

Ethical Approval: "For this type of study formal consent is not necessary."

Informed consent: This study does not contain any studies with human participants or animals, conducted by any of the authors.

**References:**

1. Umeda M, Takeuchi Y, Noguchi K, Huang Y, Koshy G, Ishikawa I. Effects of nonsurgical periodontal therapy on the microbiota. Periodontol 2000. 2004;36:98-120. DOI:10.1111/j.1600-0757.2004.03675.x
2. Xajigeorgiou C, Sakellari D, Slini T, Baka A, Konstantinidis A. Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. J ClinPeriodontol. 2006;33:254-64. DOI: 10.1111/j.1600-051X.2006.00905.x
3. Pilatti GL, Kozlowski Junior VA, Alexandrino EAN, Pinto ACEG, Gervasoni C R, Filho-Azim LC. Estudo clínico do uso coadjuvante de clorexidina ou de metronidazol na forma de gel durante a instrumentação subgengival. Rev Pau Odontol. 2002 jan-fev;(1):20-6.
4. Elisabtsky E, Souza GC. Etnopharmacologia como ferramenta na busca de substâncias bioativas. In: Simões CMO et al. Farmacognosia da planta ao medicamento. 5a ed. Florianópolis: Universitaria UFSC & UFRGS; 2003. p.263-288.
5. Pinho E, Ferreira ICFR, Barros L, Carvalho AM, Soares G, Henriques M. Antibacterial potential of northeastern Portugal wild plant extracts andrespective phenolic compounds. Biomed Res Int. 2014;1-8.
6. Martins N, Barros L, Henriques M, Silva S, Ferreira ICFR. Activity ofphenolic compounds from plant origin against Candida species. Ind Crops Prod. 2015;74:648-70.
7. Kuda T, Iwai A, Yano T. Nutrition and sciences. Food Chemical Toxicol. 2004;5:1695- 700.
8. Surh YJ, Lee E, Lee JM. The Capsaicin Study. Mutat Res. 2002;41:259-67.
9. Ganji V, Kafai MR. Capsaicinoids in vegetative organs of Capsicum annuum L. in relation to fruiting. Am J Clin Nutrition.

2004;6:1500-7.
10. Dima C, Coman G, Cotârlet M, Alexe P, Dima S. Antioxidant and Antibacterial proprieties of capsaicine microemulsions. Food Technol. 2013;37(1) 39-49.
11. Omolo MA, Wong ZZ, Mergen AK, Hastings JC, Le NC, Reiland HA, et al. Antimicrobial properties of chili peppers. J Infect Dis Ther. 2014;2:1-8.
12. Marini E, Magi G, Mingoia M, Pugnaloni A, Facinelli B. Antimicrobial and Anti-Virulence Activity of Capsaicin Against Erythromycin-Resistant, Cell-Invasive Group A Streptococci. Front Microbiol. 2015 Nov;6:1281.
13. Surh YJ, Lee E, Lee JM. The Capsaicin Study. Mutat Res. 2002;41:259-67.
14. Lee JJ, Crosby KM, Pike LM, Yoo KS, Leskovar DI. Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (Capsicum spp). SciHorticulturae. 2005;106:341-52.
15. Nuez F. El cultivo de pimientos, chiles y ajies. Madri: Ediciones Mundi-Prensa; 1995.
16. Molina JT, García-Chávez A, Ramírez-Chávez E. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. J Ethnopharmacol. 1999;64:241-8.
17. Zeyrek FY, Oguz E. In vitro activity of capsaicin against Helicobacter pylori. Ann Microbiol. 2005;55(2):125-7.
18. Barbosa JS, Coelho AR, Oliveira F, Piotroski DR, Machado-Lunkes A. Atividade anti microbiana de análogos da capsaicina. In: Anais do XVII Seminário de Iniciação Científica e Tecnologia (SICIT), UTFPR; 2012.
19. Santos MMP. Atividade antimicrobiana in vitro de extratos vegetais das espécies *Mangifera indica*, *Eugenia jambolana*, *Schinusterebinthifolius*, *Capsicumannuum*, e de análogos sintéticos da capsaicina, frente aos microrganismos da cavidade oral [dissertação]. Campo dos Goytacazes: Universidade Estadual do Norte Fluminense Darcy Ribeiro; 2010.
20. Santos MMP, Motta OV, Vieira IJC, Braz-Filho R, Gonçalves OS, Maria EJ, et al. Antibacterial activity of Capsicum annuum extract and synthetic capsaicinoid derivatives against Streptococcus mutans. J Natural Med. 2012;66(2):354-56.
21. Tsuchiya, H. Biphasic membrane effects of capsaicin, an active component in Capsicum species. J Ethnopharmacol. 2001;75:295-9.
22. Zhou Y, Guan X, Zhu W, Liu Z, Wang X, Yu H, et al. CapsaicininhibitsPorphyromonasgingivalis growth, biofilmformation, gingivomucosalinflammatorycytokinesecretion, and in vitroosteoclastogenesis. Eur J ClinMicrobiol Infect Dis. 2014 Feb;33(2):211-9. Doi: 10.1007/s10096-013-1947-0.
23. CLSI. Clinical and Laboratory Standards Institute. Antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Norma aprovada. 3a ed. Wayne, PA, CLSI document M31-A3; 2008.
24. CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility test. 11a ed. USA: 2012.
25. CLSI. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Test; Approved Standand-Tenth Edition. Wayne, CLSI document M02-A10; 2009.
26. Azzimonti B, Cochis A, Beyrouthy ME, Iriti M, Uberti F, Sorrentino R, Landini MM, Rimondini L, Varoni EM. Essential Oil from Berries of Lebanese Juniperusexcelsa M. Bieb Displays Similar Antibacterial Activity to Chlorhexidine but Higher Cytocompatibility with Human Oral Primary Cells. Molecules. 2015 May 21;20(5):9344-57.
27. Vitt A, Sofrata A, Slizen V, Sugars RV, Gustafsson A, Gudkova EI, Kazeko LA, Ramberg P, Buhlin K. Antimicrobial activity of polyhexamethylene guanidine phosphate in comparison to chlorhexidine using the quantitative suspension method. 2015 Jul 17;14:36.
28. Akca AE, Akca G, Topçu FT, Macit E, Pikdöken L, Özgen IŞ. The Comparative Evaluation of the Antimicrobial Effect of Propolis with Chlorhexidineagainst Oral Pathogens: An In Vitro Study. Biomed Res Int. 2016;2016:3627463.

29. Najafi S, Khayamzadeh M, Paknejad M, Poursepanj G, KharaziFard MJ, Bahador A. An In Vitro Comparison of Antimicrobial Effects of Curcumin-Based Photodynamic Therapy and Chlorhexidine, on Aggregatibacter actinomycetemcomitans. J Lasers Med Sci. 2016 Winter;7(1):21-5.
30. Varoni, E.; Tarce, M.; Lodi, G.; Carrassi, A. Chlorhexidine (CHX) in dentistry: State of the art. Minerva Stomatol. 2012, 61, 399–419.