ORIGINAL RESEARCH

Development of New Experimental Dentifrice of Peruvian *Solanum tuberosum* (Tocosh) Fermented by Water Stress: Antibacterial and Cytotoxic Activity

Frank Mayta-Tovalino[1], Gabriela Sedano-Balbin[2], Percy Romero-Tapia[2], Daniel Alvítez-Temoche[3], María Álvarez-Paucar[4], Luis Gálvez-Calla[4], Sonia Sacsaquispe-Contreras[5]

Abstract

Aim: "Tocosh" is a potato that has undergone a process of hydraulic oxidation that enhances its antimicrobial properties so that this natural resource can be used in medical sciences. The aim of this study was to develop and evaluate the antibacterial and cytotoxic activity of a new experimental tooth based on *Solanum tuberosum* "Tocosh" on the cell lines 3T3 and DU145.

Materials and methods: To evaluate the cytotoxicity, cell cultures 3T3 and DU145 were used. Cell viability was determined by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in a medium containing 10% calf serum where the cells were preincubated at a concentration of 1×10^6 cells/mL in culture medium for 3 hours at 37°C and 6.5% CO_2. Then, the absorbance was measured using a microplate reader where the formazan crystals were diluted with acidic and cold isopropanol, and quantified in an ELISA reader. To evaluate the antibacterial effect, the Kirby Bauer inhibition halos method was used on strains of *S. aureus* (ATCC 25923), *S. mutans* (ATCC 25175), and *S. mitis* (ATCC 49456).

Results: *Solanum tuberosum* (tocosh) was not cytotoxic because it only had one CC_{50} at the concentration of 0.26927 mg/mL and 0.26845 mg/mL for the cell lines 3T3 and DU145, respectively. Tocosh toothpaste (TD) has an antibacterial effect against *S. aureus* and *S. mutans*.

Conclusion: The new ecological dentifrice was not cytotoxic since it did not alter cell viability because its CC_{50} was only 0.268 and 0.269 µg/mL for the 3T3 and DU145 cell lines, respectively; however, it presented an optimal antimicrobial activity against the oral strains evaluated.

Clinical significance: This research has great potential for clinical use because this new dentifrice has antimicrobial activity against different oral germs.

Keywords: Antibacterial, Cytotoxic, Dentifrice, *Solanum tuberosum*, Tocosh.

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Introduction

Currently, there is a great biodiversity of plants that are considered as one of the most important environmental factors to control pathogenic microorganisms.[1] For instance, there are numerous studies of microbial characterization that have been carried out in association with the use of native plants;[2–5] however, only a few have been successful in the development of commercial antimicrobial products for the biological control of oral bacterial flora. The main factors that contribute to these interactions are not always well understood by most species of plants and bacteria, and it is believed that not only some functions are necessary to prevent the colonization of certain microorganisms but also specific characteristics of the side are needed of the plant, such as its genotype to contribute to the development of active ingredients.[6]

The search for the health of diseases and ailments has accompanied humanity since its inception, which is why plants have played a vital role in this activity. Modern medicine has emerged from traditional medicine and has been enriched by the discovery of new formulas through the active principles of these. Currently, the world trend for the treatment of diseases is to resume the help that medicinal plants can provide, since the exaggerated use of chemical drugs is producing side effects in humans.[7–13] There is some literature that has investigated the "tocosh" since it is a food considered as a gift from the Apus (Gods). The potato was considered with a connotation of divinity. One of the most popular desserts in the Andes of Peru is the consumption of "tocosh" that grew in the country during the colonial era.[14]

[1]Postgraduate Department, School of Stomatology, Universidad Científica del Sur, Peru

[2]Academic Department of Stomatology Medical Surgical, Faculty of Dentistry, Universidad Nacional Mayor de San Marcos, Lima, Peru

[3]Academic Department of Stomatology, Faculty of Dentistry, Universidad Nacional Federico Villarreal, Lima, Peru

[4]Department of Basic Sciences, Faculty of Dentistry, Universidad Nacional Mayor de San Marcos, Lima, Peru

[5]Academic Department of Medicine and Buccomaxillofacial Surgery, Faculty of Stomatology, Universidad Peruana Cayetano Heredia, Lima, Peru

Corresponding Author: Frank Mayta-Tovalino, Postgraduate Department, School of Stomatology, Universidad Científica del Sur, Peru, Phone: +51 1 214-2500, e-mail: fmaytat@ucientifica.edu.pe

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This natural resource "tocosh" is subjected to the oxidative stress of the river of the Peruvian Andes. Today in the heights of the Andes, ancient influences persist in rural peasants and continue to harvest the crops of their ancestors.[15] Therefore, it is important to reflect on the contribution to knowledge of the ancient Peruvians

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who, without having a modern technology until now, influence the creation of new knowledge. Unfortunately, these crops have been overlooked and are little studied at a biological and biochemical level, which leaves a world open to research in this area of phytotherapy.[16] Peruvian Andean tubers are a fascinating reservoir of biodiversity and germplasm that have a great potential as new species of introduced crops or source of genes for phytoproduction. This potato subjected to water stress tends to increase the activated forms of oxygen and the accumulation of free radicals associated with damage to the cell membranes of microorganisms, which leads us to believe that it could have a possible clinical application in dentistry.[17,18]

Therefore, the purpose was to develop a new experimental toothpaste of Peruvian *Solanum tuberosum* (tocosh) fermented by water stress and to evaluate its antibacterial and cytotoxic activity.

Materials and Methods

To determine the antibacterial effect, the sample size was determined with the formula for estimating means with Stata® software version 12.0, with an alpha of 0.05 and a power of 0.8 with the standard deviations previously obtained in a pilot study, by what was considered a $n = 14$ Whatman paper disks embedded with the extract of *Solanum tuberosum* "tocosh" for each group evaluated and formed the following groups:

- Group I: Petri dish with *S. mutans* (ATCC 25175) with tocosh ethanolic extract (TEE)
- Group II: Petri dish with *S. mitis* (ATCC 49456) with TEE
- Group III: Petri dish with *S. aureus* (ATCC 25923) with TEE
- Group IV: Petri dish with *C. albicans* (ATCC 10231) with TEE.

"Tocosh" Preparation

Solanum tuberosum was submerged in the river of the Palcamayo district which is a city located at coordinates –11.294146°, –75.779448° in the province of Tarma—Peru, where a pool was dug in the river and in it potatoes were chosen the same areas that were covered in a net of "ichu" (Andean straw); once introduced into the pools, it was pressed with many stones and the river water was allowed to run for 6–9 months. The water that runs through these wells will begin to ferment the potato. Then he removed the potatoes that were exposed to the sun for the respective drying.

Preparation of the TEE

We use 100 g of freeze-dried pulverized tocosh after it has been dried at room temperature. Subsequently, this powder was mixed with 500 mL of ethanol (Merck®) to leave it to macerate at room temperature. On the fourth day, it was filtered for the first time and the solution was evaporated by means of a rotavapor at 46°C for 1 hour; later the solvent was added to the surplus solids. Finally, on the seventh day after the extract was isolated from sunlight (covered with platinum paper), it was filtered on Whatman paper no. 4 and the residual solid was discarded thus obtaining the crude extract.

In Vitro Antimicrobial Susceptibility Testing of *Solanum tuberosum*

First, we proceeded to verify the viability of the strains through a Gram stain, then prepared broths of Mueller Hinton Broth (MHB) to perform the inoculation of *S. aureus* and *C. albicans* and thioglycollate media for inoculation of *S. mutans* and *S. mitis*. Subsequently 24 hours after the growth of the microorganisms in the respective broths, the strains of *S. mutans* and *Streptococcus mitis* were seeded in brain heart agar (BHA) plus sterile lamb blood (5%) while the *S. aureus* and *C. albicans* were inoculated in Mueller Hinton agar (MHA), all strains were seeded with sterile dacron swabs, and then the sterile 6-mm diameter Whatman paper disks were impregnated with 20 µL of the TTE which was the experimental substance evaluated. The inoculated plates were incubated at 37°C for 24 hours. The zone of inhibition produced by different concentrations was measured by the Kirby–Bahuer method, measuring the inhibition zones in millimeter with a Vernier caliper.

Preparation of "Tocosh" Dentifrice (TD)

Carbopol 940 was used and it was mixed in 50 mL of distilled or deionized water with continuous agitation with the help of a mechanical stirrer. Next, distilled water dissolved in sodium benzoate was required for heating in a water bath. Then, the solution was cooled, the glycerin was added, and mixed with the first solution. The required amount of sodium saccharin and sodium lauryl sulfate was taken to adequately dissolve with water and then mixed again with the first solution. Finally, the TEE was added and the previous mixture was mixed and the volume was added by adding the distilled water to obtain the paste in the required consistency.

Ingredients

Powdered tocosh, Carbopol 940, sodium lauryl sulfate, glycerin, saccharin sodium, methylparaben, menthol, distilled water, and sodium benzoate.

Antibacterial Activity of TD

First, we proceeded to verify the viability of the strains through a Gram stain, and then we prepared MHB broths to perform the inoculation of *S. aureus* and thioglycollate media for the inoculation of *S. mutants*. Subsequently, the nutrient agar diffusion method was used for bacterial cultures where the culture medium was inoculated with the microorganisms, suspended in corresponding broth. Then the 6-mm diameter perforations were made in the nutritive agar that were filled with TD, control toothpaste, and distilled water. Finally, the antimicrobial activity was analyzed through the measurement of the diameter of the inhibition halo through the haloes produced by toothpastes.

Cytotoxicity (MTT Test)

The cell culture was performed with trypsin and the cell count was adjusted to 3 lac cells/mL using a medium containing 10% newborn calf serum. The cells were preincubated at a concentration of 1×10^6 cells/mL in culture medium for 3 hours at 37°C and 6.5% CO_2. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µL culture medium and incubated at 37°C at 5% CO_2 for 24 hours. After 24 hours, when the monolayer was formed, the supernatant was removed and previously added diluted with 100 µL of different concentrations of the extract in the microtiter plates and kept in incubation at 37°C at 5% CO_2 for 72 hours where the cells were checked periodically to monitor granularity, contraction, and swelling. After these 72 hours, 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye was added to each well. Plates were shaken gently and incubated for 4 hours at 37°C in 5% CO_2. The supernatant was removed and 100 µL of isopropanol was added and the plates were gently shaken to solubilize the formazan form. The absorbance was measured using a microplate reader where the formazan crystals are diluted with acidic and cold

isopropanol, and the quantifications will be carried out in an ELISA reader (microplate reader benchmark of Bio-Rad) at 590 nm with a reference filter of 620 nm.

Ethics Statement

The authorization of the Ethics Committee of the Universidad Peruana Cayetano Heredia (SIDISI Code 64348) was obtained for the execution of the research project.

Statistical Analysis

For the univariate analysis of the variables antibacterial effect and cytotoxic effect, we proceeded to obtain the descriptive statistics (mean and standard deviation) of the quantitative variables. In addition, the normal distribution was determined by the Shapiro–Wilk test. Therefore, for the bivariate analysis, the Student *t* test and the ANOVA test were used. A level of significance of $p < 0.05$ was established.

Results

When comparing the antimicrobial activity of TEE against the strains of *S. aureus*, the TEE had 19.6 ± 2.2 mm while the chlorhexidine had 20.2 ± 0.8 mm, so no statistically significant differences were found ($p = 0.728$). On the contrary, when comparing the antimicrobial activity of TEE against the strains of *S. mutans*, the TEE had 13.7 ± 2.8 mm while the chlorhexidine had 18.0 ± 0.8 mm found statistically significant differences ($p \leq 0.001$). Finally, when comparing the antimicrobial activity of TEE against the strains of *S. mitis*, TEE had 16.5 ± 1.3 mm while the chlorhexidine had 18.2 ± 0.6 mm, so no statistically significant differences were found ($p = 0.988$). However, no antimicrobial effect was found against the strains of *Candida albicans* (Fig. 1 and Table 1).

The cytotoxicity of TEE was determined using the cell line 3T3 and DU145. To evaluate the cytotoxicity, increasing concentrations of the growing TEE (from 0 to 1,000 µg/mL) were used and the cell viability was determined by the MTT method, which measures the mitochondrial activity of the cell. The relative viability of the cells incubated with the extract was calculated taking as reference the untreated cultures (without extract, control, 0 µg/mL). The results indicate that TEE showed a 50% harmless effect (CC_{50}) at the concentration of 0.26927 mg/mL and 0.26845 mg/mL for the 3T3 and DU145 cell lines, respectively (Fig. 2). These values were confirmed by microscopy, observing decrease in the number of cells and cytopathic effect. This study shows that TEE can contain active compounds to conserve cell morphology, not being cytotoxic since it did not alter cell viability (Table 2).

When evaluating the antimicrobial activity of TD, it was found that for *S. aureus* it had an average of 14.7 ± 0.3 mm; however, the control dentifrice (CD) had 14.4 ± 0.7, finding no statistically significant differences ($p = 0.997$). For the *S. mutans*, the TD had 16.2 ± 0.5 mm whereas the CD only had 14.4 ± 0.5 mm being statistically significant ($p = 0.003$). Finally, for *S. mitis*, the TD had 14.4 ± 0.8 mm and the CD also had a similar result of 14.4 ± 0.2, there being no statistically significant differences ($p = 0.896$) (Table 3).

Discussion

In recent decades, there is a great increase in using herbal medicine as a natural therapy because it uses medicinal plants and their derivatives for therapeutic purposes, either to prevent or cure certain diseases. Therefore, since the tocosh is a natural resource native to the country, it is necessary to deepen the knowledge about its possible therapeutic uses. For this reason, this study aimed to evaluate *in vitro* the antimicrobial activity of TD on strains of the oral cavity. To evaluate the antimicrobial activity of the extracts on bacteria, two methods are generally used: the agar well method and the inhibition halos method. For both methods, sowing of previously inoculated bacteria is used in culture medium, although there is some research that shows that both methods have their own qualities.[19] For this reason, in this study the Kirby–Bauer method was used to determine the antimicrobial effect of TEE since it can be done through disks and was used to determine the sensitivity of a microbial agent to a substance with antimicrobial properties.

In this investigation, it was observed that the TEE had antibacterial activity on strains of *S. mutans*, *S. mitis*, and *S. aureus* of 13.7 ± 2.8 mm, 16.5 ± 1.3 mm, and 19.6 ± 2.2 mm, respectively. However, it had no inhibitory effect against the *C. albicans* strains; when looking for available scientific evidence, it was found that there are no studies that evaluate the antibacterial activity of this natural resource of Peru against oral bacteria, but there are some studies[6,12,13] that have evaluated *Solanum tuberosum* against others microorganisms. For example, in the investigation of Bontempo et al.[20] mentioned that

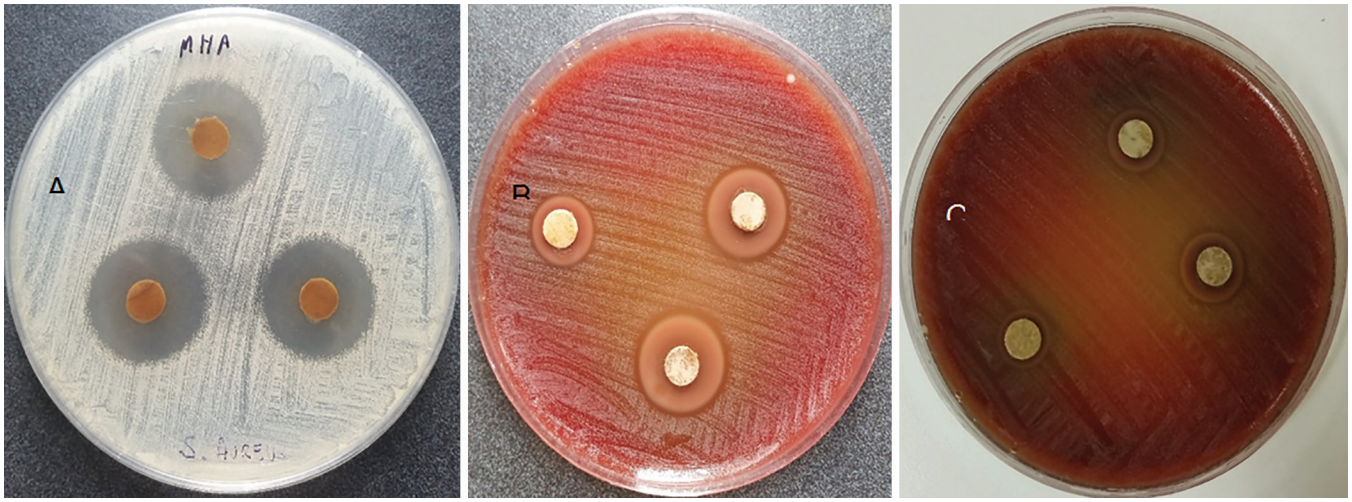


Fig. 1: Comparison of *in vitro* antimicrobial activity of TEE. Group I: *S. aureus* (ATCC 25923); group II: *S. mitis* (ATCC 49456); and group III: *S. mutans* (ATCC 25175)

Table 1: Comparison of the antimicrobial activity of tocosh ethanolic extract against the evaluated strains

Microorganisms	*TEE*	*Min*	*Max*	*CHx*	*DW*	*p**
Staphylococcus aureus	19.6 ± 2.2	16.8	22.3	20.2 ± 0.8	0	0.728
Streptococcus mutans	13.7 ± 2.8	10.8	18.3	18.0 ± 0.8	0	<0.001
Streptococcus mitis	16.5 ± 1.3	14.9	18.0	18.2 ± 0.6	0	0.988
Candida albicans	–	–	–			
*p***	0.000		0.001		–	

All values are recorded in mm. The concentrations were calculated from the dilutions of the active ingredient; the DW control group was excluded from any statistical analysis because the antimicrobial activity was not present. The strains of *Candida albicans* were excluded from the statistical analysis because it was not affected by the TEE
*Student *t* test
**ANOVA test
Level of significance ($p < 0.05$)
TEE, tocosh ethanolic extract; CHx, chlorhexidine; DW, distilled water

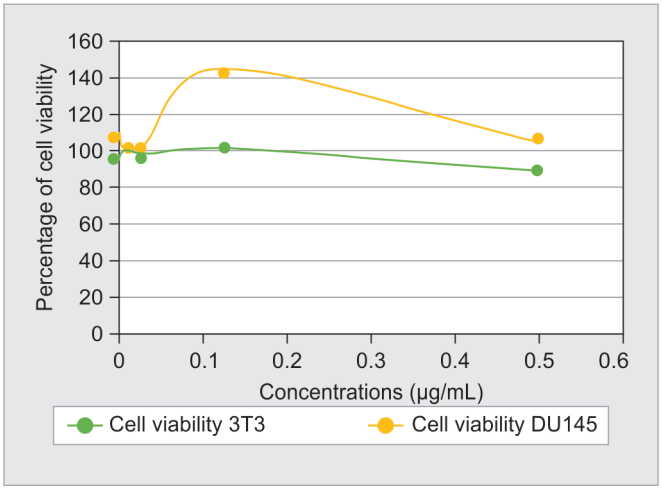


Fig. 2: Cell viability assay of ET3 and DU145 cell lines submitted to the tocosh ethanolic extract

Table 2: Evaluation of cell viability (cytotoxicity) of 3T3 and DU 145 cells against tocosh ethanolic extract

Cell line	*TEE (µg/mL)*	*Absorbance*	*Cell viability (%)*	*CC_{50}*
3T3	0.5	0.356	89	0.26957
	0.125	0.342	101.5	
	0.0312	0.32	98.2	
	0.0078	0.316	100.9	
	0.0019	0.291	99.8	
	0.0004	0.296	98	
DU145	0.5	0.128	104.6	0.26845
	0.125	0.133	145.1	
	0.0312	0.141	103.7	
	0.0078	0.147	101.3	
	0.0019	0.135	104	
	0.0004	0.123	110	

TEE, tocosh ethanolic extract

the anthocyanin extract of *S. tuberosum* var. Vitelotte showed high antibacterial activity against nine standardized bacterial strains and clinically isolated bacterial strains; Gram(+) bacteria being the most sensitive to the action of the extract; *S. aureus* from both

Table 3: Evaluation of the antimicrobial activity of tocosh ethanolic extract against oral strains

Microorganisms	*TD*	*CD*	*p**
Staphylococcus aureus	14.7 ± 0.3	14.4 ± 0.7	0.997
Streptococcus mutans	16.2 ± 0.5	14.4 ± 0.5	0.003
Streptococcus mitis	14.4 ± 0.8	14.4 ± 0.2	0.896
*p***	<0.001	0.484	

All values are recorded in mm. *C. albicans* was excluded from any analysis because there was no antifungal effect against this fungus
*Student's *t* test
**ANOVA test
Level of significance, ($p < 0.05$)
TD, tocosh dentifrice; CD, control dentifrice

standard and clinical sources showed a very high sensitivity; this is a significant fact considering that until now *S. aureus* infections are complicated due to their intrinsic resistance to multiple classes of antibiotics. However, these results disagree with what was found with Ombra et al.[21] whom showed that anthocyanins from the raw and cooked extract of *Solanum tuberosum* showed no differences in antimicrobial activity of both against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. The antimicrobial activity was almost similar for the three strains, ranging from 0.53 cm with 50 mg of anthocyanins from crude extract against *B. cereus* to 0.82 cm with 100 mg of anthocyanins from the cooked extract against *E. coli*, demonstrating a more time than the cooking of the potato does not negatively and strongly affect said activity. This is in contrast to other research[22] that ensures a strong antimicrobial activity of both. This coincides with the results obtained by Hasan et al.[23] who purified a new lectin from a potato culture of Bangladesh Deshi (*Solanum tuberosum* L.). Said lectin showed to have bactericidal properties and activities of growth inhibition against Gram(+) bacteria listeria monocytogenes and Gram(–) *Escherichia coli*, *Salmonella enteritidis*, and *Shigella boydii*. This coincides with the results of the present study, where we also found that *S. aureus* was one of the microorganisms that had the highest halo of inhibition against the TEE, also inhibiting *S. mutas* and *S. mitis*, which represents an excellent natural alternative to the chemical products that will be used as biocides and preservatives of food and human purposes.

This activity is probably due to some active principles as explained by Feng et al.[12] in their study where they mentioned that they have this natural resource. They successfully purified an antimicrobial protein (AP_1) from potato germplasm, on the basis of

its antimicrobial activity *in vitro*, but the function of this protein is still not clear, so they suggest that AP_1 belongs to a new group of proteins with characteristics of an antimicrobial. This protein is a homologue of acid phosphatase that, interestingly, is mainly related to nucleotide binding proteins.[24]

As previously mentioned, this antibacterial effect that tocosh has against the oral germs evaluated in this study represents a great potential that coincides with the popular customs of Peru, possibly the theory of the antimicrobial effect is due to the fact that this natural resource is submerged to the oxidative process of the river of the Andes is mainly colonized by fungi, surprisingly it is evident that the fungus *Penicillium notatum* among others produces "something" capable of killing oral bacteria which coincides with that described by Fleming who called this active principle *Penicillin notatum*. Which were described since 1929, although it did not arouse the interest of the scientific community in that decade. For this reason, this same possible explanation is the reason why the tocosh evaluated in this investigation had no antifungal effect against *C. albicans* because the idea that inhibits itself would be incompatible. On the contrary, it is essential to recognize that tocosh had the same antibacterial effectiveness against chlorhexidine against *S. aureus* and *S. mitis* but had less effect against *S. mutans*, which indicates the great potential it would have in future research.

In relation to the antifungal effect, an investigation[23] also showed antifungal activity against *Rhizopus* spp., *Penicillium* spp., and *Aspergillus niger* 24 hours after administration of the lectin from *Solanum tuberosum*; therefore, it could have possible applications in the clinic and in the biomedical sciences. Similarly, in the study carried out by Bontempo et al.,[20] they found that the extract of *S. tuberosum* var. Vitelotte had an antifungal effect against three strains of fungi and yeasts *C. albicans*, *B. cinerea*, and *R. solani*, although it was particularly active against *R. solani*.[12,20] However, in the present study, when evaluating the antifungal effect, no activity was found against *C. albicans*, this is probably due to the fact that the mechanism of action of penicillins is not completely known. In this way, penicillin acts by weakening the bacterial wall and favoring the osmotic lysis of the bacteria during the multiplication process, and it has been shown that some species of penicillium produce in crops; in this case coming from the fermentation of the potato tocosh; a powerful antibacterial substance that affects different bacteria in different degrees; generally, it can be said that the least sensitive are the Gram(–) negative bacteria and the most susceptible are the pyogenic cocci. Therefore, penicillin is not inhibitory to the original penicillium used in its preparation.[25]

In this study, the MTT test was used, because it is a colorimetric test that quantitatively and objectively evaluates the cytotoxicity of a substance. This investigation was used as an indicator of cytotoxicity (CC_{50}), which is an established value to determine the concentration of cytotoxicity at 50%, which is equivalent to saying the concentration of a substance to kill half of the cell lines evaluated. TEE was not cytotoxic against the 3T3 and DU145 cell lines, because its CC_{50} was 0.269 and 0.268 µg/mL, respectively. However, when looking for the literature on the cytotoxic effect of tocosh, no information was found. Although in a study conducted by Patel et al., they mentioned that *Solanum nigrum* had a strong cytotoxic activity on the HELA cell line and little activity on the Vero cell line which means that *Solanum nigrum* can be used as a possible cancer treatment. Similarly, in the study of Kim et al.[13] who measured the hemolytic activities of the peptides in the presence of heparin collected from healthy donors and found that *Solanum tuberosum* had no cytotoxic, hemolytic effect at concentrations up to 40 µM. These results are similar to those described by Munari et al.[26] who observed that the cytotoxic activity of *Solanum lycocarpum* in concentrations above 32 µg/mL did not show a genotoxic effect in chromosomal assays in V79 cells.

When comparing the antimicrobial effect of toothpastes elaborated with natural products, the study of Parthasarath et al. was found, who mentioned that the inhibitory effects of toothpastes of three plants, neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), and cinnamon (*Cinnamomum zeylanicum*) showed antimicrobial activity against *S. auricularis*, *A. lwoffii*, and *C. albicans* but not against *Micrococcus*. These results agree with that made by Jadge et al.[27] who also found that toothpaste elaborated from neem (*Azadirachta indica*) also showed antimicrobial effectiveness. These data are similar to those of the present study since TD from Peru also showed an antibacterial effect against oral strains of *S. aureus* and *S. mutans*. Therefore, the impact of this natural resource is very important in the Peruvian flora because there are few studies that evaluate the cytotoxic and antimicrobial activity of these native natural resources.[28–30]

Finally, there is currently a growing demand to test natural products in recent decades. This has resulted in the evaluation of a variety of natural products that belong to a family of polyphenols, alkaloids, and glycosides, etc. For instance, in some investigations the antiproliferative activity on squamous cell carcinoma of the oral cancer cell line was evaluated *in vitro*, and this was also tested by the same trial of the present investigation: MTT 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide only that it was on other cellular lines such as SCC-9 cells and oral cancer cell line KB 3-1.[31,32]

The main limitation of this research was the scarce scientific literature on the "Tocosh", which makes it difficult to contrast the results obtained with respect to other authors. Based on the results obtained in this research, we propose the hypothesis that the process of fermentation, oxidation, and time that *Solanum tuberosum* was immersed in the water of the Peruvian river, influencing its physical and biological properties of this natural resource. For this reason, it is suggested to carry out studies that complement the evidence on the active principle that is generating the antibacterial effect and in this way give it the clinical importance in dentistry. However, we recommend that long-term follow-up studies be carried out to verify the cytotoxicity of this natural resource.

On the contrary, when searching for information, no scientific evidence was found that studies the antimicrobial and cytotoxic activity of tocosh on oral microorganisms. Therefore, the present investigation opened a great line of research, since when determining the antibacterial effect of this natural resource of Peru against strains of *S. mutans*, *S. mitis*, and *S. aureus*, companies could create dental products based on tocosh. Probably, these would have a great acceptance on the part of the population when knowing that the main base is the tocosh that is consumed traditionally in diverse parts of the national territory. Finally, although *Solanum tuberosum* has carbohydrates, these are not fermentable by oral acidic bacteria; therefore, the risk of generating tooth decay is almost impossible. However, future studies could be carried out that add the fluorine ion to this formulation to help remineralize tooth enamel surfaces.

Conclusion

TEE has antimicrobial activity against strains of *S. mutans*, *S. mitis*, and *S. aureus*; however, there was no inhibition against *C. albicans*.

Besides TEE was not cytotoxic, since its CC_{50} was only 0.268 and 0.269 µg/mL for the 3T3 and DU145 cell lines, respectively. Finally, TD had an antibacterial effect against *S. aureus* (ATCC 25923), *S. mutans* (ATCC 25175), and *S. mitis* (ATCC 49456). It is shown that this natural resource has a potential therapeutic use in dentistry opening new lines of research in relation to this topic.

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