

Actividad bactericida de *Castela texana* sobre bacterias relacionadas a caries dental y gingivitis

*Bactericidal activity of *Castela texana* on bacteria related to dental caries and gingivitis*

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RESUMEN

Las caries dentales afectan a cualquier persona y son la causa más importante de pérdida de los dientes. Las bacterias son esenciales para el desarrollo de una lesión cariosa, en tanto que la gingivitis es una forma de enfermedad periodontal que involucra inflamación e infección que destruyen los tejidos de soporte de los dientes, esta patología se debe a la acumulación de un material adherente compuesto de bacterias, moco y residuos de alimentos que se desarrolla en las áreas expuestas del diente. Por otra parte, *Castela texana* es capaz de suprimir el crecimiento de varios microorganismos patógenos que afectan la salud del hombre y que se ha demostrado que al ingerir el extracto acuoso, este no presenta efectos secundarios nocivos al hombre. De acuerdo a los resultados obtenidos la mejor opción de estudio para bacterias relacionadas a gingivitis es el extracto metanólico de corteza y para bacterias relacionadas con caries dental es el extracto metanólico de tallo, los cuales presentan respectivamente la mayor actividad bactericida y el menor potencial tóxico sobre *Artemia salina*.

Palabras clave: caries, gingivitis, *Castela texana*, *Artemia salina*.

Abstract

Dental caries affect anyone and are the leading cause of tooth loss. The bacteria are essential for the development of a carious lesion, gingivitis is a form of periodontal disease involving inflammation and infection that destroy the tooth supporting tissues, this pathology is due to the accumulation of adherent material composed of bacteria, mucus, and food waste that develops on exposed areas of the tooth. On the other hand, *Castela texana* has the ability to suppress the growth of several pathogenic micro-organisms that affect the health of man and that it has been shown that ingesting the aqueous extract, this is free of harmful side effects to man. According to the results the best choice of study for bacteria related to the bark methanol extract gingivitis and bacteria associated with dental caries is stem methanol extract, which are respectively the most bactericidal activity and the lower toxic potential of *Artemia salina*.

Key words: caries, gingivitis, *Castela texana*, *Artemia salina*.

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Introduction

Caries

According to the World Health Organization the higher prevalence oral diseases are dental caries and periodontal disease (NOM-013-SSA2-1994).

Tooth decay is one of the most common disorders, after the common cold. It usually appears in children and young adults, but it can affect anyone, and is the most important cause of loss of teeth in younger people.

The bacteria are usually present in the mouth and make all foods, especially sugars and starches, acids. Bacteria, acid, food residues and saliva combine in the mouth to form a sticky substance called plaque, which adheres to the teeth and which is more prominent in posterior molars, just above the gum line. This is all teeth and at the edges of fillings. If plaque that is not removed from the teeth mineralizes into Tartar, it irritates the gums, causes gingivitis and periodontitis.

The bacteria are essential for the development of tooth decay. The main pathogenic microorganism in all types of dental caries is the *Streptococcus mutans*, which has several important properties such as: synthesizes insoluble polysaccharides of sucrose, is a trainer homofermentante of lactic acid, colonizes on the surface of the teeth, it is more acidurico than other streptococci. This does not mean that it is the sole trainer of polysaccharides as non-cariogenic has also been found in strains. Other microorganisms associated with tooth decay is *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mitis*, *Actinomyces viscosus* and *Lactobacillus acidophilus*.

The word caries comes from latin and means rot. It is perhaps the most common human disease. Tooth decay is a disease that, in every age and particularly in the world, shows an enormous magnitude over all possible alterations of oral health (Ministry of Public Health, 1995).

Tooth decay is a disease considered as the most common in humans due to its high prevalence, it is one of the most important etiological factors of pulp and periapical inflammation. This can be defined as a progressive and localized degeneration of the teeth, which is initiated by surface demineralization by organic acids such as lactic, produced by microorganisms of the plate (Seltzer S. y Belder I., 1987).

Among the risk factors for the disease include: high degree of *Streptococcus mutans* infection, poor resistance of enamel, dental crowding, previous experience of caries, poor oral hygiene, intake of sugary foods, among others (Rodriguez , 1997). Diets high in refined products such as sugar, cakes and highly processed foods facilitate caries (Hernández, G. 1998). The decay is

produced by some bacteria that produce acids, especially the *Streptococcus mutans*. Their food source constitute fermentable carbohydrates such as sucrose (Newbrown, 1972).

The incidence of dental caries has experienced very rapid growth due to the ongoing transformation of lifestyle and food; changing agricultural activities and, therefore, the use of grain as a basic principle of food, along with cooking and processes food processing, they have helped to increase the incidence of this disease, which has reached alarming levels, according to statistics, represent 75 to 85% (Hernandez, 1998). Little information is available on changes in rates of decay presents the Mexican population; Similarly, there are few data on the oral hygiene habits of this population (Salas L. and J. Rivas Gutiérrez, 2001). However, according to the international classification of the World Health Organization (WHO), Mexico is among the countries with a high frequency range of oral diseases (NOM-013-SSA2-1994).

The oral cavity is the first segment of the digestive tract that connects the outside world with the esophagus. The different conditions that can prevail in this cavity, along with the constant changes in lifestyle and the man's age, form an ecosystem exposed to constant changes and a variety of microbiological problems because of its open and dynamic nature (Prieto J. and A. Calvo 2004).

The occurrence of dental caries is related to oral bacteria, especially as the cariogenic *Streptococcus mutans*. Antibacterial preliminary studies revealed that extracts of nutmeg, plant species widely cultivated for its aroma and flavor, have strong inhibitory activity against *S. mutans* (Chung J.Y. et al., 2006).

Palombo E. en 2011 reported a large number of plant extracts with antimicrobial activity against bacteria that affect the oral cavity and Eguizábal et al., (2001) reported the antibacterial activity of the ethanol extract of Peruvian propolis solution 0.8% finding that has better antibacterial action *Streptococcus mutans* and *Lactobacillus casei*, chlorhexidine 0.12%.

Gingivitis

Gingivitis is the inflammation or degeneration painless the gum tissue. In this, the tissue between the teeth becomes swollen and uneven; the tissue at the gum line (the point where the

tooth meets the gums) becomes darker, and the gum bleeds easily (Ferri, 2005). Gingivitis is due to the long-term effects of plaque deposits a sticky material made of bacteria, mucus, and food debris that develops on the exposed areas of the tooth. Plaque is the major cause of dental caries and, if not removed, it becomes a hard deposit called tartar that becomes trapped at the base of the tooth. Plaque and tartar irritate and inflame the gums.

Bacteria and the toxins they produce cause the gums to become infected, swollen, and tender. An injury to the gums from any cause, including brushing and flossing too hard, can cause gingivitis. It is mainly associated with a defective or incorrect oral hygiene, which facilitates the formation of the so-called dental plaque, which by the accumulation of food debris, dead skin cells and mucin forms. This plaque provides an ideal for the development of numerous microorganisms responsible for causing gingivitis and caries means.

The bacteria in plaque are key players in the pathogenesis of periodontal disease (Nombelli, 1994). In numerous research it suggests that gum bleeding can be reduced or eliminated by careful control of the plaque (Catón, 1998).

Studies in dark field microscope and phase, noted the superiority of coconuts and immobile forms in healthy gingival sites in patients mobile and spirochetes, medium and long forms (Santamarina, 1988) predominate.

Loe in 1964 showed that by eliminating oral hygiene, gingivitis patients developed which increased in severity with each passing day in this study. Loe bacterial flora changed by a more aggressive anaerobic Gram negative type and hygiene restore gingival health recovered. This demonstrated the reversibility of gingivitis (http://www.radiodent.cl/periodoncia/clasificacion_y_caracteristicas_de_gingivitis_y_%20periodontitis.pdf, accessed September-2013).

Immunosuppression is considered as the predominant factor in the etiology of ulcerative gingivitis (NUG). A host response altered by systemic predisposing factors allows an increase in bacterial growth and tissue invasion (Y. Murayama et al., 2000).

But there are side predisposing factors necessary for the manifestation of the disease, such as emotional stress, anxiety, malnutrition, systemic diseases (endocrine, blood, venereal diseases,

HIV), diseases convalescence, metal poisoning, smoking, alcoholism, sleep disorders, tissue trauma , poor oral hygiene with high levels of plaque, gingivitis and periodontitis history (Rowland, 1999).

Gingivitis in its early is easy to control, just correct brushing technique and the use of mouthwash with chlorhexidine as an adjunct to improve hygiene and destroying bacteria. Proper and regular use of calendula in treating gingivitis is effective because it lessens the signs of disease, such as bleeding, swelling and discoloration of the gums (MA Machado et al., 2010).

Current treatment includes topical application of povidone-iodine solution, combined with meticulous local treatments, root and removal of material debridement followed by mouthwash with chlorhexidine (Lucht, E. et al., 1998).

Besides treatment, including scaling and root, perhaps drugs are used, but these can not always replace surgery. In some cases, depending on the severity of gum disease, the trained professional may recommend a surgical treatment. It will require long-term studies to determine whether the use of drugs reduces the need for surgery and if these are effective for extended periods.

Phytotherapy

Nature is a great source of wealth, plants represent a potential source and the exploitation of new antimicrobial agents (Haslam et al ., 1989). Medicinal plants are considered a potential source of new chemotherapeutic drugs because of its phytochemical content and little or no toxic effect (Beg, 2000).

Plant products have numerous pharmacological properties also including other properties as antimicrobial, antimutagenic, antiviral, antifungal; inter alia., are also used in the treatment of boils, acne, gingivitis, vaginal candidiasis and to prevent the formation of dental plaque and the ability to promote wound healing (Dunsmore, KE 2001 and Chen, X . 2003).

It is common to use plant parts in order to obtain various therapeutic effects and have been supported by scientific studies. Among the many therapeutic applications of plant includes the antibacterial action. The findings of the study of plants with therapeutic potential can serve as

a tool for medical and social support for a larger sample, mainly the lacking, the pharmaceutical industry also being interested in the knowledge of this area (De Paula J. y Martínez A. 2000).

Castela texana

Texana Castela, commonly known as "bitter little guy" has been used in Mexico for many years to treat amoebic dysentery type. It is used for lack of appetite, fever, amebiasis and as a decongestant. Bitter squat can be used for a long time without causing harmful side effects, or gastric or intestinal irritation and also is used primarily to treat diarrheal amebiasis caused by protozoan parasite *Entamoeba histolytica*.

Texana Castela (bitter squat) is a woody, branchy, thorny shrub with gray bark and bitter; ovate or elliptical leaves alternate; lonely, red or purple flowers and globose fruits; It belongs to the family of Simarubáceas. Flowers from August to September and fruiting from September to October.

It is distributed from Texas, United States to Oaxaca in Mexico and northern South America. In Mexico it is in the states of Chihuahua, Coahuila, Durango, Nuevo Leon, Oaxaca, Puebla, San Luis Potosi and Tamaulipas. In South America, it has been found north of Colombia and Venezuela in adjacent areas.

The therapeutic action of *Castela texana* is well established, as Maximino Martinez "has a remarkable action against amoebic dysentery, especially in chronic cases, amoebas are immobilized and die shortly thereafter. It is 25 times less toxic than emetine (as Gretchen M. Sprecher, University of Nebraska) and used in the treatment against amoebae. Medical and pharmacological studies in Mexico between 1910 and 1920, found on this floor three glycosides: Castilian, castelagemina and castelamarina, which have proved effective as a potent specific antidiarrheal action against amoebas, with the advantage of being 25 times less toxic than chemical and drug irritant effects present (<http://adrianguzman.com.mx/index.php?option>, accesado nov-2013).

In 1847, when US troops under General Zachary Taylor had invaded the country through Texas and into the state of Tamaulipas, some military said army doctors noticed that some Indians used the bitter brew squat to treat fevers, skin diseases, especially diarrhea and dysentery (Lopez, 1928).

Texana Castela is an alternative treatment of the disease when there is resistance to other drugs and also in women with trichomoniasis during the first trimester of pregnancy (Shoe-Flowers 1998a). It has also ruled out experimentally the toxic effect of the plant (Shoe-Flowers. 1998b).

Among the properties tested experimentally *C. Texan* has been postulated to inhibit the entrenchment of the amoeba *Entamoeba invadens* which has been used as a study model to extrapolate results *Entamoeba histolytica* (footwear-Flores, 1998). It was also found that the aqueous extract has antioxidant and possibly shield the liver and also to combat parasite *Trichomonas vaginalis*, the most prevalent in the world sexually transmitted disease which affects both sexes, but mainly to the woman.

The methanol extract of Texas Castela, presents under axenic conditions in vitro activity against *Entamoeba histolytica* amebicide both the trophozoite stage and cyst or cyst-like structure, being 50 times more effective than the drug of choice against *E. histolytica*: metronidazole (Barron et al; 2007).

At present, it is recommended to prepare an infusion of leaves and stems against diarrhea, dysentery and amebiasis, like as an astringent. To treat amoeba Boil in 1/4 liter of water a few pieces of stem and a cup of tea is taken fasting for 9 mornings. It is also found in capsules in stores.

Essential oils and extracts cover a wide spectrum of pharmacological effects, showing various properties as anti-inflammatory, antioxidant and anticancer. Other biological activities are reported as biocides against a wide range of microorganisms including bacteria, fungi, viruses, protozoa, insects and plants (Kalemba and Kunicka, 2003).

Artemia salina

Artemia salina is a small crustacean that lives in places with high salt concentration (35 g per liter) that is brackish, their rights protected by cysts eggs are incubated at a temperature of 28 ° C and requires a constant oxygenation. A. The adult salt reaches a centimeter in length on average and their average life is one year. This rapid development and the ability of eggs to withstand long periods under unfavorable conditions, have made an invaluable model in biological research, some even developed in outer space. Within the phytochemical research, Dr. Jerry L. McLaughlin et al give beginning to a new stage, with the introduction of some primary bioassays such as *Artemia salina*, thus beginning the era characterized by "screenings" and "division" (Meyer et al, 1982;. McLaughlin et al, 1988)..

Recently published studies suggesting assessment as a first step, using a toxicological screening with evidence of acute toxicity in several species, allowing preliminarily determine the toxicity of new chemicals in different mammals (Guilhermino et al., 2000); One advantage is that it is economical and, therefore, the amount of mammals (mice) that are used are reduced.

The bioassay "larval lethality *Artemia salina*" is the determination of the LD50 (lethal dose) of the extracts of plants, those having an LD50 <1000 mg / L is very likely to contain one or more active compounds , so fraccionarlos necessary to repeat bioassays at lower concentrations (McLaughlin et al., 1988). It is also a preliminary test, which detects a wide range of active compounds (antitumor, antibiotic, etc.); is a simple, rapid and reproducible used as a selective method to determine the toxicity of extracts of plants before moving to testing method cell lines, inexpensive.

Justification Research

Dental cavities and gingivitis are among the most common disorders in humans; after the common cold, they can affect anyone and are the major cause of tooth loss. In Mexico caries prevalence is 48% and 23.8% of tooth loss in younger people, so this paper considers important scientific validation of the evaluation of crude extracts obtained from Texas Castela, shrub traditionally used in herbal Mexican to treat various conditions in humans. The results obtained in this research will serve in the future to elucidate and know the characteristics of

the molecules or compounds that have activity against bacteria of dental caries and gingivitis from *Castela texana* extracts.

ASSUMPTIONS

The methanol extracts of bark, leaf and stem of *Castela texana* inhibit the growth of bacteria related to dental caries and gingivitis.

GOALS

General purpose

To determine the biological activity of the methanol extracts of bark, leaf and stem of Texas *Castela* on bacteria associated with gingivitis and tooth decay.

Specific objectives

Castela texana

- Collect field *Castela texana* plant.
- Get the methanol extracts of bark, leaf and stem Texana *Castela*.
- Conduct tests to identify functional groups (colorful testing) for each extract.
- Prepare the stock solution of methanol extracts of *Castela texana*.

Bacteria

- Take demonstrates dental caries and gingivitis.
- Isolate bacteria related to dental caries and gingivitis.
- Set the kinetics of growth of bacteria related to dental caries and gingivitis.
- Identify bacteria associated with gingivitis and tooth decay.

Bioassays

- Determine the LD50 [mg / mL] of the methanol extracts of bark, leaf and stem of Texas *Castela* on *Artemia salina*.
- To determine the biological activity of the methanol extracts of Texas *Castela* on bacteria associated with dental caries and gingivitis.

Statistic analysis

- Perform Probit analysis using statistical linear regression study using the Microsoft Excel 2007 program, to determine the LC50 of each corresponding extract on in vitro axenic cultures of bacteria related to dental caries and gingivitis.

METHODOLOGY

Biological material

- a) Stem, leaves and bark texana Castela.
- b) Bacteria isolated from samples from patients with gingivitis and tooth decay.
- c) Artemia salina.

Texana Castela bush was collected in the community of San Isidro, Villa Hidalgo, San Luis Potosi.

Once the plant material collected proceeded according to the following steps:

1. Washing the plant material to running water, are rinsed to remove any residue outside the vegetative material.
2. Dried plant material is spread and dried at room temperature.
3. Grinding The dried material was ground using a hand mill and accurate.
4. Extraction Obtaining extracts from Castela texana was performed as described

below:

- a) Methanol extracts. Thus 200 g of bark, leaves, roots and stems of Castela texana was weighed and were added 300 mL of methanol. Flasks were kept on a shaker shaker for 7 days at room temperature. After this time each extract was filtered on Whatman No. 2. The filtrate was evaporated to dryness at room

temperature. The extract was obtained by scraping waste and stored in glass bottles until used.

- b) Partial chemical screening: Each extract was performed tests proposed chemical identification by Dominguez (1973). The extract obtained in each plant was subjected to the "colorful evidence" or chemical identification tests.

Unsaturation: Test KMnO₄: was dissolved 1.2 mg sample in 1 mL of water, acetone or methanol and dropwise a solution of 2% KMnO₄ in water is added. The test is positive if discoloration or brown precipitate formation within 1 minute, resulting in the formation of manganese dioxide is observed.

Carbonyl group: test 2,4-dinitrophenylhydrazine: 1 to 10 mg, of the sample are dissolved in ethanol, is added a saturated 2-4-dinitrophenylhydrazine in 6N HCl solution; the formation of a yellow or orange precipitate indicates the presence of the carbonyl group.

Phenolic hydroxyls (vegetable tannins): Test FeCl₃: 1-2 mg of sample was dissolved in 1 mL of water or ethanol and then a few drops of ferric chloride are added to 12.5% water. The appearance of a red, blue-violet or green precipitate is considered positive.

Sterols and triterpenes: Liebermann-Burchard test 1 ml of acetic anhydride is mixed and one of chloroform, cooled to 0 ° and is added one drop of sulfuric acid. Dropwise, this reagent is added to the sample or chloroform solution. If no formation of blue, green, red, orange colors, etc., which change over time, the test will be positive. The order and time of occurrence (0, 1, 5, 20, 60 minutes) has some diagnostic value; and, a yellow color after 15 minutes, seems to correspond to C-14-methyl -7 unsaturation and a variation. The test is positive sterol containing two conjugated double bonds, which may form one or two dehydrations with isomerization. Salkowski test: Similar to the Liebermann-Burchard, sample (1-2 mg) in contact with 1.0 ml of sulfuric acid, yellow or red colors for sterols and methylsterols develop.

Carbohydrates: Molish Test: A 1-2 mg. Sample is added dropwise, Molish's reagent (alpha-naphthol 1% in ethanol), then 1 mL of sulfuric acid by the walls. The test is positive when a

colored in purple ring interface is formed. Coumarins test: 1-2 mg of sample was dissolved in 10% NaOH; If a yellow color appears which disappears acidify it is positive.

Lactones: Dissolve 1-2 mg of sample in an alcohol solution of 10% NaOH. A yellow or orange color lost or disappears by adding a few drops of HCl indicates the presence of a lactone ring.

Sesquiterpenlactones: Test Baljet: 2-3 mg of the compound are added 3-4 drops of the mixture solution to be positive if it turns orange to dark red. The mixture 1: 1 solution consists of a solution A containing: 1% picric acid in ethanol and a B: 10% NaOH.

Flavonoids: H₂SO₄ Test: A small amount of sample is dissolved in H₂SO₄ and yellowing for flavonols observed; orange-icing for flavones; bluish-red to red-purple chalcones and to quinones.

Alkaloids: Dragendorff Test: Modifying Munier and Machelobuf. 2 solutions will be made. To prepare the solution A, was dissolved in 0.85 g of bismuth nitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. For solution B, 8 g of potassium iodide are dissolved in 20 mL of water. The reagent is prepared by mixing 5 mL of solution A, 4 mL of solution B and 100 mL of water. The reagent is stable for one year and the test is positive for alkaloids plate to red or orange color persistent for 24 hours (Pérez-Cepeda, 2000).

Saponins: sodium bicarbonate test: The salt is prepared in 10% water. Dissolve 1-2 mg of the sample dissolved in water or ethanol are added 2-3 drops of concentrated sulfuric acid. Stir slightly, then 2-3 drops of sodium bicarbonate solution are added. Bubbling and retention for more than 1 minute indicating the presence of saponins. Salkowski test for saponins: Dissolve 1-2 mg of sample in 1 mL of chloroform is added 1 mL of sulfuric acid. The test is positive if red appearance.

Aromaticity: Test sulfuric acid-formaldehyde: a mixture of 1 mL of concentrated sulfuric acid with a drop of formaldehyde is prepared. Are added from 1 to 5 mg of the sample dissolved in non-aromatic solvent, a few drops of the above mixture are added and if a red-violet color appears, the test is positive.

Sampling and maintenance of the bacteria: The sampling was carried out at the premises of the Faculty of Dentistry by a specialist. The samples immediately after being taken were transported to the laboratory, where they were transferred to a tube containing nutrient medium (average MPTT); subsequently, under anaerobic conditions, reseeded were performed. Immediately is between MPTT-agar is made striated into four quadrants, and incubated at 37th C for 24 hours, then a sample of each colony was collected, inoculated into a tube through MPTT and incubated at 37th C for 24 h, and once it had already established in vitro culture, it was carried out the kinetics of growth of each single colony.

Growth kinetics of bacteria related to dental caries and gingivitis: A bacteria isolated from patients with dental caries and gingivitis underwent growth kinetics in order to meet one of the phases to perform the bioassay. A 9 MPTT broth tubes with a volume of 9 mL and 30 mL of inoculated bacteria were incubated at 37 ° C, the absorbance of each tube was determined in a spectrophotometer at 635nm for 24 h.

Partial identification of bacteria associated with gingivitis and tooth decay: Once the kinetics of growth of isolated bacteria is established, the morphology of the bacteria is determined by simple staining, and be determined by Gram-established technique; Moreover, partial identification will be made by the API-A system 20 and API-20.

Bioassays

a) Preparation of stock solution: The stock solution was prepared as detailed in Table 1, and from this stock solution were taken amounts necessary to obtain the desired concentration.

Table 1

Preparation of stock solution extractos metanólicos de *C. texana*
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	Hoja	Tallo	Corteza
Gramos	1.14	3.72	1.21
DMSO (mL)	10	10	10
Medio MPT-T (mL)	4	4	4

b) Evaluation of the biological activity of methanol extract of *C. texana* on bacteria associated with dental caries and gingivitis

Spectrophotometry: To perform the bioassay of different methanol extracts of *C. texana* (root, stem, leaf and bark) in 72 13x100 tubes containing 8 ml of culture medium, were added various concentrations were performed the methanol extracts of *C. texana*. Each tube was inoculated with 30µl of bacteria isolated from patients with dental caries and the same amount for the bacterium isolated from gingivitis, then incubated at 37 ° C for 24 h; to each tube absorbance at 635 nm was read. Then the colony forming units per mL (CFU / mL) using the technique of bacterial plate count (RBP) was determined.

Bacterial count technique Device (RBP): From each containing culture of bacteria related to dental caries or gingivitis and in the presence of each extract corresponding tube, previously incubated at 37°C for 24 hours, one milliliter be taken and dilutions were made already up 10⁻⁹ from 10⁻⁵ to 10⁻⁹ dilution milliliter be taken and be placed in a Petri dish, which will immediately add 15 mL MPTT-agar medium, it is left to solidify and then incubated to 37°C for minimum 24 hours, and subsequently the plate count is performed to determine the CFU / mL (Table 3).

c) Determination of the LD50 of the methanol extracts of *C. Texas*: According to the results obtained by the absorbance technique and technique of RBP, the LD50 was determined using the method for each replica PROBIT using statistical linear regression analysis with Microsoft Excel 2007 program.

d) Determination of toxicity of methanol extracts of *C. texana* on salt A.: For the incubation of eggs of *A. saline* sea water artificially prepared as follows: 40 g of sea salt

(Instant Ocean are weighed, Aquarium System), 0.006 g of brewer's yeast (Mead Johnson) is afora in one liter of bidistilled water, the pH adjusted to 7.8. The method is performed by incubating 0.1 g of eggs of *A. saline* in artificial sea water, placed in a plastic container divided by an intermediate wall with a space at the bottom of 2 mm; They are kept in the dark and oxygenation. One compartment is kept illuminated with a 20 watt lamp to hatch as nauplii are attracted to light.

After 24 h, the nauplii are placed with a pipette to another vessel and kept in temperature and oxygenation conditions 22-29 ° C for 24 h. In a 96-well microplate they are added 100 mL of the suspension of nauplii / well (about 10 nauplii) plus 100 mL of the dilutions of the extracts tested. Assessing concentrations will range from 10 to 1000 mg / mL. As a positive control Potassium dichromate was used at a concentration of 400 ppm; and DMSO at the same doses that are handled in the bioassay and sea water as a negative control. After 24 h of extracts and applied with the aid of a stereoscopic microscope, counting living nauplii per dose was performed.

e) Statistical analysis: To determine the biological activity of methanol extract of Texas Castela on axenic culture in vitro of bacteria related to dental caries and gingivitis, the data obtained from the absorbance in triplicate and colony forming units (CFU) in duplicate they were averaged and compared to control cultures by analysis of variance with a $P < 0.05$, using the Dunnet test-T (2-side) with SPSS for Windows, version 2007.

RESULTS

Identification of functional groups in the methanol extracts of *C. texana* results of chemical tests to identify functional groups and secondary metabolites present in the methanol extracts of leaf, stem, root and bark of *C. texana* are presented in Table 2.

Table 2

Functional groups and secondary metabolites present in the methanol extracts stem, leaf and bark *Castela texana*

Grupo funcional	Pruebas	Tallo	Hoja	Corteza
Insaturaciones	KMnO ₄	-	+	-
Grupo carbonilo	2,4-Dinitrofenilhidracina	+	+	+
Oxhidrilos fenólicos	FeCl ₃	+	+	-
Esteroles y triterpenos	Liebermann-Burchard	+		
	Salkowski	+	+	+
Carbohidratos	Molish	+	+	+
	Cumarinas	+	-	+
Lactonas	Lactonas	+	+/-	+
Sesquiterpenlactonas	Baljet	A + B+	A - B-	A - B+
Flavonoides	H ₂ SO ₄	Flavonas	Flavonas	Flavonas
Alcaloides	Dragendorff	A +	A -	A +
Saponinas	Bicarbonato de Sodio	+	+	+
	Salkowski	+/-	+/-	+
Aromaticidad	Ácido Sulfúrico- formaldeído	+	+	+

Toxicity bioassay brine shrimp *Artemia salina*: Toxic activity of the methanol extracts of the stem, root, bark and leaves of *C. texana* was evaluated on *A. salina* nauplii. With the results the LD50 (Lethal Dose) of each of the extracts, the experiment based on the Probit analysis was designed by SPSS version 17 program was calculated.

In Fig.1 the results obtained for the activity on brine shrimp lethality for each of the extracts, a marked toxic activity was observed on *A. salina* is because the extracts showed lower doses of 500 mg / mL.

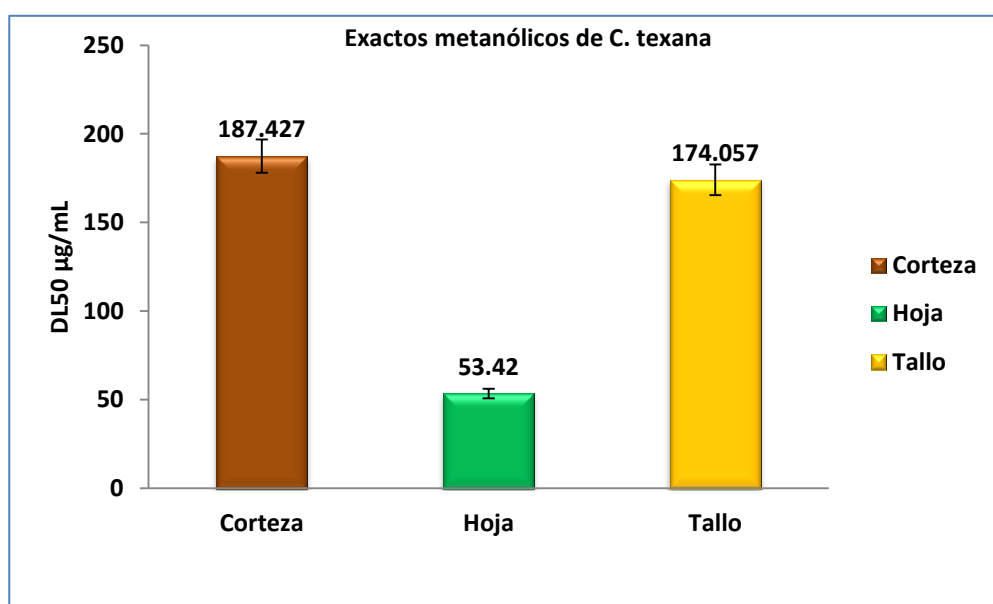


Fig. 1. Comparison of the toxic potential of the methanol extracts of bark, leaves and stems of *C. texana* sobre *A. salina*

Growth kinetics: The growth kinetics of the bacteria isolated from patients with gingivitis shown in Figure 2, in which a long lag phase is observed during the first hours, and then logarithmic growth from las10 h to 25 h . A typical stationary phase is observed, either the death phase is observed as the growth of this bacteria was very slow. Each plotted point represents the average of triplicate reading of seven tubes.

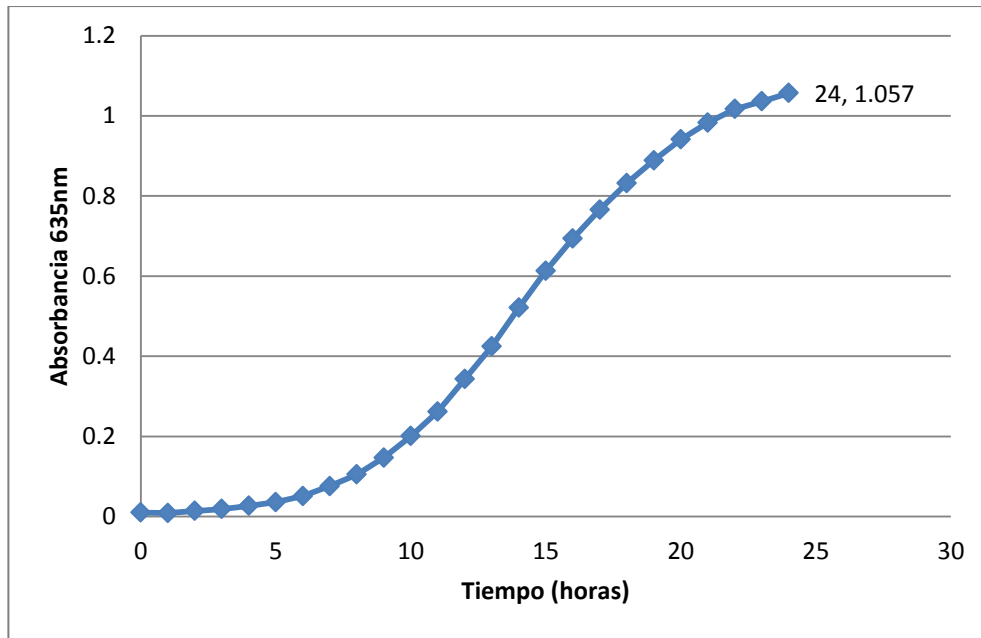


Figure 2. Kinetics of growth of bacteria isolated from patients with gingivitis, is observed at 24 h presents the maximum cell yield.

The growth kinetics of the bacteria isolated from a patient with dental caries is shown in Figure 3. It can be seen a long period of adaptation during the early hours, and then a logarithmic growth from 10 h to 18 h. Also, a typical stationary phase, and this kinetic either the death phase observed since the growth of this bacterium was also a little slow. Each plotted point represents the average of triplicate reading of seven tubes.

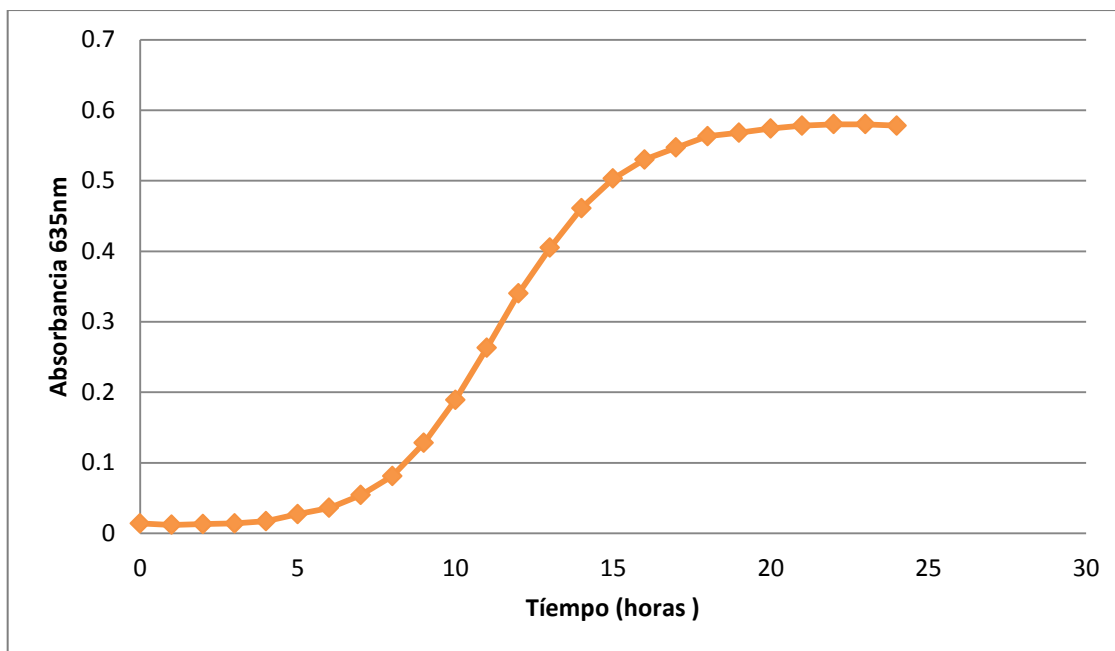


Figure 3. Growth kinetics of bacteria isolated from patients with dental caries. Approximately 18 pm maximum cell yield was observed.

Identification of bacteria associated with dental caries and gingivitis: Once isolated and the kinetics of growth of each bacterium from cultures established from gingivitis and / or dental caries, biochemical tests for partial identification of the sample bacterial isolation. The morphology indicates the presence of cocci in chains and that Gram staining observed red, ie are Gram (-), the best cell yield was obtained under conditions of microaerophilic and biochemical tests indicate that both bacteria are *Streptococcus* sp.

Biological activity of the methanol extract of bark, leaves and stems of *C. texana* on bacteria associated with dental caries and gingivitis

Turbidimetry method: To evaluate the biological activity of the methanol extracts of bark, leaves and stems of *C. texana* on gingivitis related bacteria (Figure 4) and dental caries (Figure 5), we observed a marked inhibition of cell performance patients in asylum bacteria of dental caries and gingivitis. This methanol extract in the presence of stem, root, leaf and bark of *C. texana* by using 0.1 mg / mL, 1mg / mL and 10mg / mL of extract, bactericidal results indicating the ability of these extracts on the above bacteria.

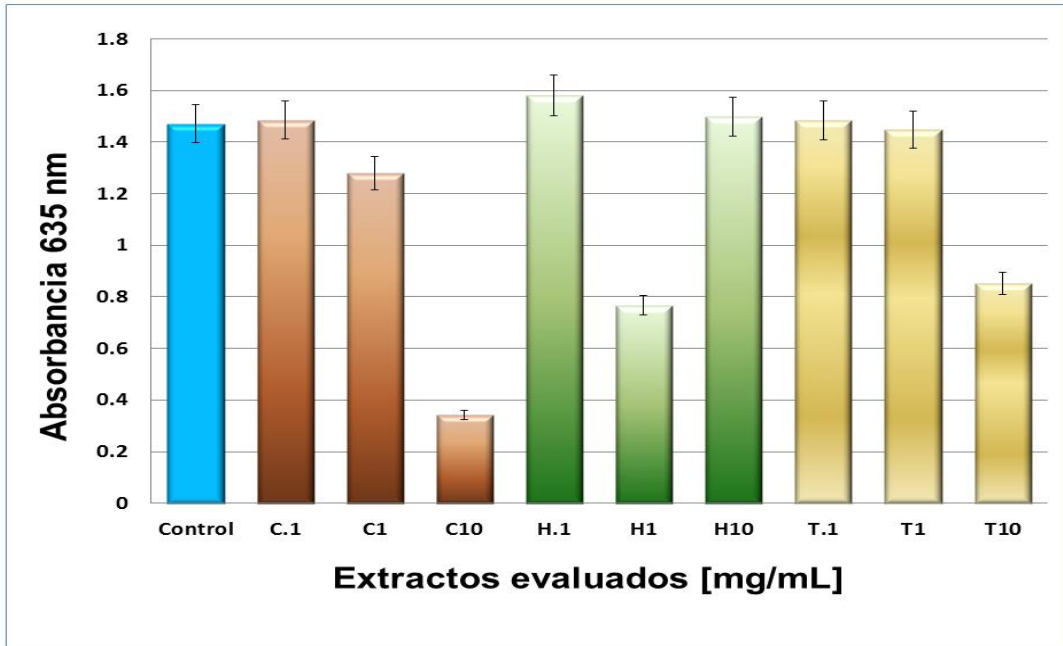


Figure 4. Graph comparing the biological activity of the extracts of *C. texana* on gingivitis related bacteria (C=corteza, H=hoja y T=tallo).

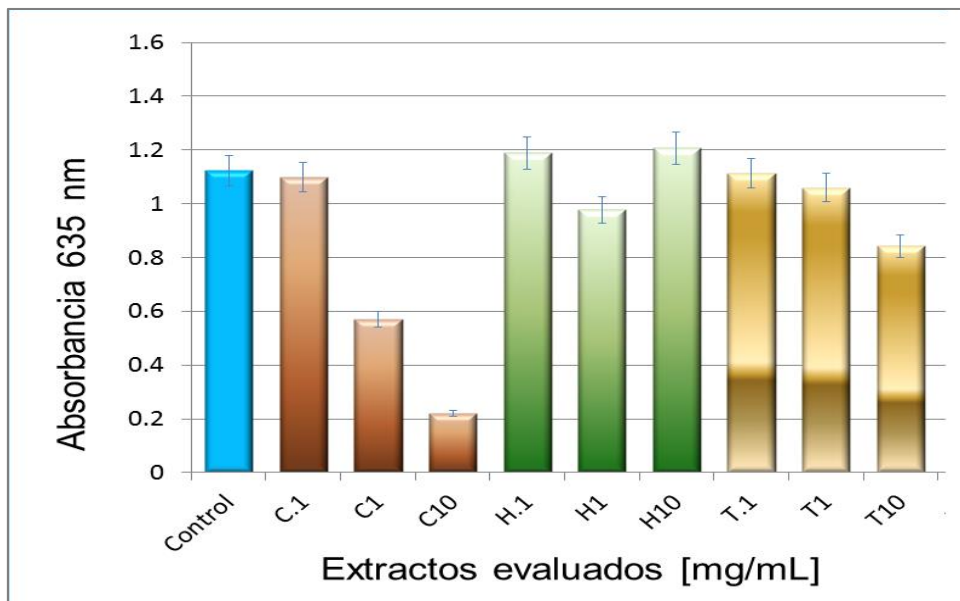


Figure 5. Biological activity of extracts of *C. texana* on bacteria related to dental caries (C=corteza, H=hoja y T=tallo).

Technical bacterial plate count (CFU / mL): The direct method to quantify the biological activity of the methanol extracts of *C. texana* on bacteria associated with gingivitis and dental caries

(Table 3) was determined by plate count technique (RBP) to determine the colony forming units.

Table 3
 Determination of CFU / mL of bacteria associated with gingivitis and tooth decay cultured in the presence of methanolic extracts of *Castela texana*

	Control	Extracto metanólico de <i>C. texana</i>								
		Corteza			Hoja			Tallo		
		0.1*	1*	10*	0.1*	1*	10*	0.1*	1*	10*
Gingivitis	830,000,000	I	6,850,000	490,000	299,550	210,000	380,000	I	3,200,000	8,650,000
Caries	178,000,000	I	179,500	I	89,000	32,500	7,900	I	I	245,000

*mg/mL I=Incontable

DISCUSSION

In this work the chemical screening of *Castela texana* plant and the determination of the biological activity of the methanol extracts on the cultivation of bacteria related to dental caries and gingivitis was evaluated. The results of the tests colorful methanol extracts of leaf, stem, root and bark of *C. texana* indicates the presence of the following secondary metabolites: for unsaturated sheet was the only one tested positive in this test while others came negative test on flavonoids in leaf was the only one different from the others went with a score of flavones, while other parts of *C. texana* contain flavones. As aromaticity, all were positive for this test; test in carbohydrates, Molish test on leaf and root were negative while the other tests to detect carbohydrates were positive (Table 2).

In evaluating the toxic activity of the methanol extracts of the stem, bark, roots and leaves of *C. texana* on *A. salina nauplii*, we note that the methanol extract of the leaf of *C. texana* has a LD50 53.42 ug / mL on *A. salina*, which indicates a high toxic activity compared to the rest of the extracts (Figure 1). The methanol extract of bark had the lowest LD50 with 187 427 mg / mL, which indicates that showed less toxic activity against *A. salina* is noteworthy that the minimum value and the maximum value for the LD50 correspond to leaf extracts *C. bark* and

Texas, however, the variant of the bush is used for extraction, which indicates that each of the bush has different compounds.

As for the antibacterial activity, we proceeded to determine by absorbance and CFU inhibition on the growth of bacteria related to dental caries and gingivitis. We evaluated only the methanol extracts of leaf, stem and bark of *C. texana* at different concentrations, the methanol extract selected to carry out this evaluation of antibacterial activity, as this type of extraction can extract more compounds from each plant sample.

The results indicate that the methanol extract of *C. texana* sheet concentration of 1 mg / mL, the cultures inhibited both isolated bacteria of dental caries and gingivitis in the 80% and 78% respectively, showing greater inhibition extract *C. texana* sheet in the bacteria associated with gingivitis. Also the antibacterial activity of methanol extract of leaf was higher in Texas Castela related bacteria dental caries (Table 3).

Bush evaluated in this study, there are widely reported work only the amoebicidal activity of methanolic extracts of *C. texana* to inhibit in vitro the growth of *E. histolytica* and *Entamoeba* inhibit encystment *invadens* (Footwear et al. , 2007), it has also been reported inhibition in vitro enquistamiento axenic *E. histolytica* (Barron et al, 2007).

The methanolic leaf extract *C. Texas* had the highest bactericidal activity, however, also it had the highest toxic potential of biological study model (*Artemia salina*). This toxicity is different to that found in murine models, no toxicity reported (Footwear et al, 1998). The potential for toxicity indicates that it is not the best choice of study to obtain metabolites with bactericidal activity, so it is concluded that the best option to study bacteria associated with gingivitis is the methanol extract of bark, and bacteria related to dental caries, the methanol extract of the stem, which respectively have the highest bactericidal activity and lower toxic potential of *Artemia salina*.

The extracts with antibacterial activity could be used in future research aimed at inhibiting both the growth of bacteria that cause dental caries and gingivitis.

CONCLUSIONS

The methanol extract of the bark of *C. texana* has low toxic potential of *A. salina* and has the highest growth inhibitory activity of bacteria associated with gingivitis.

Best methanol extract to inhibit the growth of bacteria related to dental caries is the methanol extract of the stem because it has low toxic potential of *Artemia salina*.

Bibliography

- Beg, A. (2000). Effect of *Plumbago zelanica* extract and certain curing agent on multidrug resistant bacterias of clinical drugs. *Word Journal of Microbiology and Biotechnology*, 16, 841-864.
- Calzado-Flores, C. (1998). In vitro anti-trichomonoc activity of *Castela texana*. *Proceedings of the Western Pharmacology Society*, 41, 173-174.
- Caton J., (1988). Cell populations associated with conversion from bleeding to non bleeding gingiva. *Journal of Periodontology*. 59, 7-10.
- Domínguez X.A. (1973). Métodos de Investigación Fitoquímica. México: Ed. Limusa.
- Hernández G. (1998). Cuide su boca. España:Editorial Everest.
- Kalemba, D. & A. Kunicka., (2003). Antibacterial and antifungal properties of essential oils. *Journal of Microbiology*. 10(10), 813-829.
- Murayama, Y., Kurihara, H., & Nagai, A. (2000). Acute necrotizing ulcerative gingivitis: Risk factors involving host defense mechanisms. *Periodontol*. 6, 116-124.
- Prieto-Prieto J, & Calvo. A., (2004). Bases microbiológicas en las infecciones bucales y sensibilidad en los antibióticos. *Medical Oral Patology*. 9, 8 - 11.

Rodríguez Calzadilla A. (1997). Enfoque de riesgo en la atención estomatológica risk approach in dental care. *Revista Cubana Estomatológica*. 34(1), 40-49