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Journal of Natural and Agricultural Sciences

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Presentation of content

In the fist article we present, *Antibacterial activity of Costus pulverulentus (Costaceae) C. Presl*, by RAMIRO-BAUTISTA, Luis Rodrigo, HERNÁNDEZ-MORALES, Alejandro, CARRANZA-ÁLVAREZ, Candy and MALDONADO-MIRANDA, Juan José, with adscription in the Universidad Autónoma de San Luis Potosí, in the netx article we present, *Review of floral polymorphism in chía (Salvia hispanica L.): Modified cause*, by CALDERÓN-RUIZ, Alberto, VARGAS-ESPINOZA, Everardo, GAYTÁN- RUELAS, Marina y MARTÍNEZ-CAMACHO, Adriana Paola, with adscription in the Universidad Tecnológica del Suroeste de Guanajuato, in the netx article we present, *Comparative characterization of starch biopolymers extracted from cereals using two different techniques*, by RIVERA-ARREDONDO, Marisa, RODRÍGUEZ-ÁNGELES, Mario Alberto, MORALES-FÉLIX, Verónica de Jesús and GAYTÁN-RUELAS, Marina, with adscription in the Universidad Tecnológica del Suroeste de Guanajuato and the Universidad Politécnica de Juventino Rosas in the last article we present, *Use bioregulators in cluster ToV (Salanum lycopersicum L.)*, by CALDERÓN-RUIZ, Alberto, MARTÍNEZ-CAMACHO, Adriana Paola, ESPINOZA-ZAMORA, Jesús and RIVERA-ARREDONDO, Marisa, with adscription in the Universidad Tecnológica del Suroeste de Guanajuato.

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Antibacterial activity of *Costus pulverulentus* (Costaceae) C. Presl

Actividad antibacteriana de *Costus pulverulentus* (Costaceae) C. Presl

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Abstract

Antimicrobial resistance is a great concern in public health. Therefore, it is necessary to obtain new compounds to treat diseases caused by bacteria. Medicinal plants are an alternative to search natural compounds to improve human health, including antioxidants, anti-inflammatory, and antimicrobials compounds. *Costus pulverulentus* (Costaceae) C. Presl is a plant used traditionally in Huasteca Potosina to treat bacterial infections. However, the compounds involved in this activity remain poorly understood. To determine the antibacterial activity of *C. pulverulentus*, an ethanolic extract was obtained. Plant stem was macerated in ethanol and then was fractionated with hexane, chloroform, ethyl acetate, acetone, ethanol, and methanol. Ethanolic extract and derived fractions were tested against bacteria by the disk-diffusion agar method. The results showed that the ethanolic extract of *C. pulverulentus* exerted activity against *Chromobacterium violaceum* CV026 and *Bacillus* sp. at 10 and 30 µg/disk, whereas only the methanolic fraction showed similar activity to complete extract. Fraction 8 obtained from methanolic fraction showed inhibitory activity against *Bacillus* sp., *S. aureus*, and *S. aureus* Oxacillin resistant. Gas Chromatography-Mass Spectrometry characterization of active fraction 8 showed that it contains vanillic acid and p-coumaric acid suggesting that they are involved in the antibacterial activity of *C. pulverulentus*.

Antimicrobials, *Costus pulverulentus*, *p*-coumaric acid

Resumen

La resistencia a los antimicrobianos es una gran preocupación en la salud pública. Por tanto, es necesario obtener nuevos compuestos para tratar enfermedades provocadas por bacterias. Las plantas medicinales son una alternativa para buscar compuestos naturales para mejorar la salud humana, incluidos compuestos antioxidantes, antiinflamatorios y antimicrobianos. *Costus pulverulentus* (Costaceae) C. Presl es una planta utilizada tradicionalmente en la Huasteca Potosina para tratar infecciones bacterianas. Sin embargo, los compuestos implicados en esta actividad siguen siendo poco conocidos. Para determinar la actividad antibacteriana de *C. pulverulentus* se obtuvo un extracto etanólico. El tallo de la planta se maceró en etanol y luego se fraccionó con hexano, cloroformo, acetato de etilo, acetona, etanol y metanol. El extracto etanólico y las fracciones derivadas se probaron contra bacterias mediante el método de agar de difusión en disco. Los resultados mostraron que el extracto etanólico de *C. pulverulentus* ejerció actividad contra *Chromobacterium violaceum* CV026 y *Bacillus* sp. a 10 y 30 µg / disco, mientras que solo la fracción metanólica mostró una actividad similar al extracto completo. La fracción 8 obtenida a partir de la fracción metanólica mostró actividad inhibidora frente a *Bacillus* sp., *S. aureus* y *S. aureus* resistente a oxacilina. La caracterización por cromatografía de gases y espectrometría de masas de la fracción activa 8 mostró que contiene ácido vainílico y ácido *p*-cumárico, lo que sugiere que están involucrados en la actividad antibacteriana de *C. pulverulentus.*

Antimicrobianos, *Costus pulverulentus*, Ácido *p*-cumárico

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Introduction

Currently there is a huge and serious problem caused by the increase in the resistance of microorganisms to antimicrobial treatments. Resistance occurs when microorganisms undergo changes that make the drugs used to cure infections stop being effective, as a result, infections persist in the body, increasing the risk of spreading to other people (WHO, 2017). This represents one of the greatest threats to global health, food security and development. In addition, it jeopardizes the medical capacity to treat common infectious diseases, with the consequent increase in hospital stays, deaths, and the prolongation of the disease and with it medical costs (WHO, 2016).

Among the resistant microorganisms with a wider distribution and that represent a serious health problem because they are important etiological agents of infections of the urogenital and gastrointestinal tract are: the non-encapsulated enterobacterium *Escherichia coli*, the encapsulated enterobacterium *Klebsiella pneumoniae*, the Gram-positive cocci Staphylococcus aureus , the non-relevant enterobacterium *Pseudomonas aeruginosa*, parasites such as *Entamoeba histolytica*, and yeast fungi such as *Candida albicans* (Cohen and Tartasky, 1997; Bansal, Malla, and Mahajan, 2006; Calderon and Aguilar, 2016).

The problem becomes more relevant when it is considered that most of the countries worldwide, mainly those in the developing world, do not have one hundred percent guaranteed access to health services, for which strategies based on traditional medicine are sought and complementary (MTC), among which the use of medicinal plants stands out (WHO, 2013).

In Huasteca Potosina, the enormous existing biodiversity makes it possible to have a great variety of therapeutic herbal resources both in rural and suburban areas (Alonso-Castro, et al., 2015). Research has been carried out on some plants, among them *Costus pulverulentus* stands out for the ethnopharmacological applications that are given to it (treatment of infections of the urinary and gastrointestinal system) and the studies previously carried out that showed various beneficial effects of the plant.

However, validation tests are still lacking for different activities assigned to *C. pulverulentus*, among them is the antibacterial capacity, which is suggested derived from phytochemical studies previously carried out in which compounds reported with this effect were found. Therefore, this study evaluated the antimicrobial effect on bacteria of clinical importance of the complete ethanolic extract and organic fractions obtained from *C. pulverulentus* (Costaceae) as well as the identification of the bioactive metabolite.

Methodology

Vegetal material

The plants were collected in August 2017 in Ciudad Valles, San Luis Potosí at coordinates 21 ° 49'39.4 "N 99 ° 05'18.9" W. A specimen with collection number SLPM-033070 was deposited in the Herbarium Isidro Palacios Desert Zones Research Institute, Autonomous University of San Luis Potosí.

Preparation of the ethanolic extract (E.E)

100 g of powdered dried stem were macerated in 1000 mL of ethanol for seven days in the dark. The extract was filtered and concentrated to dryness on the R-100 rotary evaporator (Büchi). The dry residue was fractionated into hexane, chloroform, ethyl acetate, acetone, ethanol, and methanol. Subsequently, the active fraction was separated by column chromatography packed with Silica gel 70-230 mesh, 63-200 µm (Sigma 60741) using a solvent system hexane: ethyl acetate: methanol in increasing polarity.

Fractionation of the extract

The E.E. It was fractionated using ACS grade solvents of different polarity starting from lowest to highest, hexane <chloroform <ethyl acetate <acetone <ethanol <methanol. The dry residue of E.E. It was dissolved in 50 ml of hexane, two fractions were obtained, soluble and insoluble. The soluble fraction was recovered and brought to dryness in the convection oven. The dry residue was weighed to determine the yield, subsequently it was dissolved in absolute ethanol and the concentration was calculated in mg / mL.

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On the other hand, the fraction insoluble in hexane (residue) was brought to total dryness at 45 ° C for 48 h and dissolved in chloroform, again two fractions (soluble and insoluble) were obtained. The soluble fraction was recovered and dried in the convection oven and weighed to calculate the yield. The chloroform fraction was dissolved in absolute ethanol and the concentration was calculated in mg / mL. The same procedure was repeated with each of the remaining solvents, thus obtaining a total of six fractions of known concentration in mg / mL.

Column chromatography

A 22 x 450 mm glass column was used, packed with a suspension of 21.5 g of Silica Gel 70-230 mesh, 63-200 µm (Sigma 60741) in 100 mL of hexane. The column was allowed to stand for one hour at room temperature and was subsequently loaded with 250 mg of the sample. The mobile phase consisted of the polarity gradient solvent system formed by Hex: AceOEt: MeOH (Table 1). The resolution of the sample was carried out with a flow of 60 drops / min. 50 ml fractions were collected, which were concentrated using the Rotavapor R-100 (Büchi).

Hexane (ml)	Ethyl acetate (ml)	Methanol (ml)
100	0	0
90	10	0
80	20	0.1
70	30	0.2
60	40	0.3
50	50	0.4
40	60	0.5
30	70	0.6
20	80	0.7
10	90	0.8
0	100	0.9

Table 1 Solvent system used in Column Chromatography

Gas Chromatography coupled to Mass Spectrometry

The extracts and organic fractions were derivatized using the following methodology. 50 µL of extract was dried under a nitrogen flow. Subsequently, 20 µL of pyridine (Sigma 270407) and 80 µL of BSTFA reagent with 1% TMCS (Sigma 15238) were added and incubated at 85 ° C for 25 min. The reaction mixture was cooled to room temperature, Isooctane (Sigma 650439) was added to a final volume of 200 µl.

Finally, 100 µL of the clear mixture was transferred to a vial and analyzed by GC-MS. For the analysis, a Gas Chromatograph model GC9090A (Agilent Technologies) coupled to a mass spectrometer model 5975 (Agilent Technologies) was used. 1 µL of the derivatized was analyzed in pulsed split injection mode with a ratio of 5: 1. The injector temperature was 250 ° C. Chromatographic separation was performed on an Agilent J&W DB-1MSUI capillary column (60m x 250 µm x 0.25 µm) and helium was used as carrier gas at a constant flow rate of 1 mL / min. The GC oven program started at an initial temperature of 45ºC, was maintained for 5 minutes and then increased at a rate of 10ºC per minute until a final temperature of 300ºC was obtained, which was maintained for 25 minutes.

The temperature of the transfer line was adjusted to 250 ° C. Mass spectra were obtained at 70 eV electron energy. Measurements were made in scan mode with a m / z range set to 40-550. The ion source and the temperature of the quadrupole analyzer were 230 ºC and 150 ºC respectively, they kept running at 2.9 scans per second.

Data obtained by GC-EM were examined with MassHunter Workstation software version B.06.00 (Agilent Technologies). The AMDIS software (http://www.amdis.net/) was used for the determination of the retention time and the extraction of the mass spectrum of each component of the chromatograms. The mass spectral library software and the NIST MS database were used for the identification of compounds.

Determination of antimicrobial activity

Microorganisms

Five Gram positive bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus*, *S. aureus* OxR, *Enterococcus faecalis*, *Bacillus* sp. and five Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Proteus mirabilis*, *Citrobacter freundii*, *Chromobacterium violaceum* CV026.

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Disk diffusion test

10 and 30 µg of ethanolic extract, organic fractions were placed on sterile filter paper disks. Subsequently, the discs were placed on a bacterial lawn inoculated on Mueller Hinton Agar (MH) plates, left to rest at 4ºC for one hour and subsequently incubated at 37ºC for 24 h. After the incubation time, the diameter of the inhibition halos generated by the evaluated extracts was measured. Three replications were performed per treatment, and the experiments were repeated three times.

Determination of the Minimum Inhibitory Concentration

The M100 protocol proposed by the CLSI (Coyle, 2005) and the guidelines established by Cos et al. (2006) and Balouiri (2016) were used. The plate microdilution method was used, for this, the compound solution to be evaluated was diluted 1: 2 in Mueller Hinton broth. The wells were inoculated with a bacterial suspension at turbidity equivalent to the McFarland standard 0.5 to have 1 x 10^8 CFU / mL in each well of the microplate (Cos, et al., 2006; Balouiri, et al., 2016). The plate was incubated at 37 ° C for 24 h and subsequently visually inspected. The lowest dilution of the compound where no microbial growth was observed was considered the minimum inhibitory concentration (MIC). To check MIC, 5 µl of a 0.05% tetrazolium chloride indicator solution was added to each well. Wells where the reduction of tetrazolium chloride was not observed indicated absence of microbial activity.

Anti Quorum sensing activity

Chromobacterium violaceum CV026 was inoculated in LB broth supplemented with Kanamycin (100 µg / mL) and incubated at 30 ºC for 24 h. Subsequently, 500 µl of the culture was mixed with 100 ml of LB agar melted at 45 ° C supplemented with Kanamycin (100 µg / mL) and 1 µM of N-butanoyl-l-homoserine lactone (C4-HSL). The mixture was poured into Petri dishes and gently shaken for uniform distribution. For the anti quorum sensing activity, discs were placed on the surface of the agar impregnated with Ethanolic Extract and organic fractions. The absence of purple pigmentation in *C. violaceum* CV026 colonies indicates anti quorum activity.

The appearance of colonies with purple pigment indicates that the extracts and fractions lack activity

Determination of toxicity with Artemia salina

Eggs of *A. salina* were hatched in artificial seawater, incubated at 28 ° C for 24 h, placed under a yellow light lamp and fed fish diet. To evaluate the toxicity, 20 nauplii of *A. salina* were placed in a plastic container and the doses of extracts, methanolic fraction and F8, etc. were added. The tests were incubated under the same conditions and mortality was determined at 24 h. Three repetitions were performed per treatment and the experiment was repeated three times (Rajabi, et al., 2015)

Results

Preparation of the ethanolic extract of C. pulverulentus

From 100 g of fresh plant, 7.86 ± 0.77 g of dry ground plant were recovered, which was macerated in ethanol to obtain the ethanolic extract. 0.39 ± 0.01 g of dry ethanolic extract were obtained, which corresponds to 5.10 ± 0.58% of yield based on the dry weight of the plant. The extract was analyzed by GC-MS and 94 peaks were detected, 74 peaks (79%) were identified in the NIST database, while 20 peaks (21%) did not show similarity with reported compounds.

Most of the compounds identified in the extract correspond to carbohydrates, organic acids and sterols. The most abundant compound in the ethanolic extract of *C. pulverulentus* was D-psychofuranose with a retention time (RT) of 26.46 m and relative abundance of 9.74% (Table 3), while, among the organic acids, the p-hydroxy-cinnamic with TR of 27.41 was the most abundant with a relative abundance of 6.79%.

This compound has been previously reported in ethanolic extract of *C. pulverulentus* in 7.38% (Alonso-Castro et al. 2016). Hydroxycinnamic acid is a phenolic compound that acts as an antioxidant and inhibitor of the action of free radicals. It is found in a wide variety of vegetables, fruits, and grains; and it presents antioxidant, antimicrobial, and immunomodulatory activity.

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In addition, in the analysis of the compounds of the ethanolic extract of *C. pulverulentus*, stigmasterol with TR of 46.33 and sitosterol with TR of 48.16 were identified, to which anti-inflammatory activity has been attributed (Liu et al., 2019). In previous studies, stigmasterol and sitosterol have been identified in an ethanolic extract of *C. pulverulentus* and could be responsible for the anti-inflammatory activity attributed to this species (Alonso-Castro et al. 2016).

Determination of the antimicrobial activity of the Ethanolic Extract (E.E.) of C. *pulverulentus*

C. pulverulentus is used in traditional medicine to treat "bad urine", a disease with symptoms that suggest a urinary tract infection (UTI). UTIs are caused by Enterobacteriaceae, the most frequent being *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* that colonize the urinary tract causing a variety of symptoms compatible with "urine disease". In addition to Enterobacteriaceae, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus* and *Enterococcus faecalis* are also etiological agents of UTIs.

To determine the antimicrobial effect of *C. pulverulentus*, the E.E. against five bacteria, 3 Gram negative *Escherichia coli and Klebsiella pneumoniae*, related to bad urine; and *Aeromonas hydrophila*, which has also been reported as an etiologic agent in the urine of patients with cystitis and asymptomatic bacteriuria (Tena et al. 2007). It was also evaluated against 2 Gram positive *Enterococcus faecalis* and *Bacillus* sp.

For evaluation, filter paper discs were impregnated with 10 μg of the E.E. and were placed on a lawn of bacteria inoculated on the MH agar. The results show that the E.E. of *C. pulverulentus* forms a growth inhibition halo on the plates where the two Gram positive bacteria, *E. faecalis* and *Bacillus* sp., respectively, were inoculated. The results show that the E.E. has significant activity against *Bacillus* sp, generating a halo of 16 ± 0.06 cm, this value is close to the established value (20 mm), by the CLSI with which it is indicated that the microorganism presents intermediate sensitivity to E.E. of *C. pulverulentus*. Regarding the effect against *E. faecalis*, E.E. generates a halo of 7 ± 0 mm;

However, this value has no clinical significance and is classified as resistance of the bacteria to E.E. of *C. pulverulentus.* Regarding Gram negative bacteria, no growth inhibition was observed in any of the evaluated species.

Determination of the Anti-Quorum Sensing Activity of E.E. by C. pulverulentus

The quorum sensing (QS) is the communication system in most bacterial species, it regulates the expression of genes involved in pathogenicity and virulence at the transcriptional level (Oliveiro et al., 2011). In Gram negative bacteria, QS is mediated by inducing molecules called acyl-homoserin lactones (AHL) that are produced in response to population density and are specific for each species of microorganism (Oliveiro et al., 2011).

Currently, QS is considered one of the therapeutic targets to control infections caused by Gram-negative bacteria (Oliveiro et al., 2011). For this reason, the search for QS inhibitor compounds is carried out in extracts, essential oils and pure compounds.

The most widely used model is *Chromobacterium violaceum* CV026, which produces a pigment called violacein. In this work, the effect of E.E. to determine if it possesses metabolites capable of blocking the QS mechanism in *C. violaceum* CV026 by inhibiting the synthesis of violacein.

The results obtained show that the production of violacein is not affected in the presence of E.E., indicating that the extract of *C. pulverulentus* does not have anti-QS compounds. However, the E.E. has a growth inhibitory effect at a dose of 10 μg / disc, generating a halo of inhibition of 12 mm in the plate inoculated with *C. violaceum* CV026

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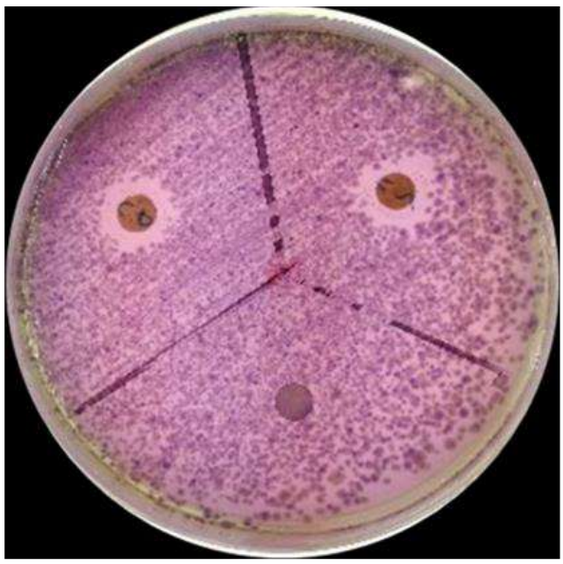

Figure 1 Effect of E.E. against *C. violaceum* CV026. All tests were done in triplicate

Fractionation of the Ethanolic Extract of C. pulverulentus

Once the inhibitory effect of E.E. against *Bacillus* sp., *E. faecalis* and *C. violaceum* CV026, the crude extract of *C. pulverulentus* was fractionated, in order to isolate and / or purify the metabolite with inhibitory activity. To isolate the compounds present in the E.E. of *C. pulverulentus*, the extract was brought to total dryness and the dry residue was fractionated with solvents of increasing polarity. 6 fractions were obtained in which the partitioning of the compounds present in the E.E. The compounds of E.E. were distributed in each fraction according to the polarity of the solvent used

The organic fractions were concentrated to dryness to obtain the residue, they were weighed and the yield was determined (Table 2).

Fraction	Weight	Performance %
Hexanic	0.4651 g	0.4651 %
Chloroform	0.0180 g	0.0180 %
AceOEt	0.0090 g	0.0090 %
Acetone	0.0128 g	0.0128 %
Ethanolic	0.1294 g	0.1294 %
Methanolic	0.3054 g	0.3054 %

Table 2. Yield of organic fractions obtained from E.E *.
* Yield based on 100 g dry weight of the plant

Antimicrobial activity of the organic fractions obtained from E.E.

To determine the effect of the organic fractions, 30 µg per disk were used to make the comparison with the effect of the reference antibiotic (Kanamycin).

It was tested against the bacteria from the first screen to identify if the fractions have an effect. The results obtained show that the hexane, chloroformic, AceOEt and acetonic fractions are inactive against the bacteria evaluated. However, the ethanolic and methanolic fractions generate a concentration-dependent halo of inhibition. In the case of *Bacillus* sp. the ethanolic fraction generates a halo of 8 ± 0 mm, while the methanolic fraction 15 ± 0.5 mm, indicating that the bioactive compound of E.E. is in the last fraction. In *E. faecalis*, an inhibition halo similar to that generated by E.E. complete, which according to CLSI indicates that the bacterium has resistance to the extract (Table 3).

Bacterium	Fraction / Inhibition mm		
	EtOH	MeOH	Control
Escherichia coli	0	0	20
Klebsiella pneumoniae	0	0	20
Aeromonas hydrophila	0	0	20
Enterococcus faecalis	7 ± 0	6 ± 0	15
Bacillus sp.	8 ± 0	15 ± 0.5	19
Chromobacterium violaceum CV026	0	12 ± 0	0
Kanamycin control	Bacteria-dependent inhibition according to international standards (BSAC, 2013).		

Table 3 Antimicrobial effect of *Costus pulverulentus* fractions at 30µg / disc (Concentration established for kanamycin discs, which is the reference antibiotic)

Open Column Chromatography of the Methanolic Fraction

Once the bioactive fraction had been identified, it was separated by open column chromatography. 200 mg of the methanolic fraction were used and eluted with a polarity gradient with the Hexane: AceOEt: MeOH solvent system. 32 (F1-F32) fractions were obtained and brought to dryness on the Rotavapor R-100, the fractions were resuspended in methanol and analyzed by TLC. The process was repeated 10 times obtaining the same pattern of color and bands, by TLC.

Antimicrobial Activity of the Fractions (F1-F32)

To determine the antimicrobial activity of the 32 fractions, 10 µg of fraction per disc were placed and evaluated against microorganisms that previously showed greater sensitivity to the methanolic fraction, *Bacillus* sp.

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The results obtained show that Fraction 8 (F8) generates an inhibition halo of 18 ± 0 mm in the culture of *Bacillus* sp., Indicating that F8 possesses it or the metabolites responsible for the inhibitory effect generated by the methanolic fraction. The rest of the fractions did not show an inhibitory effect at the dose evaluated.

Antimicrobial activity against clinically important microorganisms

Once the F8 was obtained, inhibition tests were carried out against bacteria of priority importance according to the World Health Organization. For this, the guidelines established by the CLSI (Cos, Vlietinck, Berghe, and Maes, 2006; Balouiri, Sadiki, and Ibnsouda, 2016) were used. 30 µg of F8 per disc were used and evaluated against representative bacteria of each microbial group (Table 4).

Group	Representative bacteria	Inhibition (mm)	
		F8	Km*
Gram-positive cocci	*Staphylococcus aureus* ATCC 29213	18±0	19
	Staphylococcus aureus Ox^R	18±0 (Het)	19
	Staphylococcus epidermidis	0	25
Non-encapsulated Enterobacteria	*Escherichia coli* ATCC 15597	0	20
	Escherichia coli BLEE +	0	20
	Escherichia coli BLEE -	0	20
Encapsulated Enterobacteria	*Klebsiella pneumoniae* ATCC 22895	0	20
No enterobacteria	*Pseudomonas aeruginosa* ATCC 30298	0	22
Chromobacterium violaceum CV026		0	0

Table 4 Representative bacteria by group of microorganisms evaluated against fraction 8 (F8) of C. pulverulentus

The results obtained show that F8 inhibits the development of *S. aureus* ATCC 29213 and *S. aureus* OxR and has no effect against the Gram negative bacteria evaluated. For the case of *S. aureus* ATCC 29213, a halo of 18 ± 0 mm was obtained (Figure 2), which according to the CLSI criteria indicates sensitivity to F8. Regarding *S. aureus* OxR, F8 generates a halo of 18 ± 0 mm in diameter (Figure 3); however, there is development of colonies within the halo suggesting that the strain has heterogeneous susceptibility.

Similar results were obtained with the antibiotic used as reference (Km). In the case of Enterobacteriaceae and the *Pseudomonas aeruginosa* strain ATCC 30298, F8 does not exert an inhibitory effect. Regarding the effect against Gram negative bacteria, no significant effect was observed.

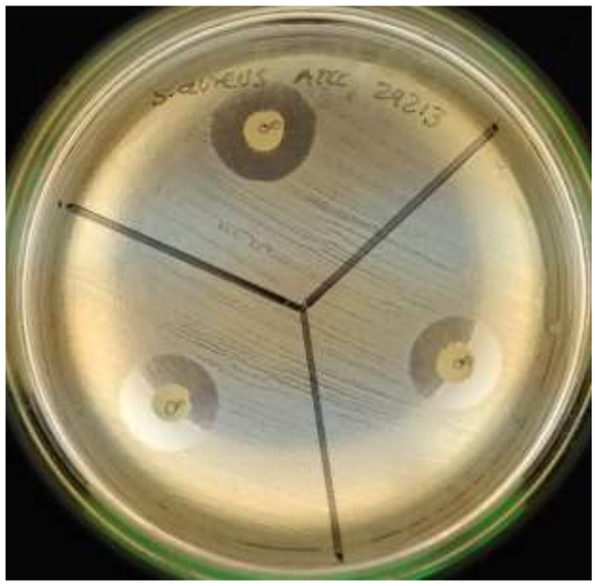

Figure 2 F8 inhibitory effect against: A) *S. aureus* ATCC 29213

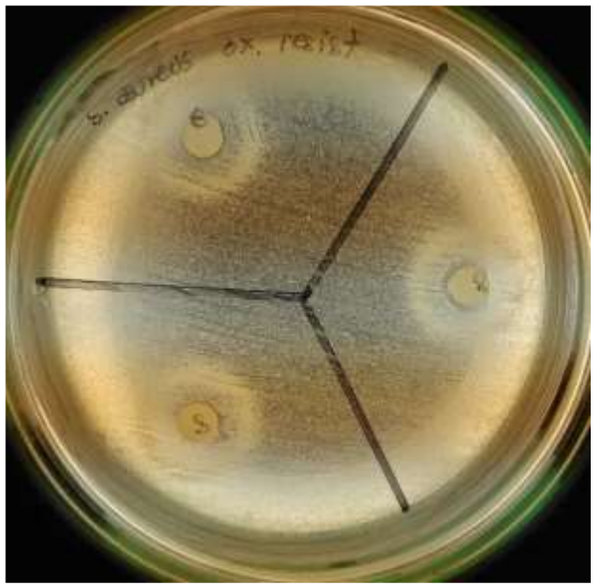

Figure 3 F8 inhibitory effect against: A) *S. aureus* oxacillin resistant (heterogeneous susceptibility)

Analysis and characterization of F8

The F8 was analyzed by GC-MS to determine the compounds responsible for the inhibitory activity. The chromatogram analysis revealed the presence of 16 peaks, of which 13 peaks (81.25%) correspond to organic acids, 2 (12.5%) were not identified in the NIST library and 1 (6.25%) was identified as glycerol (Table 5).

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T.R	Area	AR %	Compound
12.47	182229996	3.18	Lactic acid
12.89	49836550	0.87	Hydroxyacetic acid
18.86	2194509791	38.33	Glycerol
19.39	32683803	0.57	Maleic Acid
19.64	344369982	6.02	Succinic acid
20.29	53985329	0.94	Glyceric acid
20.49	14528588	0.25	Fumaric Acid
20.95	20439199	0.36	NI
22.93	72596375	1.27	3,4-Dihydroxy butanoic acid
24.32	55911259	0.98	Malic Acid
27.25	88872730	1.55	P-Hydroxy benzoic acid
30.38	1158551128	20.24	Vanilic acid
30.81	101690192	1.78	P-Cumaric acid isomer 1
30.99	16801180	0.29	Azelaic Acid
32.45	29039520	0.51	NI
33.86	1309135295	22.87	P-Cumaric acid isomer 2

Table 5 Chromatographic profile of F8
NI = Compound not identified in the database

Among the most abundant compounds, glycerol is found in 38.33% relative abundance. Glycerol at concentrations of 50 and 80% has been shown to alter the viability of *P. aeruginosa*, *Staphylococcus* sp., *Bacillus* sp., This effect depends on the concentration and the exposure time (Saegeman et al. 2008). Among the organic acids identified, the most abundant are p-coumaric acid in 24.65% and vanilic acid in 20.24%; these belong to the group of polyphenols, and could be responsible for the antimicrobial effect of F8 against *S. aureus* ATCC 29213 and *S. aureus* OxR (heterogeneous). In addition, a third polyphenol was identified, p-hydroxybenzoic acid, which could exert a synergistic effect with the major acids in F8.

Antibacterial effect of p-coumaric acid and vanillic acid

To determine the effect of the major F8 compounds, p-coumaric acid (Sigma C9008) and vanillic acid (Sigma 94770) were obtained from Sigma Aldrich and the antimicrobial activity was evaluated by plate microdilution. The MIC of p-coumaric acid and vanillic acid against *S. aureus* ATCC 29213 was determined because it was the bacteria most sensitive to F8.

To determine the potential of the pure compounds, the analysis included the representative bacteria of each group according to WHO, the non-capsulated enterobacterium *E. coli*, the capsulated enterobacterium *K. pneumoniae* and *P. aeruginosa* that does not belong to the enterobacteria. In addition, the effect was evaluated against *S. epidermidis*, *Proteus mirabilis* and *Citrobacter freundii*. The results obtained show that p-coumaric acid has an antimicrobial effect against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *Citrobacter freundii* and *K. pneumoniae* (Figure 4); while vanillic acid does not show an inhibitory effect at the concentrations evaluated (Table 6).

In order to determine if the combination of acids could enhance the antimicrobial activity, mixtures of p-coumaric acid with vanillic acid were made in a ratio of 1: 0.88 (The relationship is based on the results obtained from fraction 8 in the gas chromatography). Similar doses to individual compounds were evaluated. The results obtained showed that the inhibition pattern was not enhanced with the mixture of compounds. On the contrary, the effect in mixing decreased, because the amount of p-coumaric acid was reduced.

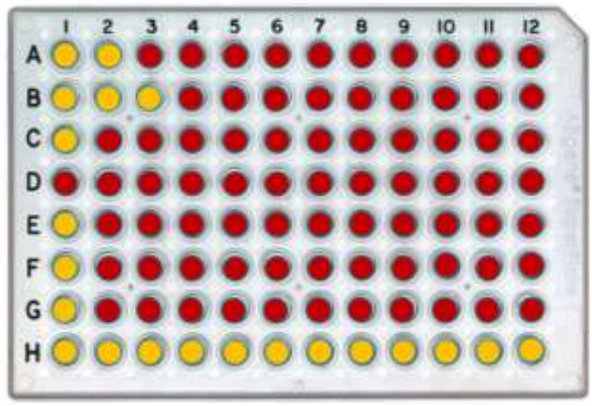

Figure 4.*Rows with MH medium were inoculated with A) P. aeruginosa, B) S. aureus, C) E. coli, D) P. mirabilis, E) S. epidermidis, F) C. freundii, G) K. pneumoniae, H) Negative control, MH medium. In each column the concentration of p-coumaric acid 1) 1000 µg / mL, 2) 500 µg / mL, 3) 250 µg / mL, 4) 125 µg / mL, 5) 62.5 µg / mL, 6) 31.25 µg / mL, 7) 15.62 µg / mL, 8) 7.82 µg / mL, 9) 3.90 µg / mL, 10) 1.95 µg / mL, 11) 0.97 µg / mL, 12) 0 µg / mL. The well in red color indicates bacterial activity by reduction of tetrazolium chloride.*

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Microorganism	CMI (µg/mL) P-coumaric acid	Vanillic acid	Acid mixture
P. aeruginosa ATCC 30298	500	> 1000	> 1000
S. aureus ATCC 29213	250	> 1000	> 1000
E. coli ATCC 15597	1000	> 1000	> 1000
P. mirabilis	> 1000	> 1000	> 1000
S. epidermidis	1000	> 1000	> 1000
C. freundii	1000	> 1000	> 1000
K. pneumoniae	1000	> 1000	> 1000

Table 6 Minimum inhibitory concentration

Toxic activity against A. salina

Artemia salina is one of the most valuable test organisms available for toxicity testing. Due to the speed, convenience and low cost of Artemia-based assays, the different extracts and organic fractions of *C. pulverulentus* were evaluated for toxicity. The results show that there is no toxic effect of the complete extract (750-30000 ppm), methanolic fraction (375-15000 ppm) and fraction 8 (25-1000 ppm) on nauplii of *A. salina* (Table 7) for the toxicity test acute in a 24-hour period (Rajabi, 2015).

Complete extract *	Methanolic fraction *	[F 8]*	Mortality (%)
750	375	25	0
1500	750	50	0
3000	1500	100	0
7500	3750	250	0
15000	7500	500	0
30000	15000	1000	0
Saline solution control			0
Saline + solvent control			0

Table 7 Toxicity test of E.E of Costus pulverulentus fractions on Artemia salina. * Concentrations expressed in ppm (µg / mL)

Discussion

Studies on *C. pulverulentus* have focused mainly on its distribution and biology (Guzmán, 2015; Sytsma et al., 1985). However, there are few studies of the biological and chemical activity of the plant. Regarding the pharmacological activity, it has been shown that the Ethanolic Extract of the stems of *C. pulverulentus* has cytotoxic activity against the PC3 prostate cancer cell line, anti-inflammatory and antinociceptive activity in mouse models. Likewise, it has been shown that it does not exert toxic effects in the evaluated models (Alonso-Castro et al., 2016).

With regard to phytochemistry, studies have focused on the characterization of the Ethanolic Extract of the stems in which the presence of organic acids and sterols has been identified (Alonso-Castro et al., 2016), many of which possess antinociceptive, anti-inflammatory, antiproliferative and antimicrobial activity (Valerio and Awad, 2011; Liu et al., 2019; Santos et al., 2013; Walker et al., 2017; Novotny et al., 2017).

In the Huasteca Potosina, *C. pulverulentus* (Costaceae) is used in traditional medicine for the treatment of a condition colloquially called urine disease, whose symptoms correspond to a urinary tract infection (UTI) (Andreu et al., 2011) . UTIs are a serious public health problem and are caused by a variety of pathogens, commonly *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus*, affecting approximately 150 million people each year worldwide (Flores-Mireles et al., 2015).

The traditional use of *C. pulverulentus* (Costaceae) for the treatment of "bad urine" suggests that the plant possesses bioactive metabolites with antimicrobial activity. Therefore, in this work, the antibacterial activity of the plant was evaluated against bacterial strains that commonly cause UTIs. Additionally, the effect against bacterial species whose resistance to antibiotics makes it necessary to search for therapeutic alternatives was evaluated.

To carry out this study, the Ethanolic Extract (EE) of the stems of *C. pulverulentus* was elaborated, in order to identify the bioactive metabolite, and the antimicrobial effect against *Escherichia coli*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Enterococcus faecalis* was determined. and *Bacillus* sp. The results obtained show that *E. faecalis* and *Bacillus* sp. show moderate sensitivity to E.E. of *C. pulverulentus*, while Gram negative bacteria are resistant to the doses evaluated.

In most antimicrobial studies, Gram negative bacteria appear to show greater resistance to natural products compared to Gram positive bacteria. This fact could be related to the complexity of the membrane of Gram negative bacteria (de la Fuente-Salcido et al., 2015).

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In the case of this bacterial group, the alternative has been to search for bioactive metabolites with anti-quorum sensing activity (Olivero et al., 2011). Therefore, in this study, the anti-QS activity of E.E. of *C. pulverulentus* in the inhibition of the production of the pigment violacein in *C. violaceum* CV026. The results obtained showed that the production of violacein is not affected by E.E., indicating that the extract of *C. pulverulentus* does not have anti-QS compounds. However, it shows growth inhibitory effect of *C. violaceum* CV026 at a dose of 10 μg / disc, generating a 12 mm inhibition halo in the inoculated plate.

The chemical characterization of the extract shows the presence of carbohydrates, organic acids and sterols, in a similar way to that previously reported (Alonso-Castro et al., 2016). Phenolic compounds were identified among organic acids, to which antimicrobial activity has been attributed (Creus, 2004). In this work, the evaluated doses of E.E. of *C. pulverulentus* exert moderate antimicrobial activity against *E. faecalis*, *Bacillus* sp. and *C. violaceum* CV026.

To determine the bioactive metabolite (s) responsible for the antimicrobial effect, the E.E. of *C. pulverulentus* (Costaceae) using solvents of different polarity (hexane, chloroform, ethyl acetate, acetone, ethanol and methanol) in order to purify and separate the compounds contained in the mixture, subsequently the antimicrobial activity of the fractions was determined. The results obtained show that the methanolic fraction preserves the antimicrobial activity against *E. faecalis*, *Bacillus* sp. and *C. violaceum* CV026, while the rest of the fractions did not show inhibitory effects. Results suggest that bioactive compounds are polar in nature.

The methanolic fraction was purified by column chromatography in order to obtain the bioactive metabolite responsible for the antimicrobial activity. 32 fractions were obtained and antimicrobial activity was evaluated. The results obtained showed that only fraction 8 (F8) conserved the biocidal activity against *Bacillus* sp., Indicating that the bioactive metabolite is found in this fraction. Regarding *C. violaceum* CV026, none of the fractions showed an inhibitory effect, suggesting that the biocidal activity of F8 is due to the synergistic effect of the compounds present in it.

Additionally, F8 inhibits the development of *S. aureus* ATCC 29213 and *S. aureus* OxR, however, at the doses evaluated it lacks an inhibitory effect against the Gram negative bacteria used in the study.

Analysis of F8 by GC-MS shows the presence of 16 peaks, indicating that column chromatography does not resolve the metabolites present in this fraction. According to the analysis, F8 mainly contains three compounds from the group of polyphenols, p-coumaric, vanillic and p-hydroxybenzoic acids, which could be responsible for the antimicrobial effect of F8 against *S. aureus* ATCC 29213 and *S. aureus* OxR.

To determine the individual effect of the compounds present in F8, the two major acids, p-coumaric acid and vanillic acid, were selected. The MIC for organic acids was determined by 1: 2 dilutions according to Cos et al. (2009). The results showed that p-coumaric acid has antimicrobial activity against *S. aureus* ATCC 29213 at a MIC of 250 μg / mL. The effect of p-coumaric acid against *S. aureus* is controversial, because studies in this regard report different MIC doses from each other, which may be due to the technique used and the strains evaluated in the determinations. Alves et al. (2013) suggests that p-coumaric acid exerts antimicrobial activity against sensitive (MSSA) and methicillin resistant (MRSA) *S. aureus* at a MIC greater than 1000 μg / mL, while Kozyra et al. (2019) found no activity of p-coumaric acid against the type strains of *S. aureus* ATCC 6538 and *S. aureus* ATCC 25923.

On the other hand, Krishna et al. (2014) shows that 200 μg of p-coumaric acid generate an inhibition halo in the development of *S. aureus* by means of disk diffusion tests. In *S. epidermidis*, a MIC of 1000 μg / mL was determined, similar to that previously reported for the strain type *S. epidermidis* ATCC 12228 (Kozyra et al. 2019), and lower than that reported by Alves et al. (2013) who suggest that the MIC for *S. epidermidis* is greater than 1000 μg / mL.

Regarding Gram negative bacteria, for *P. aeruginosa* ATCC 30298 a MIC of 500 μg / mL was determined. So far there are no reports where the activity of p-coumaric acid against this bacterium is determined.

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However, p-coumaric acid at 61.0 μM has been shown to inhibit the development of *Pseudomonas putida* by 33% (Rühl et al. 2009). Regarding the rest of the Gram negative microorganisms, a MIC of 1000 μg / mL was determined for *E. coli* ATCC 15597, *C. freundii*, *K. pneumoniae*, while *P. mirabilis* was the most resistant bacterium with a MIC greater than 1000 μg / mL. In previous studies, the antibacterial potential of p-coumaric acid against *E. coli* was demonstrated with a MIC of 1000 μg / mL, similar to the dose obtained in this work (Alves et al. 2013).

In general, p-coumaric acid has been shown to exert antibacterial activity by significantly increasing the permeability of the outer membrane and the plasma membrane (Lou et al., 2012). Likewise, it has been suggested that it binds to bacterial genomic DNA to inhibit cellular functions (Lou et al. 2012). Due to its antibacterial properties, p-coumaric acid has been evaluated in food systems as a preservative agent and growth inhibitor of *S. aureus*, the main food contaminant (Stojković et al., 2013). P-coumaric acid at concentrations of 0.23 to 1.87 μg / mL has been shown to inhibit *in situ*, in prepared chicken broth and pork, the growth of *S. aureus* for a period of 72 h. In addition, the inhibitory effect of p-coumaric acid improves the organoleptic characteristics of food, which shows its usefulness as a preservative and flavor enhancer for the food industry (Stojković et al. 2013).

Regarding vanillic acid, it was determined that the concentrations used did not inhibit the development of the Gram-positive microorganisms evaluated, suggesting that the MIC is higher than 1000 μg / mL. The doses determined in this study are similar to those reported by Kozyra et al. (2019) for *S. epidermidis* ATCC 12228, who show that the MIC is greater than 1000 μg / mL. On the other hand, it has been shown that vanillic acid does not inhibit the development of the type strains, *S. aureus* ATCC 6538 and *S. aureus* ATCC 25923 (Kozyra et al. 2019). However, Alves et al. (2013) demonstrated a MIC of 1000 μg / mL and 500 μg / mL of vanillic acid for sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus*, respectively. *S. aureus* MRSA is a causative agent of difficult-to-treat nosocomial infections due to the emergence of resistant strains and the limited availability of efficient antibiotics (Chambers 2001).

Therefore, it is necessary to search for alternatives to control this microorganism. In this sense, it has been shown that vanillic acid acts as an inhibitor of beta-lactam-resistant peptidoglycan PBP2a MecA transpeptidase in *S. aureus* MRSA (Alves et al. 2013), being an excellent alternative for antibacterial treatment. Despite the fact that in this study no significant antibacterial activity of vanillic acid was found against the available strains of *S. aureus*, our results show that *C. pulverulentus* is a potential source of vanillic acid that can be used for the treatment of infections caused by MRSA.

Regarding Gram negative bacteria, the MIC for vanillic acid is higher than 1000 μg / mL, these results differ from the inhibitory effect reported by Alves et al. (2013) with a MIC of 1000 μg / mL for *E. coli* and *P. mirabilis* (Alves et al. 2013).

Additionally, a mixture of p-coumaric acid and vanillic acid was made in a proportion similar to that identified in F8. The mixture was evaluated in order to determine if the combination of compounds enhanced antimicrobial activity. However, the activity decreased because the concentration of p-coumaric acid was reduced. This suggests that p-coumaric acid is primarily responsible for the antibacterial activity of F8.

Conclusions

The complete extract and ethanolic fraction of *Costus pulverulentus*, in addition to the activities previously reported, shows the ability to inhibit the growth of bacteria of medical importance, mainly on *S. aureus*. In the subsequent column fractionation, only fraction number 8 maintains activity against *Bacillus* sp. and *S. aureus* ATCC 29213 and resistant Ox in concentrations of 30 μg / disc, showing a medium susceptibility according to the CLSI criteria. After gas chromatography coupled with mass spectrometry, the antimicrobial activity is attributed to the major metabolites of fraction 8, which are vanillic acid and p-coumaric acid, which are polar chemical compounds from the secondary metabolism of the plant and which can be classified as phenolic compounds. The microdilution plate test shows that the compound with the highest activity is p-coumaric acid on *S. aureus* ATCC 29213 at a concentration of 250 mg / L.

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The evidence allows us to suggest the absence of acute toxicity of the complete extract and the fractions evaluated in the standardized test with *A. salina*.

The Ethanolic Extract and the fractions obtained from *C. pulverulentus* did not show anti-QS activity on *C. violaceum* CV026 at concentrations of 10 and 30 µg / disc, however, if growth inhibition is observed despite the fact that the strain has genes resistance to kanamycin, the antibiotic used as a control.

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Review of floral polymorphism in chía (*Salvia hispanica* L.): Modified cause

Revisión del polimorfismo floral en chía (*Salvia hispanica* L.): Causa modificada

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Abstract

This article reviews the current state of knowledge about the color of the flower of the chia (*Salvia hispanica* L.) plant because it presents different colors, within a population that was the basis for the generation of five internationally marketed varieties. After a historical review of the most influential studies on floral color polymorphism, the different types of pigments involved and other anthropogenic manipulations such as the application of ionizing radiation that may affect the final color of flowers are analyzed; the latter is involved in the current polymorphism of this species. Although there is a great diversity of types of floral polymorphism, those related to the loss of anthocyanic pigments are the most frequent in wild species. On the contrary, in chia it is the opposite, so far there are no study reports on chia cultivation that support this relationship, in this article we propose a possible hypothesis in relation to a genetic mechanism that is the modified cause of the floral polymorphism it presents.

Polymorphism, Ionizing radiation, *Salvia hispanica* L.

Resumen

En este trabajo se revisa el estado actual del conocimiento sobre la coloración de la flor de la planta de chía (*Salvia hispanica* L.) dado que presenta diferentes coloraciones, dentro de una población que fue base para la generación de cinco variedades comercializadas internacionalmente. Tras un repaso histórico de los estudios más influyentes sobre polimorfismo en el color floral, se analizan los diferentes tipos de pigmentos involucrados y otras manipulaciones antropogénicas como la aplicación de radiación ionizantes que pueden afectar al color final de las flores; este último involucrado en el polimorfismo actual de la chía. Aunque existe una gran diversidad de tipos de polimorfismo floral, los relacionados con la pérdida de pigmentos antociánicos son los más frecuentes en especies silvestres. Por lo contrario, en la chía es lo inverso, hasta el momento no existen reportes de estudio en el cultivo de la chía que sustente esta relación, en este trabajo se propone una posible hipótesis en relación con un mecanismo genético que sea la causa modificada del polimorfismo floral que presenta.

Polimorfismo, Mutación por radiación, *Salvia hispanica* L.

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Introduction

Studying the structures of flowers has fascinated many botanists such as Lineo, who used structures to classify different species, and evolutionary biologists such as Ronald Fisher who laid the mathematical foundations for the study of their evolution (Barrett, 2002). On the other hand, the color of flowers has been a subject of study that has fascinated many; For example: Aristotle, Mendel, Batenson among others, currently continues to generate a large amount of bibliography, being a model system that allows the depth of the study of ecological and evolutionary processes.

For example, flower color constitutes a simple model for studying the adaptation of plants to new pollinators (Hoballah et al., 2007). and the knowledge of the genetic basis that determine floral color has allowed integrating molecular genetics with evolutionary ecology (Álvarez-Buylla et al., 2017). The floral color polymorphism is the discrete and qualitative variations of floral color between individuals of the same species both at the population level and between populations (Narbona, 2014).

Color in flowers

Although Gregor Mendel (1866) is considered the father of genetics, for his contribution to his famous laws of inheritance. A Mendel observation that is probably the least known was that the absence of pigments in the white petals was associated with the lack of pigmentation in the seed coat and in the leaf axilla (Ellis et al. 2011; Reid and Ross 2011). In 1916, the book "Anthocyanin pigments in plants" by Muriel Whledale was published, where numerous cases of polymorphisms based on anthocyanin compounds were documented and the patterns of variation and their inheritance were analyzed in detail.

Perhaps one of the most studied polymorphism cases is that of Linanthus parryae, this species has an interesting distribution pattern of floral color morphotypes, since there are monomorphic populations and dimorphic populations, with white and blue individuals (Epling and Dobzhansky, 1942).

Pigments are chemical compounds that, when light falls on them, absorb certain wavelengths and reflect the rest (which is what we perceive as color). Humans detect colors with wavelengths between 380 and 730 nm (nanometers); however, pollinators, mainly insects and birds, also perceive shorter "ultraviolet" wavelengths (Kelber et al., 2003). Pigments in plants are classified into four large groups: chlorophylls, carotenoids, flavonoids and betalains. Chlorophylls, which provide a green color, are a constitutive part of vegetative tissues and sepals. That rarely accumulate in the petals or tepals, although there are exceptions such as in the Orchidaceae family (Vignolini et al., 2012).). Carotenoids are isoprenoids and generate the colors yellow, orange and red in some flowers (Lee, 2007). Flavonoids are the pigments that provide the most color diversity to flowers (Lee 2007; Miller et al. 2011). Among them, anthocyanins are the most important flavonoids and provide an orange, red, blue, pink, purple color, and in some cases almost black (Shikazono et al. 2008). The white color in the petals generally responds to the lack of pigments; As there are no pigments that absorb a part of the light spectrum, it is completely reflected. Only one type of pigment accumulates in the petals (Glover 2007; Lee 2007).

Not all color polymorphisms are equally frequent or come from the evolution or co-evolution that has been given to cultivated species. In wild species, more diversity of polymorphisms can be found; For example, we can cite various cases of variation in the color of the flowers, for example in the Lycoris longituba species, the color of the petals of its leaves can vary purple, red, orange-yellow (He et al. 2011). In Ursinia calenduliflora we can find orange, red and black, although in this one, it is due to the presence of macules on the petals. (From Jager and Ellis, 2014). The pink to white Mimulus lewisii species (Wu et al., 2013). and Parrya nudicaulis purple to white (Dick et al. 2011). This may possibly be related to what can be found in S. hispanica L. On the other hand, there are other types of polymorphism that affect the uniformity of the color of the petals. Thus, there may be a variation in the presence or absence of macules or venation on the petals.

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Mutagenesis in plant breeding.

The discovery of X-rays, radioactivity and the handling of radioactive elements, gave rise to the deliberate induction of mutations in the genetic structure in plants. These mutations have generated a number of successful and promising varieties.In 2017, more than 700 varieties were registered in the database of mutant varieties of the joint agency of the Food and Agriculture Organization of the United Nations, and the International Atomic Energy Agency. Among the main reported genera, Chrysanthemum, Rosa, Dahlia and Alstroemeria stand out, with 283, 67, 35 and 35 registered varieties, respectively (Hernández-Muñoz, 2020).

It should be noted that the demand for novel varieties continues to grow and implies a challenge for plant breeders. One of the techniques that dramatically induces mutagenesis in plants is ionic irradiation. When plant material is irradiated, due to its characteristics and its high penetration range, it can cause changes in important molecules such as deoxyribonucleic acid (DNA), which translates into variation in plant species.

Chia and its appearance

The research on chia is recent compared to other crops that are the basis of food, it began in the 90's, the chemical composition of the seed being the main focus of research centers, in this sense science began to determine the stability of the oil production present in the grain and its composition in general; Thanks to this, today chia is considered one of the most important sources of polyunsaturated fatty acids (AGPINs or PUFAs in English) Omegas 3 and 6, proteins, oil and fiber (Baginsky et al., 2016; Ullah et al. ., 2017) .The consumption of fatty acids (Omega 3) favors the deformation of erythrocytes and the decrease of viscosity in the blood, this effect provides greater oxygenation to the tissues, as well as reduces cholesterol and triglycerides in blood also reduces blood pressure, which is associated with cardiovascular diseases (Coelho and Salas, 2014). Chia seed contains between 9 and 23% protein, 26 to 41% non-fibrous carbohydrates and 30 to 33% of the total weight of the seed is oil. That is why it is considered as a functional food and hence its importance today (Oliveros and Paredes, 2013; Olivos-Lugo et al., 2010).

Regarding the floral characteristics, few studies speak of the relationship of the flowers in relation to the color of the seeds. Since the importance falls on the color of the seed, which is currently an influence on commercialization. At present, only five commercial registered varieties of chia are reported (Sosa et al., 2016), the first developed in the United States (Hearthland, 2016; Jamboonsri et al., 2012), being available to the public in white and black seed, being the Hearthland company that has the breeder's right (Hildebrand et al., 2016). The three remaining genotypes were registered in Argentina, which are named Sahi Alba 911, Sahi Alba 912 and Sahi Alba 914 (Bochicchio et al., 2015). Currently Mexico already has a high-yield line, being the first variety of white chia (Rovati et al., 2012). Similarly, in 2019 the patent “Patent No .: US20190183084A1 chia variety designated rehnborg” is obtained (Sosa-Baldivia et al 2019).

It is necessary to mention that to generate the five previous varieties of chia; A variety of Mexican called "Pinta" that comes from the state of Jalisco, specifically from the Acátic municipality, was used as a genetic source. Which is a mixture of black and white seeds in a 9: 1 ratio (Coates and Ayerza, 1998; Jamboonsri et al., 2012; Sosa et al., 2016).

Methodology

Project location and plant material

In 2017, the chia variety "pinta" was established in the experimental fields of the Sustainable and Protected Agriculture career of the Technological University of the Southwest of Guanajuato, in Valle de Santiago. In which floral polymorphism was identified in the population, they were selected and protected.

The protected seeds were established in 2019, in the same fields of the University. Showing the same polymorphism. In the literature, the existence of a great diversity of types of floral polymorphism is reported, most of them are related to the loss of anthocyanin pigments and are the most frequent in wild species, which is why it is striking in the cultivation of chia. The variety "pinta" is cultivated in different parts of the country.

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Results and discussions

The polymorphism presented in the evaluated population of the most representative chia "Pinta" was the following:

Figure 1 Floral polymorphism in chía "Pinta"

As can be seen, the variation of these colors in chia is diverse, it was reviewed in the literature, with the possibility of finding an explanation for this variation presented by the "Pinta" population, and its possible anthropogenic management, the following was found:

The Mexican chia and its appearance in the world

The integration of chia into modern agriculture began in 1991 with the ¨Western Argentina Regional Project¨. The results obtained from this project were so impressive that the implementation of the technology generated led to the area cultivated with chia going from 500 hectares in 1994 to 370,000 hectares in 2014 (Orozco. 1993, Weber et al. 1991). But as the interest in the cultivation of chia arises, perhaps the base report in the bibliographic review carried out is the work is by Orozco De Rosas (1993), who in his thesis reports that the seeds used in his work were wild chías, the Which are obtained from generation to generation and from his account he mentions that for years this plant is cultivated in the town of Acatic.

There is little information regarding what happened in this period; but the history of chia was written a few years ago by Ricardo Ayerza and Wayne Coates (2006) responsible for the ¨Western Argentina Regional Project¨, the two foreign researchers (Canadian and Argentine nationals, respectively) who have conducted the most research on this culture.

Generating five varieties registered internationally and which the first place in the world production of this crop is Argentina; while Mexico is in third place (Peperkamp, 2015).

What is striking about the local varieties, there was a record, it was improved or because there is only a record of this plant outside of Mexico, since it is considered a center of origin in Mexico, are some of the questions that we can generate.

There is a report of what happened and a possible theory that is in relation to the Mexican chia and the success of the project "Western Argentina Regional Project" recovered from the website: https://web.archive.org/web/20140321145146/http : //chiablanca.com.mx: 80 / investigation.

According to the information prepared by G. Orozco De Rosas, he mentions the following: “Acatic, Jalisco. located in western Mexico, where the natives were of Nahua origin, they conserved the cultivation and it has been the center of development of this variety until today. Not only has it been cultivated since time immemorial, but all the technology for producing, harvesting and processing the grain for Central, South America and Australia was generated here. All the varieties of chia that are currently planted in the world have their origin in Acatic, Jalisco since the native variety of Acatic is a mixture of 90% black seeds and approximately 10% white seeds.

Due to its characteristics of Precocity and performance adapted to the agroecological conditions of Acatic, where the plant produces the highest amount of Omega 3 fatty acids and antioxidants. Pioneers in research and development of this crop since 1987, currently the result has been that CHIABLANCA SC DE RL, a family cooperative, has the largest collection of Agronomic research on this important crop".

On the same page, Orozco De Rosas describes that this crop has been planted since time immemorial. Regarding the maternal relatives, there is no exact date of the initiation of his cultivation, only the reference of his great-great-grandparents.

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He initiates experimental work on the selection of lines of plants with contrasting characteristics, varieties with black seeds, white seeds, blue pubescence, pigmentation on the stem, and wild species with open calyx. On the other hand, in search of precocious silvers that were not affected by the lack of humidity and frost, he decided to look for diversity. With the application of X-rays to the seeds, as a result of that work in 1991, he already had several pure lines of plants of: white seeds, black seeds, light blue flowers. strong blue flower (almost purple), blue pubescence, pigmentation on the stem.

Although, this is clear that on the one hand C. Orozco de Rosas is a key element in the generation of genetic diversity of this species of chia and perhaps the use of X-rays is the answer to the polymorphism of chia. Since this happened before the entire report from the great project "Western Argentina Regional Project" was released. The question is why other researchers from other countries made it known and not the Mexicans.

There is a large number of scientific articles and studies in magazines, books and web pages, and according to countless authors, on floral polymorphism and its possible causes, which help us document and compare them with each other, and thus be able to generate An overview of the floral polymorphism that occurs in the flowers of various species and botanical families, using this as a reference to propose a possible cause of the floral polymorphism in chia (S. hispanica L.), we know that the existence of the white chia that has white flowers and black chia that has blue-purple flowers that are the characteristics of internationally registered chias.

In relation to the literature, in the case of plants with white flowers they produce white seeds and the plants with blue or colored flowers produce black seeds, so it differs from what Orosco de Rosas mentions, it should be noted that perhaps this morphology that It occurs in the flowers in chia can prompt a more specific research topic, for example, the study of the plant-insect relationship. One answer to what may have happened in this culture of S. hispanica L. is to compare it with the work of Hellens et al. (2010), which identified the genes A and A2 of the pea.

A is the factor that determines anthocyanin pigmentation in pea that was used by Gregor Mendel 160 years ago in his study of heredity. Gene A encodes a transcription factor bHLH. The white flower mutant allele most likely used by Mendel is a simple transition from Guanine to Adenine at a splice donor site leading to a mis-spliced mRNA with a premature codon, and a rare second mutant allele has been identified. The A2 gene encodes a WD40 protein that is part of an evolutionarily conserved regulatory complex.

Conclusions

The flower color polymorphism of chia (Salvia hispanica L.) with respect to its generation of diversity has been documented by the use of X-rays, which gives us an overview of the possible cause of floral polymorphism in this species. Compared to the work of the pea, it can be investigated that possibly a gene that controls color in flowers may have been silenced or activated with the application of X-rays, hence the difference in color in the petals of the flowers in Chia, remains to be verified. and there is a high probability that the flower polymorphism in chia is due to anthropogenic modification.

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Comparative characterization of starch biopolymers extracted from cereals using two different techniques

Caracterización comparativa de biopolímeros de almidón extraídos de cereales mediante dos técnicas diferentes

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Abstract

In the field of research and consequent elaboration of biopolymers it has been growing in the last decade, either due to the hardening of the environmental legislation of each country or due to ecological awareness, in any case the term biopolymer is quite broad, these being used as material premium in medicines, food supplements and of course in the production of bioplastics. Since the topic of interest is the comparative characterization of biopolymers using starch extracted from two different cereals, oats and rice, using the alkaline technique with NaOH treatment and the milling technique, assuming that the starch extracted from the *Oryza sativa* cereal presents chemical characteristics, geometric and mechanical superior in both techniques used unlike the polymers made with *Avena sativa* starch. These results suggest that the starch quality of each cereal is relevant for the production of biopolymers [1]. The commercially significant properties of starch, such as its mechanical strength and flexibility, depend on the strength and character of the crystalline region, which depends on the ratio of amylose and amylopectin.

Resumen

En el campo de investigación y consiguiente elaboración de biopolímeros ha ido creciendo en la última década, ya sea debido a endurecimiento de las legislaciones ambientales de cada país o por conciencia ecológica, en todo caso el término biopolímero es bastante amplio, siendo estos utilizados como materia prima en medicamentos, suplementos alimenticios y por supuesto en la elaboración de bioplásticos. Puesto que el tema de interés es la caracterización comparativa de biopolímeros usando almidón extraído de dos cereales diferentes avena y arroz mediante la técnica alcalina con tratamiento de NaOH y la técnica de molienda, presumiendo que el almidón extraído del cereal de *Oryza sativa* presenta características químicas, geométricas y mecánicas superiores en ambas técnicas utilizadas a diferencia de los polímeros elaborados con almidón de *Avena sativa*. Estos resultados sugieren que la calidad de almidón de cada cereal es relevante para la elaboración de biopolímeros [1]. Las propiedades comercialmente significativas del almidón, tales como su resistencia mecánica y flexibilidad, dependen de la resistencia y de carácter de la región cristalina, la cual depende de la relación de amilosa y amilopectina.

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Literary Review

Structural properties of natural starch.

Starch grains are made up of macromolecules organized in layers. Two different polymer structures make up starches: amylose and amylopectin. About 20% of most starches is amylose and 80% amylopectin. Amylose molecules, located in the inner layers, are composed of approximately 200 to 20,000 glucose molecules linked by α-1,4 glucosidic bonds (Figure 1) in unbranched or helix-wound chains. Many amylose molecules have some α - D- (1,6) branches, approximately 0.3 to 0.5% of the total bonds. These are generally neither very long nor very short and are separated by great distances allowing the molecules to act, essentially with a linear polymer, forming strong films and fibers, and easily retrograded. As a consequence of the formation of helix-shaped chains, amylose fibers and films are more elastic than cellulose. Amylose is soluble in hot water which is due to the formation of a colloidal suspension. [2]

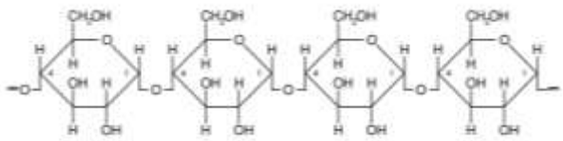

Figure 1 Segments of an amylose molecule [5]

The structure of amylopectin, located in the outer layers, is different from that of amylose. Amylopectin molecules contain α-1,4 and α-1,6 glycosidic linkages, as shown in Figure 2. Glycosidic linkages link glucose molecules in the amylopectin backbone. Branches of the main chain are frequently found, which are due to α - 1,6 glycosidic linkages with other glucose molecules. The connection points of the branches constitute between 4 and 5% of the total of the links. [6]

Amylopectin molecules are significantly larger than amylose molecules; some contain between 10,000 and 20 million glucose units [7]. The molecular weight of amylose is between 0.1 and one million g / mol. And that of amylopectin is between (10,000 to 1,000 million) g / mol [5]. One of the most important properties of natural starch is its semi-crystallinity where amylopectin is the dominant component for crystallization in most starches.

The amorphous part is made up of branched regions of amylopectin and amylose. The commercially significant properties of starch, such as its mechanical strength and flexibility, depend on the strength and character of the crystalline region, which depends on the ratio of amylose and amylopectin and therefore on the type of plant, the distribution of the molecular weight, the degree of branching and the shaping process of each polymer component. [8]

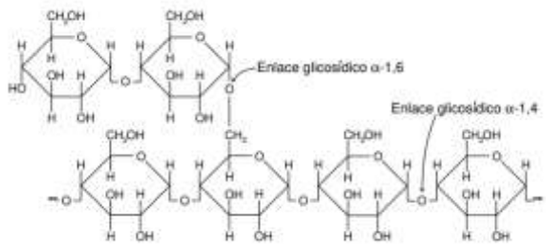

Figure 2 Segment of an amylopectin molecule [5]

Gelatinization

It is defined as the loss of crystallinity of starch grains in the presence of heat and high amounts of water with little or no depolymerization. Starch grains are insoluble in water and organic solvents. In aqueous suspension, the grains swell due to the action of heat, they tend to lose the properties that their semi-crystalline structure gives them and at a critical temperature they form a gel. [8]

During gelatinization, water initially penetrates the amorphous regions initiating swelling, which is seen by the decrease in birefringence. Then the water dislodges the starch chains from the surface of the crystals as the temperature increases; the thermal mobility of the molecules and the solvation produced by the swelling forces cause a decrease in crystallinity due to the unwinding of the double helices, until the granular structure is fragmented almost completely, obtaining a solid-gel.

The main difference between the preparation of thermoplastic starch (TPS) gels, foods, films, or processed materials is the amount of water or plasticizer during gelatinization or melting of the starch granules.

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To obtain thermoplastic starch, the starch is melted with the help of a relatively low amount of water during the extrusion, pressure molding or injection molding process, where the amount of water is below 20% in most of the cases. Some of the water is usually replaced by small amounts of glycerin. Differences in water and glycerin content and processing conditions such as: the speed of Obtaining a biodegradable polymer from corn starch, shear and temperature, produce differences in the formation of the starch network and in the morphology of the material produced. [9]

Starch, as a raw material for the production of a bioplastic

To convert a dry starch into a bioplastic material, it is necessary to break and melt its semicrystalline granular structure [10]. Starch without adequate additives (plasticizers) does not have the necessary properties to work as a thermoplastic. Plasticizers increase the flexibility of starch due to its ability to reduce the interaction of hydrogen bonds, in addition to increasing the molecular space [12]. Two types of ordering can be distinguished in thermoplastic starch after processing: residual crystallinity classified into type A, B and C forms caused by incomplete melting during plasticization and crystallinity induced during processing, according to the arrangements generated in the polymer chains according to [11] type A is common in cereal starches, B in tubers and C in certain roots and seeds.

The amount of residual crystallinity is related to the temperature and shear stress applied during processing; likewise the composition of the feed mixture also indirectly influences this amount of remaining crystallinity. Depending on some processing and storage conditions such as temperature and humidity, amorphous starch undergoes structural changes after cooling, based on: recrystallization of amylose and amylopectin in different crystal structures, phase separation and reorientation of the polymer. The molecular interactions (mainly hydrogen bonds between starch chains) that occur after cooling are called retrogradation [10]. This retrogradation also refers to the changes that take place in gelatinized starch from an initial amorphous state to a more ordered crystalline state. It occurs because starch gels are not thermodynamically stable.

According to the amylopectin chains, they are responsible for the retrogradation phenomena that are generated in the long term, while amylose is related to changes at shorter times. [10]

Materials and Methods

Starch extraction

The starch extraction method is carried out by the method used by Yamamoto, 1973 and some modifications. Where two different methodologies were used in each cereal [5]. In the first methodology, 100 grams of Oryza sativa and 100 grams of Avena sativa were subjected to a series of washes, firstly with 500 ml of distilled water each, then 500 ml of 0.1 N NaOH were placed, continuing with water distilled, and finally a neutralization with 0.5 M HCl, finally a washing with distilled water and centrifuged at 3000 rpm for 20 minutes and drying at 60 ° C for 48 hours.

The process concluded with grinding and sieving. It should be mentioned that between each wash a 24-hour rest was maintained at 6 ° C. The second methodology used the same quantities of 100 g of each cereal and consisted of letting both cereals rest with water for 24 hours. Subsequently, a simple grinding was carried out, it was centrifuged at 3000 rpm for 20 minutes. Excess water was removed and the residue was dried in porcelain capsules in an oven whose parameters were 60 ° C for 48 hours. The process concluded with grinding and sieving. [13]

Preparation of biopolymers

For the elaboration of the biopolymers by means of an acid hydrolysis, it was carried out using the methodology of Rosales 2016 and adjustments. Where the same methodology was carried out, the variant was cereal starch. Table 1, later the mixture of each of the components was made and they were subjected to heating on a thermal plate at 80 ° C for 15 minutes, it was poured on waxed paper to later be spread by metal plates and allowed to dry for 48 hours at a temperature of 30 ° C. This was done with each starch sample in triplicate. [14]

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Cereal and forms extraction of starch	Quantity (g)	Water distilled (ml)	Acid citric (g)	Glycerin (ml)	Nomenclature of the treatment
Oryza sativa NaOH	5	15	2.5	1.5	T2SO
Oryza sativa Molienda simple	5	15	2.5	1.5	T1MO
Avena sativa NaOH	5	15	2.5	1.5	T2SA
Avena sativa Molienda simple	5	15	2.5	1.5	T1MA

Table 1 Manufacture of biopolymers
Own Source

Chemical and mechanical analysis

The moisture determination was carried out by the method described by NMX-F-083-1986. Likewise, the ash determination was carried out according to the guidelines described by NMX-F-066-S-1978. The resistance determination was carried out in a texture analyzer, 0 to 1500g / 0.20g, Brookfield, CT3-1500, where a biopolymer with dimensions of 15 cm long and 15 high was placed. The tensile strength was obtained by dividing the maximum force before the film broke by the transverse thickness of the film. The cutting force in N.

Geometric analysis

The thickness determination (mm) using a 0-150mm Vernier caliber consisted of placing a sample fragment in the tips for external measurements and subsequently the reading was taken on the digital marker.

Results

Manufacture of biopolymers

In Figure 3 shows the Oryza sativa biopolymers with the alkaline starch extraction technique (T2SO) and simple grinding (TIMO) and with promising characteristics in terms of their breaking strength and starch quality, where one of the most important properties of natural starches, which is their semicrystallinity where amylopectin is the dominant component for their crystallization, which is attributed to a favorable mechanical resistance due to the amylose and amylopectin ratio with a value of (36.41 ± 2.2 and 62.75 ± 2.2). [15] According to their starch gelatinization temperature they vary from 70 to> 75 ° C. [16]

Figure 3 Oryza sativa biopolymers. A biopolymer by extraction of starch in alkaline solution (T2SO). B biopolymer by simple milling starch extraction (T1MO)
Own Source

In Figure 4 shows the biopolymers of Avena satina with its two starch extraction techniques, it can clearly be seen that there is less resistance to its breaking force. The amylose content is relatively lower compared to that of other cereals [17] Oat starches had the lowest gelatinization temperatures, between 56.8 and 59.7 ° C, using the same temperature for the production of biopolymers, they lost more moisture generating little resistance mechanics.

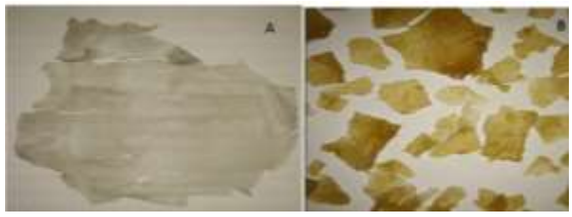

Figure 4 Biopolymer of Avena satina. A biopolymer extracted by alkaline solution (T2SA). B biopolymer extracted by simple milling (T1MA)
Own Source

Chemical and mechanical analysis

The relative humidity with the lowest percentage was presented by the T1MA polymer and following the T2SA both are the same starch different extraction technique. This indicates that Avena Sativa has a lower absorption by its semi-crystalline region with a lower ratio of amylose and amylopectin, generating a faster loss of water and generating less mechanical resistance. Table 2. Confirming with its percentage of minor ash in the starches of Avena sativa.

T1MA	T2SA	T1MO	T2SO
5.08%	7.55%	9.30%	9.32%
±0.15	±0.16	±0.05	±0.03

Table 2 Moisture values in the treatments performed
Own Source

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T1MA	T2SA	T1MO	T2SO
1.77%	0.66%	2.11%	1.33%
±0.33	±0.2	±0.33	±0.3

Table 3 Ash values in the treatments carried out. *Own source*

Determination of the breaking force

The shear force was obtained by dividing the maximum force before the film broke by the transverse thickness of the film. The cutting force in N. It was only determined with T1MO and T2SO by the appearance of their surfaces since the T1MA and T2SA treatments presented cracks in their entire surface. Confirming that it is attributed to the quality of cereal starch. Table 4.

T1MO N	T2SO N	T1MA N	T2SA N
2.70	2.90	0	0
±0.02	±0.05	0	0

Table 4 Values of the determination of the breaking force *Own Source*

Geometric analysis

Finally, the thickness determination was made, showing the treatments as well as their standard deviations, there is no significant difference with respect to the thickness that can be attributed to the resistance or to its moisture and ash content. Table 5

T1MA mm	T2SA mm	T1MO mm	T2SO mm
0.61	0.65	0.61	0.68
±0.14	±0.02	±0.06	±0.13

Table 5 Grosor de los tratamientos realizados *Own Source*

Conclusions

The proportion of amylose and amylopectin play an important role in the properties of thermoplastic starches. The gelatinization temperature is the one at which the starch grains begin to absorb water and swell irreversibly in hot water, it is associated with the amylose content.

What can be distinguished is two types of ordering in thermoplastic starch after processing: residual crystallinity classified into type A, B and C forms caused by incomplete melting during plasticization and induced crystallinity during processing, according to to the arrangements generated in the polymeric chains according to. [11] Type A is common in cereal starches, B in tubers, and C in certain roots. This indicates that even though the starch belongs to the same residual crystallinity classification.

The ratio of amylose and amylopectin is different in each type of cereal, according to the analyzes carried out it was obtained that the starch extracted by both extraction techniques from Oryza Sativa present better biopolymer properties, which attributes that the important thing is the starch and not there is a significant difference with respect to the starch extraction method.

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Use bioregulators in cluster ToV (*Salanum lycopersicum* L.)

Uso de biorreguladores en tomate (*Salanum lycopersicum* L.) de racimo ToV

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Abstract

Tomato (*Solanum lycopersicum* L.), is one of the most important vegetables that supports food and nutritional security. One of the various forms that is offered is in cluster, also known as ToV, being one of the specialties of the tomato ball. The disadvantage is the visual quality (the homogeneous color) that determines the level of consumer acceptance and the foreign market, as well as the consumption time. Due to this, the effect of two spray techniques (mopping and touch) in postharvest on the ToV fruits grown in the greenhouse was evaluated. This to favor the homogeneity of color; using bioregulators: A) 2-cloroethyl phosphonic acid y B) K_2O 20%. in three concentrations [A (T_1= 7.2 ppm, T_2= 12 ppm y T_3= 120 ppm, T_4= control H_2O); B (T_1= 66 ppm, T_2= 550 ppm, T_3= 715 ppm, T_4= control H_2O)] under a complete randomized design with three repetitions to two observations (initial - final) during seven days. The results were subjected to post hoc comparisons, for which the weight variable in both bioregulators did not show statistical effect; however, in the color variable, the spraying techniques showed statistical differences with respect to the control. Therefore, it is concluded that the use of bioregulators in post-harvest is a solution to the color homogeneity of the ToV tomato.

***Solanum lycopersicum* L., Post-harvest bioregulators, Application techniques**

Resumen

Tomate o tomatera (*Solanum lycopersicum* L.), es una de las hortalizas más importantes que da sustento a la seguridad alimentaria y nutricional. Una de las diversas formas que es ofertado es en racimo. También conocido como ToV, siendo una de las especialidades del tomate bola. La desventaja es la calidad visual (el color homogéneo) que determina el nivel de aceptación del consumidor y el mercado extranjero, al igual que el tiempo de consumo. Debido a esto, se evaluó el efecto de dos técnicas de aspersión (mopeo y toque) en postcosecha sobre los frutos ToV cultivados en invernadero. Esto para favorecer la homogeniedad de color; mediante los biorreguladores: A) Àcido 2-cloroetil fosfónico y B) K_2O 20%. A tres concentraciones: [A (T_1= 7.2 ppm, T_2=12 ppm y T_3= 120 ppm, T_4= control H_2O); B (T_1= 66 ppm, T_2= 550 ppm, T_3= 715 ppm, T_4= testigo H_2O)], bajo un diseño completo al azar con tres repeticiones a dos observaciones (inicial- final) durante siete días. Los resultados se sometieron a comparaciones *post hoc*, para los cuales la variable peso en ambos biorreguladores no mostro efecto estadístico; sin embargo, en la variable color, las técnicas de aspersión mostraron diferencias estadísticas con respecto al testigo. Por lo que, se concluye que el uso de biorreguladores en postcosecha es una solución a la homogeniedad de color del tomate ToV.

***Solanum lycopersicum* L., Biorreguladores en postcosecha, Técnicas de aplicación**

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Introduction

Tomato (*S. lycopersicum* L.) is one of the most important fruit crops in the food industry (Navarro-González, and Periago, 2016), whose main use is the preparation of traditional dishes, preparation of sauces and processed products (pasta, juice, dehydrated, among others (Leyva and Pérez, 2015). Currently, vitamins such as B1, B2, B5, E and C have been considered as an important source; also carbohydrates, phosphorus (P), potassium (K) and magnesium (Mg) (Palomo, 2010), in addition to lycopene, flavonoids, flavones and total phenolic compounds, whose consumption is related to their antimutagenic potential and anticancer properties (Luna and Delgado, 2014).

In Mexico it is one of the main vegetables that is exported, the high demand has diversified the obtaining of this fruit and to satisfy the demands, there is a great segmentation of the market and therefore a wide range of types and varieties of tomato: round or round, saladette or roma, cluster. Flu or grape, cherry, cocktail, pear and heirloom (Intagri, 2017).

The cluster tomato, also known as ToV is a specialty of ball tomatoes, which are characterized by the fact that they are harvested in bunches and the commercial presentation is in clusters of 4 or 5 fruits. Among the most important quality parameters of TOV tomato clusters are: (1) well colored, (2) bright (without marks or immature green spots), (3) uniform shape, (4) texture or strong firmness (5) taste and (6) clean and (7) free from external defects. One of the parameters that influences the marketing of Tov tomato is the visual quality (the homogeneous color), which determines the level of acceptance of the consumer and the foreign market, as well as the time of consumption this interacts with it.

There are different agronomic practices that different physiological activities canbe controlled, such as: vegetative growth control, size increase and fruit set, among others. (Ramírez *et al*., 2012). The ripening of fruits is one of the processes that receives the most attention for postharvest in tomato. The use of plant hormones is an agronomic practice that contributes to the control of physiological activities.

On the other hand, there are compounds (bioregulators) that in low concentrations promote, inhibit or modify the morphophysiological process associated with their use (Cuesta and Mondaca, 2014). Ethephon (2-chloroethyl phosphonic acid), is a phytoregulator precursor of ethylene, a plant hormone that regulates growth (Crisosto et al., 2010). Ethylene regulates the change in composition and structures during fruit ripening, an increase in carotenoids, which in the case of tomato helps to homogenize the color, it is currently the most used (Chandrika et al., 2003). On the other hand, the use of nutrients exogenously has the synergy to help physiological processes, for example, potassium (K) and boron B). These have an effect on the translocation of sugars and carbohydrates, nitrogen (N) metabolisms, protein synthesis, enzyme activation, key processes in color (Martínez, 2017).

Materials and methods

Vegetal material

The Maxesa variety (Enza Zaden), organic ToV cluster tomato, was used. The experiment was carried out in a greenhouse whose location is at 20 ° 28'56.0 "N 101 ° 10'59.6" W.

Applied bioregulators

Two products were used

A) 2-chloroethyl phosphonic acid
Using three different concentrations:
T1 = 7.2 ppm
T2 = 12 ppm
T3 = 120 ppm
T4 = H2O Control)

B) K_2O 20%.
Using three different concentrations
T_1= 66 ppm,
T_2= 550 ppm,
T_3= 715 ppm,
T_4= control H_2O)

Experimental design

The experiment was established under a complete randomized design with three repetitions of two observations (initial-final) for seven days. Through two application techniques of bioregulators.

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1) Moping: which consists of covering the cluster tomato with the product using a towel which is immersed in the treatment and rubbed on the tomatoes.
2) Touch: it consists of applying the dilution in the lower part of the calyx and the stylar region of the tomato only.

Each sample consisted of three ToV cluster tomatoes, placed in low-density polypropylene bags, with a sanitizing solution of paraacetic acid at a concentration of 80 ppm. and were placed in a cold chamber, whose conditions were relative humidity (87% to 95%) and temperature (9.5 to 10.7 ºC), during the seven days of evaluation.

Variables evaluated

Fruit weight (g)

It was obtained using a digital kitchen scale, with a capacity of 5 kg. and sensitivity of 5 g.

Fruit color

It was determined by means of a table of comparison of the states of maturation of the tomato based on Castro et al., (2009), in (figure 1) reference is made to the scale used.

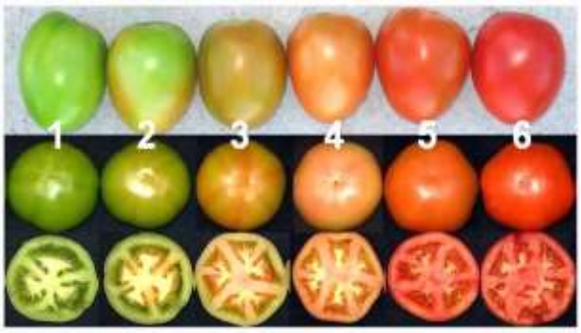

Figure 1 Tomato ripening states in relation to color:https://ricardodeleon1961.wordpress.com/2015/11/18/de-que-color-es-tu-mundo/

The treatments were selected in a range of 1-2 color (initial), for homogeneity. They were monitored for six days after selection, on the seventh day they were evaluated again (final). This parameter is important in the marketing of ToV tomatoes. In the region there are many marketers whose crops are exported to other countries, which have a record of the post-harvest time is that it is seven days.

As it is a difficult variable to control in its traceability, in experience a good ToV tomato when offered to the customer must have a grade 4-5 on the maturation scale (figure 1), a scale below or above means the rejection of it, hence the importance of this variable.

Statistic analysis

Multiple pairwise comparisons, also called post hoc, were performed using the XLSTAT Software (2019). Using an LS means test, which allows us to compare the effects of the treatments with the variables evaluated and the application method as a whole.

Results

Use of bioregulators

From the statistical analysis, from the comparison of LS means for treatment-product, there was no difference between the various treatments of the products between them, but there is significant evidence with respect to the control (Figure 2). It can be mentioned that the use of the different bioregulators favor the variables evaluated, as is the case of the Martínez-Damian et al., (2019) study, the individual use of 2-chloroethyl phosphonic acid and iodine (I), increased significantly the weight and firmness of the fruits, respectively, with respect to the control, in the tomato crop.

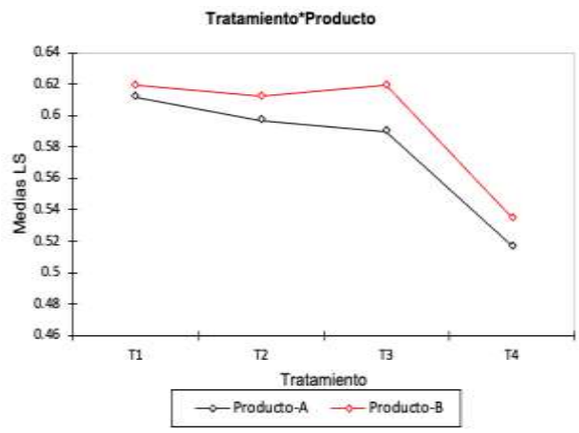

Figure 2 Analysis of LS means for treatment- product A) 2-chloroethyl phosphonic acid and B) K20

Application technique

From the analysis of multiple comparisons by pairs the statistical means LS for the application method factor, there are no statistical differences between the mopping and touch technique.

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It is concluded for this experiment the type of application does not favor the treatments (table 1). This indicates that the best application technique is the one that requires the least time resource. In some cases, the method of applying bioregulators by spraying shows a positive effect compared to the controls (Osuna-Enciso et al., 2019). so it can be an option and carry out an evaluation with this type of technique in tomato ToV.

Category	Average LS	EE	Lower limit (95%)	Upper limit (95%)
Moping	0.422	0.011	0.398	0.445
Touch	0.393	0.011	0.370	0.416

*EE= Standard error.

Table 1 LS means for the application method factor

Fruit weight

Among the weight of the fruits evaluated, there was no variation in loss or increase (Figure 3). In this sense, it cannot be compared with what happened in the work Martínez-Damian et al., (2019), in which the use of 2-chloroethyl phosphonic acid in sprayed plants had an increase of 62.5% compared to the control. On the other hand, the use of 2-chloroethyl phosphonic acid on tomato bunches increases the weight of 8% more compared to that which is not applied to it (Atta-Aly et al., 1999). Making it clear that the use of this bioregulator in other concentrations and phenological states in tomato favors a gain in fruit weight.

Fruit color

A difference is found in all treatments with respect to the control, based on the analysis of multiple comparison by pairs. The LS means of the treatments are summarized in (table 2) and for a better perception of these see (graph 3). What is confirmed visually and based on the reference of tomato maturation states, the existence of a difference in the use of bioregulators with respect to where it was not used. It should be noted that for this qualitative variable it is not controlled, since the color perspective depends on each evaluator; Therefore, it is useful to consider another way of measuring it to reduce the error in the operational part of an agricultural production unit, given the complexity of monitoring this type of important parameter in the commercialization of this crop.

It is known that the use of other bioregulators in tomato favors a homogeneous coloration. For example, the use of calcium prohexadione (Ca) (Martínez-Damian et al., 2019). However, an evaluation of Sodium Selate and Potassium Iodide in cherry tomatoes did not report color variations (Islam et al., 2018). On the other hand, the use of Selenium decreases the ripening rate by temporarily inhibiting the pathway of the ethylene biosintestines (Caffagni et al., 2012.), a phytohormone responsible for the ripening of the fruits and related to the color of the fruit..

Treatments	Weight-Inc	Weight-End	Col-Inc	Col-FIN
T1	0.402	0.399	0.509	0.833
T3	0.433	0.428	0.389	0.777
T2	0.412	0.406	0.388	0.777
T4	0.433	0.422	0.388	0.611
Pr> F (Model)	0.4509	0.4570	0.0025	< 0.0001
Significant	No	No	No	No
Pr> F (Treatment)	0.4416	0.4910	0.0006	< 0.0001
Significant	No	No	No	Sí

Of the LS means of the treatments, treatment 4 (Control) shows a significant difference in the color variable.

Table 2 Summary of the LS means of the treatments

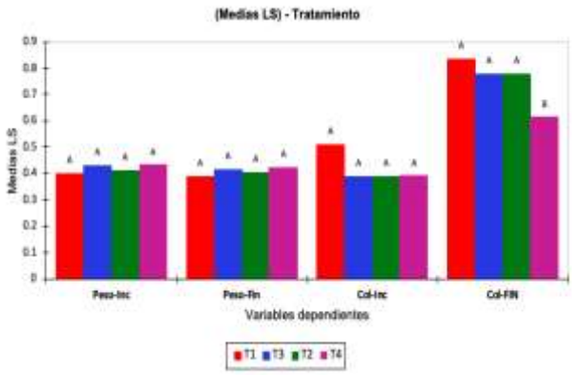

Figure 3 Graphic representation of the LS means of the experiment, weight Inc (initial), weight-end (Final), Col-inc (initial) and col-FIN (final) of the treatments as a function of the set values. LS means with the same letters are not statistically different at a 95% confidence interval.

Conclusion

It will be possible to identify that the application techniques (mopping and touching) of the evaluated bioregulators, there is not a significant difference, so any technique can be used in the process of presenting ToV tomatoes. The bioregulators evaluated A) 2-chloroethyl phosphonic acid and B) K2O 20%, had significant favorable differences for the fruit color variable, when compared with the control, but there was no effect on the fruit weight variable.

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Regarding the concentrations evaluated, no significant differences were found according to the analysis of multiple comparisons. Therefore, we can affirm that the use of these bioregulators in the concentrations evaluated favors the uniformity of the color of the fruit since at seven days of evaluation the ToV tomatoes treated with the bioregulators were on average in the five state of maturity, which is what suitable for the international market.

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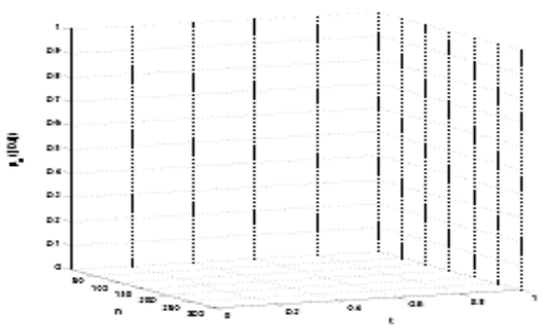

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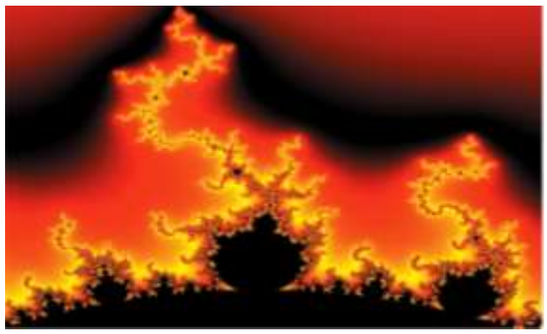

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