Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i30.4050

Article

Development of antibacterial talc from residual starch enriched with *Larrea tridentata*

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Abstract

Larrea tridentata, commonly known as 'gobernadora' or 'creosota' (creosote bush), is an endemic plant of the Zygophyllaceae family that is very abundant in northern Mexico. Due to its toxicity, its derivatives have limited applications in materials with human contact; therefore, in search of new applications, the research was conducted in 2024. The work focused on carrying out a process to extract and process creosote bush and residual starch of potato from the southeast region of Coahuila, in northern Mexico, to obtain an antibacterial talc. The product obtained through a grinding and mixing process was characterized by infrared spectrometry and thermogravimetric analysis; a hemolysis test was performed to know its compatibility with human blood and its antibacterial properties were tested by antibiogram tests with Gram-positive strains of *S. aureus* (ATCC 6538 and ATCC 33591), obtaining a product with applications as antibacterial talc for its moisture-absorbing, antibacterial, and human erythrocyte-biocompatibility properties.

Keywords:

Larrea tridentata, antibacterial talc, creosote bush.

DOI: https://doi.org/10.29312/remexca.v16i30.4050

Introduction

The north of Mexico is made up of large arid and semi-arid areas in which it is possible to find *Larrea tridentata*, an evergreen shrub (Jitsuno and Mimaki, 2010) belonging to the Zygophyllaceae family, known by different colloquial names, such as 'gobernadora, creosote, hediondilla, guamis, huamis, or falsa alcaparra' (creosote bush). It is estimated that 25% (500 000 km^2) of the Mexican territory is covered by this plant.

Its leaves have a thick resin that behaves as an antiperspirant to prevent water losses through its leaves, which contains a wide variety of compounds, such as lignans, flavonoids, saponins, tannins, polyphenols, terpenes, amino acids, essential oils, minerals, phenols, and nordihydroguaiaretic acid, which makes some of its biological activities antibacterial and antifungal (Vassão *et al*., 2007; Osorio *et al*., 2010; Martins *et al*., 2013; Hernández-Zamudio *et al*., 2018; Núñez-Mojica *et al*., 2021; Bamidele *et al*., 2024).

It has been used for centuries by Native Americans to treat infections, kidney problems, kidney stones, rheumatism, arthritis, diabetes and tumors (Martins *et al*., 2012), and respiratory tract infections; likewise, it has anti-inflammatory, antiviral, and anti-cancer properties (Schmidt *et al*., 2012). However, this plant is relatively cytotoxic due to the lignans it contains (Lambert *et al*., 2005).

Other studies have found that *Larrea tridentata* also reduces plasma triglycerides, cholesterol, and insulin levels, and reduces liver weight, possibly due to decreased lipid peroxidation and increased antioxidant activity in the liver (Chaudhary *et al*., 2025). Other applications it has include corrosion inhibitor (García Inzunza *et al*., 2013) and insect repellent, and its resin can be used as an antibacterial and antifungal product (Saldívar *et al*., 2003). Nevertheless, it is necessary to seek alternative uses of this shrub.

Moreover, potato starch is a biopolymer with biodegradability and biocompatibility properties and is non-toxic, cheap, and very abundant in nature. It can be used for product packaging, pharmaceuticals, textiles, and water treatment, among others (Sosa-Santillán *et al*., 2022; Valdez-Valdés *et al*., 2024). Nonetheless, in the southeastern region of the state of Coahuila, in northern Mexico, when potatoes produced in the region do not pass quality control for trade, they are discarded, which encourages the search for a new use for this waste material.

Due to the above, this work has focused on looking for other applications of the creosote bush and the residual starch of the southeast region of Coahuila; therefore, an antibacterial talc based on these materials has been developed, and its degree of hemolysis has also been studied to know if it is biocompatible upon contact with human blood.

Finally, in light of the newly sparked controversy over whether the mineral talc from the Johnson and Johnson company (made mainly from hydrated magnesium silicate) could cause cancer problems, since it has been classified as a possible carcinogen for humans due to its asbestos residue content (Johnson and Johnson, 2019; Casey *et al*., 2025), it is essential to look for new alternatives to partially replace this product; therefore, an antibacterial talc based on residual starch mixed with creosote bush to combine the biocompatibility properties of the biopolymer with the antibacterial properties of the plant may be a good choice.

Materials and methods

Materials

Several potatoes measuring approximately 3 to 8 cm in length and irregular in shape (*Solanum tuberosum*) were donated by local farmers in the region of Arteaga, Coahuila, Mexico, and were used the day after their harvest (March 14, 2024).

A *Larrea tridentata* plant with a height close to 1.5 m was selected, and approximately ⅓ of its volume was pruned and collected (Arteaga, Coahuila) to be used on the same day of its harvest (March 12, 2024). The strains of *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus aureus (ATCC 33591)* were acquired from the American Type Culture Collection. Müller-Hinton agar and broth were purchased from BD Bioxon®, Mexico.

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Equipment

An 8 L capacity ball mill, filled to 40% of its total volume with 4 cm diameter metal spheres and a sieve with a #150 mesh, both from the Denver Equipment Division of the Joy Manufacturing Company, were used. The characterization was carried out with an FT-IR spectrometer from PerkinElmer, USA, and a Hi-Res TGA Q5000 thermogravimetric analyzer from TA Instruments, USA. A microplate reader (Agilent BioTek, Sinergy HTX) was used for the hemolysis test.

Obtaining powder from the creosote bush

From the branches of a creosote bush plant, the leaves (441.2 g) were removed by hand and dried in an oven at 80 °C for 72 h to remove water and the most volatile compounds and minimize the process time. The leaves were then introduced into a ball mill, which was loaded to 30% with 2 cm diameter steel balls and rotated at 30 rpm for 25 min. At the end of the milling, the product was passed through a #150 mesh, obtaining 218.3 g of fine creosote bush powder. The rest of the dust that did not pass through the mesh was discarded.

Extraction of potato starch

The extraction of potato starch was carried out as follows: the potatoes were peeled and cut into pieces, and 1 kg of them was ground in a domestic blender with enough water to cover them. The suspension was passed through a #150 mesh sieve and washed with distilled water. Then, 500 mL of a NaOH solution (0.25% w v^{-1}) was added to disperse and break down the starch granules (and make extraction more efficient) and kept for 24 h in a refrigerator. The top layer was removed, and the precipitate was centrifuged, washed with distilled water, and dried at 50 °C for 72 h. A white powder was obtained.

Obtaining talc from the creosote bush and starch

Different mixtures of creosote bush powder and potato starch were made, varying the percentage of creosote bush added to the starch. The mixtures were 0, 5, 10, 15, 25, 50, and 100% of creosote bush (% of total weight) (Table 1); the percentages of each sample are shown below.

Table 1. Composition of creosote bush and starch in talc mixtures.

Num. of sample	(%) Creosote bush	(%) Starch
1	0	100
2	5	95
3	10	90
4	15	85
5	25	75
6	50	50
7	100	0

Evaluation of the antibacterial effect

In this study, *S. aureus* strains (ATCC 6538 and ATCC 33591) were used to evaluate the antibacterial activity of the different starch and creosote bush mixtures. The standardized single-disc method was chosen for antibiotic susceptibility; for this purpose, a sterile Müeller-Hinton agar medium was prepared and distributed in Petri dishes.

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Discs measuring 6 mm in diameter were prepared with compressed talc and a drop of water. These were spread over the surface of the Müeller-Hinton agar using a sterile cotton swab to obtain uniform microbial growth on the plates. Then, the compressed talc discs were placed, under aseptic conditions, on the agar plates. The plates were incubated at 37 °C for 24 h to obtain reliable microbial growth, and finally, the inhibition zones were measured. These tests were performed in duplicate (Hernández *et al*., 2016).

Determination of hemolytic activity in isolated human erythrocytes

The hemolytic activity or hemolysis assay is used to evaluate the potential toxicity of a compound to erythrocytes. In this way, if the compound or extract breaks down blood cells, hemoglobin will be released, which can be quantified at 415 nm. The amount of hemoglobin released is proportional to the damage produced in the cells tested.

Venous blood was extracted from several adult donors, healthy and non-smokers, by arm puncture, following the guidelines for studies where human samples are used. Blood was collected in Vacutainer tubes with sodium citrate as an anticoagulant. The blood was centrifuged at 2 500 rpm for 4 min at 25 °C. The erythrocyte pellet was washed three times with an Alsever solution (pH 6.4).

The Alsever solution was prepared by mixing dextrose (10.45 g), sodium citrate (3.97035 g) and sodium chloride (2.07462 g) in distilled water to obtain 500 ml of solution; all the reagents used were of analytical grade.

Once the washed erythrocyte pellet was obtained, a 1:99 dilution was prepared. It was gently shaken to form a homogeneous suspension that served as the basis for hemolysis tests. For each test, two experiments were carried out, each one in triplicate, and in addition to the study samples, there were also negative and a positive control.

Negative control

Blood (150 µl) was used together with the Alsever solution (1 350 µl).

Positive control

Blood (150 µl) was used together with distilled water (1 350 µl). The absorbances obtained corresponded to 100% hemolysis.

Experimental groups with extracts

Blood samples were incubated with increasing doses of starch and creosote bush-starch samples (10%) at concentrations of 0, 1.25, 2.5, and 5 mg ml^{-1}, at 37 °C for 60 min, with a final volume of 1 500 µl. Once the stirring time was over, the tubes were centrifuged to separate the supernatant and 1 ml of it was placed in multi-plate cells for the determination of absorbance at 415 nm in the microplate reader. To determine the (%) of hemolysis, the following formula was used: >

$$\%\ \text{Hemolysis:}\frac{\left|(\text{Experimental group-Control}^{-})\right|}{(\text{Control}^{+}\text{-Control}^{-})}\times 100.$$

Characterization

An infrared (IR) spectroscopy analysis was performed on the 10% creosote bush-starch mixture with a Frontier Fourier-transform infrared spectrometer, which was used in reflection mode and equipped with a universal ATR sampling accessory (FTIR-ATR). FTIR-ATR spectra were collected at a resolution of 4 cm^{-1} and 128 scans per run. Thermogravimetric analysis (TGA) was performed in an air atmosphere with a TGA 4000. The samples (10-30 mg) were heated from 30 to 800 °C at a rate of 20 °C min^{-1}.

Revista Mexicana de Ciencias Agrícolas
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Time-dependent moisture absorption tests

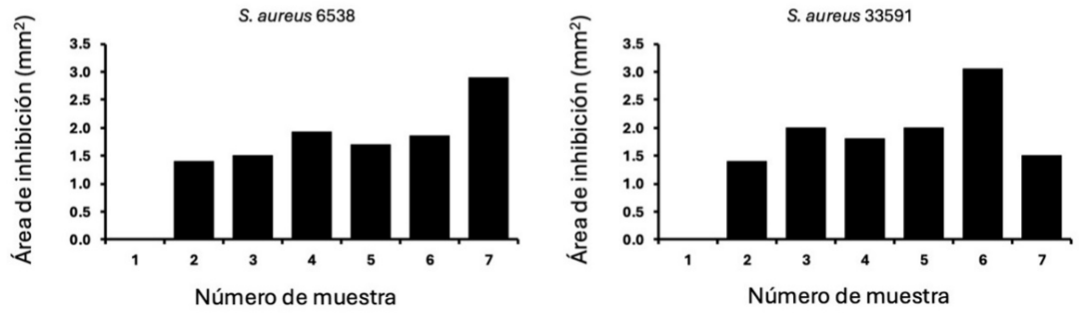
To determine the degree of moisture absorption, samples 1-7 were weighed and wrapped in filter paper in triplicate and then immersed in distilled water; after 30, 60 and 120 min, the sample was removed and weighed to determine how much water it absorbed.

Results and discussion

Antibacterial analysis

Once the different creosote bush-starch mixtures were obtained, their antibacterial activity was measured by the antibiogram method using compressed tablets of fine powder products. Figure 1 shows the graphs of the results of these tests. In this assay, the results were expressed as the mean ± standard deviation (n= 3). The data were analyzed through Anova and Tukey tests ($p \leq 0.05$) with the statistical program GraphPad Prism version 8.

Figure 1
Areas of inhibition of antibacterial tests against *S. aureus*.



The results show that the residual starch samples (Figure 1) in both cases fail to inhibit the *S. aureus* strain, because the starch does not have this biological characteristic; however, from the second sample (5% of creosote bush), significant inhibition of the strains is observed. In the case of the *S. aureus* strain 6538, with 100% creosote bush, it shows its maximum inhibition potential; this differs from the *S. aureus* strain 33591, whose maximum inhibition capacity was reached with 50% creosote bush, and then it decreased when having only the powder of this plant (Vassão *et al*., 2007).

This is partly because strain 33591 is used for antibiotic resistance research, but it is more sensitive to other compounds present in *Larrea tridentata*. Figure 2 shows the photographs of the results of the antibacterial activity tests using the disk diffusion method against the two strains of *S. aureus*. In this, it is possible to visually appreciate the inhibition halo of the different mixtures, except for the pure starch in both strains. Samples 3 to 7 show the inhibition halos clearly visible, both in the ATCC 6538 and ATCC 33591 strains.

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Figure 2. Photograph of the antibiograms of the antibacterial tests against *S. aureus*.

Samples 5, 6, and 7 have broader and more defined halos and show a greater antibacterial efficacy due to their higher content of *Larrea tridentata*. Sample 1, made up of residual starch, does not present an inhibition halo in any of the strains. This study demonstrates that talc formulated with residual starch and *Larrea tridentata* has effective antibacterial activity and has the potential to act even against multidrug-resistant bacteria, such as *S. aureus* ATCC 335991.

For subsequent experiments, sample 2 (10% creosote bush - 90% starch) was chosen because, in the results of the antibacterial studies, sample 2 presents a good inhibition against the strains of *S. aureus* without requiring a high percentage of creosote bush. This was done to use a greater amount of agro-industrial waste (residual starch) instead of an endemic plant (*Larrea tridentata*).

Hemolytic assay of the talc

The results of the hemolytic assay, presented in Table 2, show that none of the samples evaluated (starch and 10% creosote bush-starch mixture) caused a degree of hemolysis greater than 2% at any of the concentrations evaluated. According to the ASTM F756-08 standard, a hemolytic percentage of less than 2% indicates that talc based on a mixture of residual starch and 10% creosote bush can be considered safe to be in contact with erythrocytes of human blood.

Table 2. Percentage of hemolysis caused by samples in isolated human erythrocytes.

Sample (mg ml^{-1})	Negative control 0 (Alsever solution)	Concentration			Positive control Distilled water
		1.25	2.5	5	
Starch	0	0	0	0	100
Sample 2	0	0	0	0	100

Thermal analysis of the talc

The thermal stability of the talc obtained is of utmost importance to consider other possible applications where thermo-mechanical processes are needed in this talc, such as the possibility of plasticizing it. For this purpose, the thermogravimetric analysis was carried out in an air atmosphere.

Figure 3 shows the result of this analysis, where 4 main stages can be seen; in the first stage, from 100 to 150 °C, the first decrease in weight is due to the elimination of water; in the second

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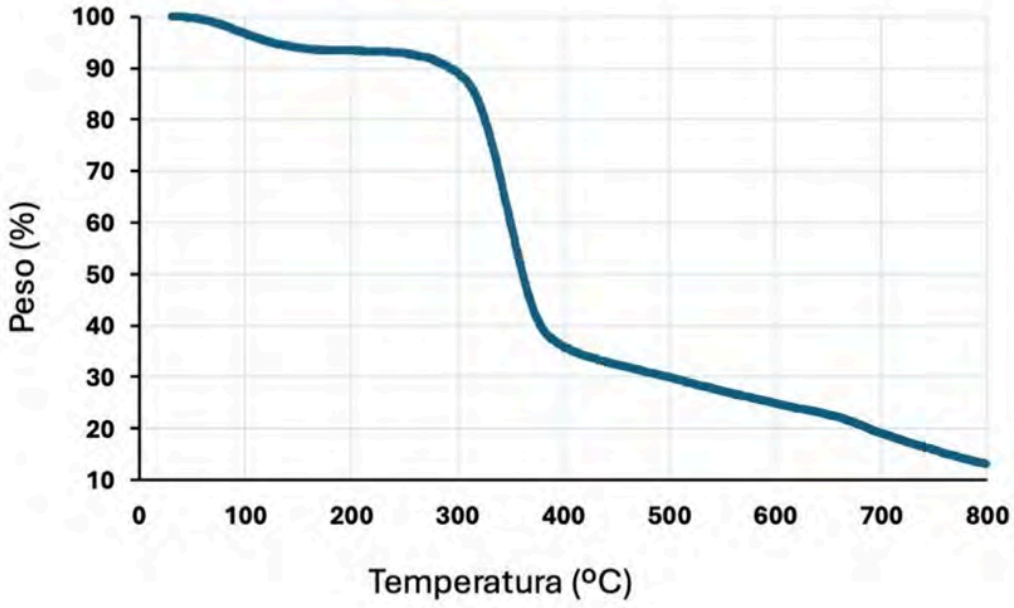
stage, from 200 to 350 °C, the polymer chains of starch and other carbohydrates of the creosote bush are decomposed. Subsequently, in the third stage, from 350 to 500 °C, the lignin and other compounds of the creosote bush are decomposed, and finally, in the last stage, from 500 to 800 °C, both components of the talc are fully decomposed (Izquierdo, 2022).

Figure 3. Thermogram of the talc sample at 10% creosote bush

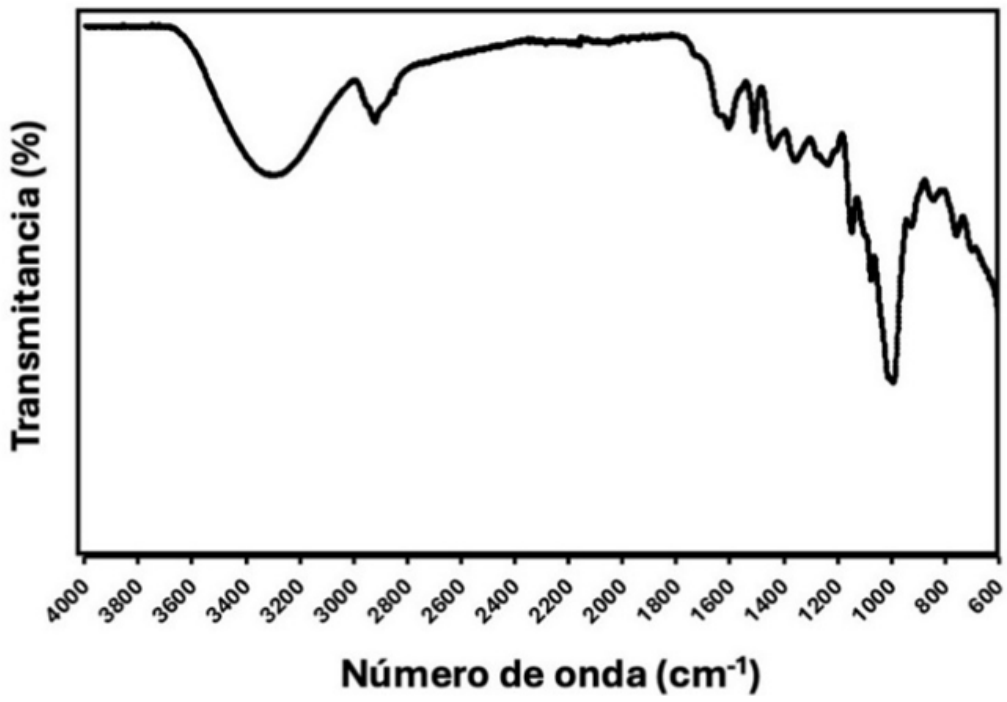


FT-IR characterization of the talc

To determine the functional groups that make up the talc, an infrared spectroscopy study was conducted, as shown in Figure 4.

DOI: https://doi.org/10.29312/remexca.v16i30.4050

Figure 4. FTIR spectrum of the talc with 10% creosote bush.



In the image, it is possible to see, from 3 600 to 3 200 cm^{-1}, the signals attributed to the O-H stretching of the hydroxyl groups of the starch and polyphenols of the creosote bush; from 3 000 to 2 800 cm^{-1}, the signals of the methyl and methylene groups of the organic compounds of the creosote bush appear.

The signals from 1 800 to 1 500 cm^{-1} correspond to the vibrations of the carbonyl groups and aromatic rings of the flavonoids and lignans of the creosote bush; finally, the signals from 1 200 to 800 cm^{-1} are associated with the C-O-C and C-O-H vibrations of the starch. These signals confirm the presence of the functional groups of the creosote bush and starch, with the latter signals being more intense due to its greater presence in the mixture.

Time-dependent moisture absorption tests

To find out if talc can absorb moisture, time-dependent absorption tests were conducted. The results of these tests are shown in Table 3.

Table 3. Results of time-dependent moisture absorption tests.

Sample	0%	5%	10%	15%	25%	50%	100%
Time							
0 min	0.5	0.5	0.5	0.5	0.5	0.5	0.5
30 min	0.75	0.826	0.908	0.953	0.937	1.015	0.852
60 min	0.912	1.188	0.978	1.061	0.995	1.21	1.198
120 min	0.935	0.973	0.985	1.029	1.047	1.056	1.11

The sample results show that creosote bush powder alone has a higher water absorption capacity over time compared to pure starch. Nevertheless, the sample chosen to make talc (10% creosote bush-90% starch) has a water absorption capacity only a little lower than the pure creosote bush; this shows that the talc obtained can absorb moisture from the feet and maintain it over time.

DOI: https://doi.org/10.29312/remexca.v16i30.4050

Conclusions

In this study, it was possible to develop an antibacterial talc using residual potato starch as the main matrix, enriched with extracts of *Larrea tridentata*. The extraction and grinding process made it possible to obtain a homogeneous mixture with physical and chemical properties suitable for topical application. The results of the antibacterial tests showed that the different mixtures of residual starch and *Larrea tridentata* have antibacterial properties against two strains of *S. aureus* (ATCC 6538 and ATCC 33591), confirming the antimicrobial potential of the bioactive compounds present in *Larrea tridentata*.

Hemolytic testing indicated that the mixture composed of residual starch and 10% by weight of *Larrea tridentata* does not generate significant hemolysis in human erythrocytes, suggesting that this product can be used in applications such as talc for feet or body, even in the presence of superficial wounds. This can be attributed to the fact that *Larrea tridentata* is found in a lower proportion than residual starch, which is biocompatible.

In addition, the FTIR characterization showed the characteristic functional groups of the creosote bush and starch, the most intense being those of starch due to its higher proportion in the mixture. The thermogravimetric study shows that the talc is thermally stable at temperatures below 150 °C, which guarantees its integrity during storage and conventional use. Finally, the absorption test showed that this mixture of residual starch and *Larrea tridentata* can absorb approximately its weight in moisture, which gives it the possibility of being applied as an antibacterial talc, biocompatible with the advantage of being derived from natural and sustainable sources.

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DOI: https://doi.org/10.29312/remexca.v16i30.4050

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Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i30.4050

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Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 00 June 2025
Date accepted: 00 August 2025
Publication date: 15 October 2025
Publication date: Sep-Oct 2025
Volume: 16
Issue: esp30
Electronic Location Identifier: e4050
DOI: 10.29312/remexca.v16i30.4050
Article Id (other): 00013

Categories

Subject: Articles

Keywords

Keywords

Larrea tridentata

antibacterial talc

creosote bush

Counts

Figures: 4

Tables: 3

Equations: 0

References: 19