

Antibacterial Effect of Coconut Oil (*Cocos nucifera*) on *Streptococcus mutans* ATCC 25175: An *In vitro* Study

Efecto Antibacteriano de Aceite de Coco (*Cocos nucifera*) Sobre *Streptococcus mutans* ATCC 25175: Un Estudio *in vitro*

Grecia Vásquez Vereau¹ & Gustavo Guardia Méndez^{1,2}

VÁSQUEZ, V. G. & GUARDIA, M. G. Antibacterial effect of coconut oil (*Cocos nucifera*) on *Streptococcus mutans* ATCC 25175: an *in vitro* study. *Int. J. Odontostomat.*, 15(4):922-927, 2021.

ABSTRACT: The present *in vitro* study determined the antibacterial effect of Coconut (*Cocos nucifera*) oil on *Streptococcus mutans* ATCC 25175. Cross-sectional and experimental study. *Streptococcus mutans* strain ATCC 25175 was seeded on Müller-Hinton Blood Agar. The effect was determined by inhibition halo and the minimum inhibitory concentration, using procaine penicillin G as a positive control and standard suspension of *Streptococcus mutans* as a negative control. Twelve replicates per Coconut oil concentration (25 %, 50 % and 75 %) were performed. Coconut oil concentration at 25 % generated an inhibitory mean of 17 mm and 2.23×10^2 CFU, at 50 % a mean of 21.75 mm and 0.17×10^2 CFU, at 75 % a mean of 22 mm and 0 CFU, procaine penicillin G a mean of 14.25 mm and 0 CFU, the negative control gave a mean of 2.8×10^5 CFU. The Kruskal-Wallis non-parametric test detected a highly significant statistical difference of the three concentrations of Coconut oil ($p < 0.01$). The Mann-Whitney test with Bonferroni adjustment determined that the 50 % and 75 % concentration had similar inhibitory action and that both the 75 % concentration and procaine penicillin G gave a mean of 0 CFU. All concentrations of Coconut oil showed inhibitory action on *Streptococcus mutans* ATCC 25175. 75 % concentration showed the highest inhibitory mean and was the minimum inhibitory concentration that completely eliminated CFU.

KEY WORDS: coconut oil, *Streptococcus mutans*, antibacterial agents.

INTRODUCTION

Currently, there is a growing interest based on the development of techniques and the search for products for the treatment and prevention of dental caries, which is highly prevalent worldwide and in which *Streptococcus mutans* is the cause due to its ability to synthesize extracellular polymers, aciduricity and acidogenicity (Lemos *et al.*, 2019; Romero-González., 2020). Although the use of drugs helps to treat various oral conditions, some of these have adverse effects (Tartaglia *et al.*, 2019). For this reason, there is a constant search for and use of natural resources to help prevent disease and maintain oral health, especially in developing countries such as India, where people use natural oils as oral rinses through a technique called "Oil Pulling" (Naseem *et al.*, 2017; Rupani *et al.*, 2018). One of the most commonly used oils in this technique is coconut (*Cocos nucifera*) oil (Woolley *et al.*, 2020), which contains predominantly

medium-chain saturated fatty acids (Chatterjee *et al.*, 2020) that are metabolized more easily and quickly than long-chain fatty acids because they are smaller, more soluble and more stable against oxidation (Restrepo *et al.*, 2020). Coconut oil is 50% lauric acid (Ghani *et al.*, 2018), to which antibacterial, antiviral and antiprotozoal properties are attributed, especially to its monoglyceride, monolaurin (Joshi *et al.*, 2020; Silva *et al.*, 2018). In Peru, little is known about the antibacterial action of Coconut oil on *Streptococcus mutans* and, since this is related to the high prevalence of dental caries, it is necessary to include easily extractable, low-risk natural substances in daily oral hygiene products that help to slow the development and progression of dental caries. The aim of the present study was to determine the *in vitro* antibacterial effect of Coconut (*Cocos nucifera*) oil on the growth of *Streptococcus mutans*.

¹ Dental surgeon, Peru.

² Senior lecturer National University of Trujillo, Peru.

MATERIAL AND METHOD

Experimental cross-sectional "*in vitro*" study carried out at the Pharmacognosy and Microbiology Laboratory of the National University of Trujillo. The handling and disposal of the *Streptococcus mutans* ATCC 25175 strain was carried out according to the Biosafety manual of the Microbiology and Medicine Laboratories. Authorisation was obtained from the Permanent Research Committee of the Faculty of Stomatology of the National University of Trujillo and the principles of the Declaration of Helsinki were followed.

The study population consisted of a set of Petri dishes containing each of the different concentrations of Coconut oil with adequate seeding of *Streptococcus mutans* (3 x 10⁸ CFU/ml). Plates with adequate *Streptococcus mutans* seeding a priori to the oil challenge that showed inhibition or non-inhibition of bacterial growth were included. Petri dishes that showed contamination (other bacteria or fungi) after the incubation process were excluded.

Sample size. The calculation for the number of tests was determined by the following formula (1):

$$n = \frac{2 * (Z_{\alpha/2} + Z_{\beta})^2 * DE^2}{d^2}$$

Assuming the requirements of 90% confidence (=0.10 Z=1.64) a test power of 80 % (=0.20; Z=0.84) a ratio of SD/d=1.00 is obtained: (Formula 2)

$$n = 2(1.64 + 0.84)^2 / (1.00)^2 = 12$$

Therefore, 12 replicates per concentration were performed.

Collection and preparation of the strain. *Streptococcus mutans* strain ATCC 25175 was obtained from the cepario of the Microbiology Laboratory of the Faculty of Medicine of the National University of Trujillo, was grown in thioglycollate broth and incubated for 48 hours at 37°C. Strain growth was confirmed when a strong turbidity was observed in the test tubes. Petri dishes were prepared on Müller-Hinton Agar medium spiked with 5 % rabbit blood.

Collection and taxonomic identification of *Cocos nucifera*. The fruit *Cocos nucifera* was obtained from the district of Tarapoto, province of San Martín, San

Martin region (Peru). A complete specimen of the fruit was taken to the Herbario Trujillense (HUT) of the National University of Trujillo, for identification and subsequent taxonomic verification.

Preparation of Coconut oil. The oil was extracted by cold pressing. The rind was cut with the respective fruit hairs and then washed with distilled water and disinfected with 0.5 % sodium hypochlorite. Subsequently, the "eyes" or germination pores of the fruit were punctured to remove water and the coconuts were opened. The pulp was removed and cut into small pieces. The pulp was then grated and mixed with distilled water in a blender to form a paste and filtered through sterile gauze, separating the mass from the coconut milk. The coconut milk was then placed in a plastic bottle and left in a dark place for 48 hours. After this time, the bottle was placed in the refrigerator fridge for 24 hours, where the coconut oil and pulp formed a solid layer on top through a decanting process. With the help of scissors, the bottle was opened and the solid mixture of oil and pulp was removed. It was then placed in a water bath until it became a liquid. It was then strained and the oil was separated from the pulp. The strained oil was placed in an amber glass bottle.

Dilution of Coconut oil. Once the Coconut oil was obtained, dilutions were made using Polysorbate 80 as solvent to obtain concentrations of 25 %, 50 % and 75 %.

Inhibition halo (susceptibility test). To determine the inhibition halo, the Kirby and Bauer disc diffusion technique was used, for which sterile filter paper discs were immersed in each concentration of Coconut oil (25 %, 50 % and 75 %) for 1 hour. The discs were placed over the *Streptococcus mutans* cultures in the petri dishes and incubated at 37°C in micro anaerobiosis. Discs embedded in procaine penicillin G were used as a positive control. After 24 hours, inhibition halos were measured with a Vernier caliper. The diameters of these zones were measured in mm, according to the Duraffourd scale, which is used to qualitatively determine the inhibitory effect *in vitro* and has the following indicators:

- Null (-): Diameter less than 8 mm.
- Sensitive (+): Diameter between 8 to 14 mm.
- Very sensitive (++) : Diameter between 14 and 20 mm.
- Highly sensitive (+++) : Diameter greater than 20 mm.

Minimum inhibitory concentration. To determine the minimum inhibitory concentration, 0.8 ml of each concentration of Coconut oil was placed in test tubes

and 0.2 ml of *Streptococcus mutans* culture was added to each tube. Procaine penicillin G was used as a positive control and *Streptococcus mutans* standard suspension was used as a negative control. The tubes were placed in the Gas-Pack Jar at 37 °C for 24 hours. 0.1 ml of each suspension was dispersed in petri dishes with Müeller-Hinton Blood Agar. After 24 hours, CFU counting was performed using the viable bead method by visual inspection. It was considered as ineffective when colony formation was recorded on visual inspection, and effective when colony formation of *Streptococcus mutans* ATCC 25175 growth was not observed.

Statistical analysis. For the statistical analysis, due to the lack of fulfilment of some basic assumptions for the F-test of the analysis of variance to be valid, the non-parametric Kruskal-Wallis test for a one-way completely randomized design was used as an alternative, considering that there is a significant statistical difference if the probability of error is less than 5 % ($p < 0.05$). To complement the analysis and compare pairs of groups, the complementary non-parametric Mann-Whitney test with Bonferroni adjustment was applied with the same significance criterion.

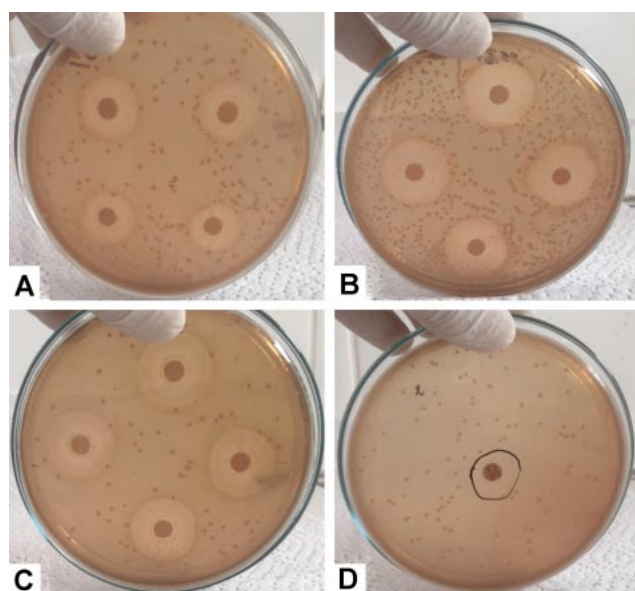


Fig. 1. A. Concentration inhibition halos at 25% concentration. B. Concentration inhibition halos at 50% concentration. C. Concentration inhibition halos at 75%. D. Inhibition halo of the control group (procaine penicillin G).

RESULTS

The Kruskal-Wallis non-parametric test revealed a highly significant difference in the inhibitory action of the three concentrations of Coconut oil ($p < 0.01$).

The 75 % concentration had the highest inhibitory mean at 22 mm, while the positive control (procaine penicillin G) had the lowest inhibitory mean at 14.25 mm (Fig. 1). The Mann-Whitney test with Bonferroni adjustment indicates that the 50 % concentration does not differ in its effect with respect to the 75 % concentration, being considered as "Very sensitive" and "Highly sensitive" for the microorganism according to the Duraffourd scale (Table I). The Mann-Whitney test with Bonferroni adjustment shows that both the positive control and the 75% concentration completely inhibit CFU (Table II), the latter being the minimum inhibitory concentration (Fig. 2).

Table I. *In vitro* antibacterial effect according to the concentration of coconut oil on *Streptococcus mutans* ATCC 25175 through the diameter of the inhibition halo and according to the Duraffourd scale.

Halo diameter indicator (mm)	Study Group: Coconut oil concentration			
	25%	50%	75%	Procaine penicillin G
Media	17.0	21.8	22.0	14.25
Standard Deviation	1.5	0.9	0.7	0.8
Test K-W +	X ² (K-W) = 40.1			
Mann-Whitney Test	A	B	B	C

+ : Non-parametric Kruskal-Wallis test

++ : Non-parametric Mann-Whitney test with Bonferroni adjustment comparing pairs of groups; Two groups with the same letter do not differ statistically.

Duraffourd scale	25%		50%		75%		Procaine penicillin G	
Null	0	0.0	0	0.0	0	0.0	0	0.0
Sensible	0	0.0	0	0.0	0	0.0	2	16.7
Very sensitive	12	100.0	1	8.3	0	0.0	10	83.3
Highly sensitive	0	0.0	11	91.7	12	100.0	0	0.0
TOTAL	12	100.0	12	100.0	12	100.0	12	100.0

Table II. *In vitro* antibacterial effect according to coconut oil concentration on *Streptococcus mutans* ATCC 25175 through the number of colony forming (CFU's) and minimum inhibitory concentration (MIC).

CFU number indicator	Study group: <i>Cocos nucifera</i> oil concentration				
	25%	50%	75%	Procaine penicillin G	
Mean	222.5	16.9	0.0	0.0	
Standard Deviation	82.7	19.1	0.0	0.0	
K-W Test +	$\chi^2(K-W) = 40.3$			p < 0.01	
Mann-Whitney Test ++	A	B	C	C	
+: Non-parametric Kruskal-Wallis test					
++: Non-parametric Mann-Whitney test with Bonferroni adjustment comparing pairs of groups; Two groups with the same letter do not differ statistically.					
Minimum inhibitory	25%	50%	75%	Procaine penicillin G	
Yes+	0	4	12	12	100.0
No	12	8	0	0	0.0
TOTAL	12	12	12	12	100.0

+: When the number of CFU's= 0

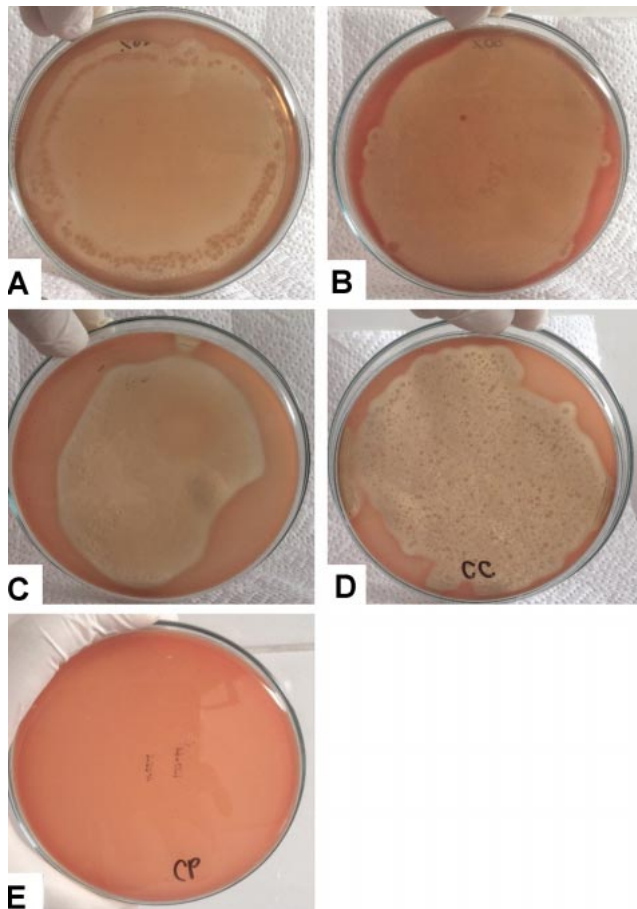


Fig. 2A. Colony-forming units of the 25 % concentration. B. Colony-forming units of the 50 % concentration. C. Colony-forming units of the 75 % concentration. D. Colony-forming units of Penicillin procaine G (Positive control). E. Colony-forming units of Penicillin procaine G (Positive control):

Colony forming units of the 75 % concentration. D. Colony forming units of Penicillin procaine G (Positive control). E. Colony forming units of *Streptococcus mutans* sample (Negative control).

DISCUSSION

It has been reported in the literature that the main component conferring antibacterial properties to coconut oil is monolaurin, a heat-stable monoglyceride produced by the esterification of lauric acid and glycerol. Monolaurin appears to act primarily by destroying a variety of lipid-coated viruses by disrupting their membranes, thus interfering with assembly and maturation (Boateng *et al.*, 2016; Hewlings, 2020). It is also attributed with the ability to permeate cell membranes by rendering the phospholipids of the lipid bilayer more fluid, inhibit membrane enzyme activities and nutrient uptake, and interfere with transduction signalling in bacteria (Khoramnia *et al.*, 2013; Liebermann *et al.*, 2006). Monolaurin also appears to have immunomodulatory effects on proinflammatory cytokine gene expression in fungi (Seleem *et al.*, 2016). Recent studies have reported that oil pulling with coconut oil appears to inhibit plaque similarly to chlorhexidine (as well as generating fewer adverse effects) (Sezgin *et al.*, 2019) and that there are no statistically significant changes in its antibacterial efficacy (Peedikayil *et al.*, 2016). Likewise, some studies have reported that sucrose monolaurate and the glycolipid component present in coconut have anticaries properties, probably due to the condensed glycolysis and oxidation of sucrose in a non-competitive method affecting *Streptococcus mutans* (Rukmini *et al.*, 2017). Coconut, sesame and olive oils together with chlorhexidine gel have also been reported to significantly reduce *Streptococcus mutans* and *Lactobacillus* values (Singla *et al.*, 2014) and Coconut oil has been reported to generate a

statistically significant decrease in plaque and gingival indices (Peedikayil *et al.*, 2015) however, the controversy lies in the fact that participants were previously exposed to mechanical brushing, attributing the reduction in bacterial numbers to the result of the mechanical removal as well as the strength of the rinsing. Faisal evaluated the inhibitory effect on *Streptococcus mutans* strains using commercial FIS brand *Cocus nucifera* oil and revealed an inhibition halo of approximately 15 mm (Faisal, 2015). Alberca Perea and Colca Solano, also using commercial Coconut oil, found no antibacterial effect on *Streptococcus mutans*, but their methanolic extract did have an effect with an average inhibition halo of 13 +/-1 mm (Alberca *et al.*, 2018). Torres found that the mean inhibition haloes resulted for chlorhexidine with a mean of 15.47 mm, *Cocus nucifera* oil at 100 % with a mean of 12.96 mm, at 75 % with a mean of 12.05 mm, and at 50 % with a mean of 11.17 mm. He determined that the microorganism is significantly more sensitive to 0.12 % chlorhexidine than to *Cocus nucifera* oil at all three concentrations (Torres, 2017). In this study, the *in vitro* antibacterial effect of *Cocus nucifera* oil on *Streptococcus mutans* ATCC 25175 was greater than that obtained in previous studies in other countries, which may be attributed to differences in the method of oil extraction, degree of maturity and origin of the fruit and the chemical compounds added to the commercial products. For this reason, studies should be carried out to compare the effect according to these variables. Likewise, inter- and intra-evaluator calibration and validation of the measuring instrument was not carried out, so it is important that this be taken into account in future research. The antibacterial capacity of coconut oil observed in this study may be due to monolaurin, its main component, which is why studies should be carried out to identify its active components and mechanism of action so that it can be included in oral hygiene products, as it is a natural, inexpensive and easily extracted product.

CONCLUSION

All concentrations of *Cocus nucifera* (coconut) oil showed *in vitro* antibacterial effect on *Streptococcus mutans* ATCC 25175 and the effect varied according to the concentration used, with the 75 % concentration producing the largest inhibition halo at 22 mm and representing the lowest inhibitory concentration at 0 CFU.

ACKNOWLEDGEMENTS

To Dr. Elva Mejía Delgado of the Microbiology Laboratory of the Faculty of Medicine of the National University of Trujillo - Peru, for processing the samples.

VÁSQUEZ, V. G. & GUARDIA, M. G. Efecto antibacteriano de aceite de coco (*Cocus nucifera*) sobre *Streptococcus mutans* ATCC 25175: Un estudio *in vitro*. *Int. J. Odontostomat.*, 15(4):922-927, 2021.

RESUMEN: El presente estudio *in vitro* determinó el efecto antibacteriano del aceite de coco sobre *Streptococcus mutans* ATCC 25175. Estudio transversal y experimental. La cepa de *Streptococcus mutans* ATCC 25175 se sembró en Agar Sangre Müeller-Hinton. El efecto se determinó mediante el halo de inhibición y la concentración inhibitoria, utilizando la penicilina G procaína como control positivo y la suspensión estándar de *Streptococcus mutans* como control negativo. Se realizaron doce réplicas por cada concentración de aceite de coco (25 %, 50 % y 75 %). La concentración de aceite de coco al 25 % generó una media inhibitoria de 17 mm y $2,23 \times 10^2$ UFC, al 50 % una media de 21,75 mm y $0,17 \times 10^2$ UFC, al 75 % una media de 22 mm y 0 UFC, la penicilina G procaína una media de 14,25 mm y 0 UFC, el control negativo dio una media de $2,8 \times 10^5$ UFC. La prueba no paramétrica de Kruskal-Wallis detectó una diferencia estadística altamente significativa de las tres concentraciones de aceite de coco ($p < 0,01$). La prueba de Mann-Whitney con ajuste de Bonferroni determinó que las concentraciones del 50 % y del 75 % tenían una acción inhibitoria similar y que tanto la concentración del 75 % como la penicilina G procaína daban una media de 0 UFC. Todas las concentraciones de aceite de coco mostraron una acción inhibitoria sobre *Streptococcus mutans* ATCC 25175. La concentración del 75 % mostró la media inhibitoria más alta y fue la concentración inhibitoria mínima que eliminó completamente las UFC.

PALABRAS CLAVE: aceite de coco, *Streptococcus mutans*, agentes antibacterianos.

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Corresponding author:

Grecia Paola Vásquez Vereau
Cirujano Dentista COP: 44830
Universidad Nacional de Trujillo
Monserrate – Trujillo 13008
PERÚ

E-mail: greciavasquezvereau@hotmail.com