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Research Article

STUDY OF THE BIOLOGICAL EFFECTS OF A HYDROALCOHOLIC EXTRACT OF *PUNICA GRANATUM* (POMEGRANATE)

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Abstract

Herbal medicines are derived from medicinal plants and/or parts with known pharmacological characteristics. The use of these plants has been encouraged by the World Health Organization, as the culture use is still beneficial due to traditional factors and curative conferred its users. The development of antimicrobial drugs is important for the development of new sources for the therapeutic treatment of infectious diseases, considering that the characteristic of bacteria have become resistant to the action of antibiotics. Thus, aim of this study was to evaluate the influence of an alcoholic extract of the fruit peel of *Punica granatum* as its possible genotoxicity, cytotoxicity and mutagenic action against *Staphylococcus aureus* (ATCC 8096). To obtain the extract, the skins were dried, crushed and subjected to extraction with ethanol and, after that, the extract was placed on rotary evaporator. To assess cytotoxicity, we employed the method of nutrient agar diffusion disk. After standardization of turbidity of saline (0.9% NaCl) for UFC comparing with the scale of 0.5 Mc Farland was rated the bacterial growth front discs impregnated the presence of different volumes of extract and antibiotics. For the determination of genotoxicity and mutagenicity, beyond this extract were used as genotoxic and mutagenic agent's hydrogen peroxide (H_2O_2) and Stannous Chloride ($SnCl_2$). The results show that the extract in question has a similar effect of the antibiotics gentamicin and chloramphenicol and also that the extract has similar activity to the stannous chloride and hydrogen peroxide, which are agents that interfere with the bacterial genome. With the analysis of the results may suggest that the hydroalcoholic extract of the fruit peel of *Punica granatum* has cytotoxic, genotoxic and mutagenic action likely, but there is a need for more specific analyzes to elucidate the presence of this last feature.

Keywords: *Punica granatum*, disk diffusion, genotoxicity, mutagenicity, *Staphylococcus aureus*

Introduction

The culture of several people using plants as curative for various diseases (Rangel *et al*., 2001). The consumption of medicinal plants has increased dramatically, due to cultural and socioeconomic factors, since there are social classes with low financial power and has no access to allopathic medicine (BRAZIL, 2006).

Due to increased public interest in the use of these plants and their therapeutic potential, the scientific community has been searching for ways to obtain new herbal (MACIEL, *et al.* 2002; CALIXTO, 2005; MICHELIN *et al*., 2005; NOLDIN *et al*., 2006).

Herbal medicines are made from medicinal plants and / or its parts with known pharmacological characteristics. According Salvagnini and colleagues (2008), the World Health Organization has supported the use of medicinal plants, it is because of this use still be traditional among various peoples and by this practice bring many benefits to its users and curative factors. There are several auxiliaries, including the social and economic spheres, which work in advancing health through the use of medicinal plants. (ELISABETSKY, 1991).

Hope to enter the medical industry characteristics that pertain to the healing potential of medicinal plants arising encouraged the research of scientists. However, incorrect utilization and consumption may allow toxic and / or action has the effect not of interest, this should occur due to lack of knowledge of these adverse effects. Therefore it is necessary first of all to know the plant, part of this being exploited, otherwise there can be side effects and even by their inappropriate use effects may occur (PEREIRA *et al*., 2004).

Among the effects of medicinal plants can be noted that micro-controller. The emergence of new drugs controlling microorganisms is a strong ally for population health and the current growth area for scientific technical area, in search of the appearance of new molecules with significant biological features such as antimicrobials. With this new reality, people have access to knowledge about medicinal plants from experiments, assisting those using natural sources such as pharmacy besides inducing the generation of new patents and / or processes (FERREIRA, *et al*., 2011).

Additionally, another relevant to the development of antimicrobial herbal medicines reason is the fact that bacteria had the hallmark of becoming resistant to antibiotics. The emergence of new therapeutic aspects possible new alternative for the treatment of infectious diseases (ANTUNES *et al*., 2006).

A large number of medicinal plants have antimicrobial activity (YARNELL, 2002), and among them, *Punica granatum* has been used by people for this purpose (WERKMAN *et al.,* 2008) including the replacement of antibiotics as chloramphenicol and ampicillin in combating Salmonella typhi is the causative agent of typhoid fever (PEREZ AND ANESINI, 1994).

In 2009, the Ministry of Health released the RENISUS (National List of Medicinal Plants of Interest to SUS) list, which is integral to *Punica granatum*, that this is a listing of currently 71 plant species of interest to the health care system with in order to encourage and support research in the field of herbal medicine in order to develop new drugs derived from medicinal plants that are safe and effective in combating diseases (PORTAL OF HEALTH, 2009). The *Costus spicatus* (cane swamp), for example, belongs to list and is able to help in cases of hypoglycaemia (NASCIMENTO, 2015).

The *Punica granatum*, known as pomegranate, is a shrub native to the Middle East. This is highly branched and slightly spiny. Its composition is divided between seeds, corresponding to 50% of fruit weight, and transparent compartments containing a spongy tissue (rag). Popularly used to treat gastrointestinal disorders, loss of dental prophylaxis, relief of pain and other ear (Navarro et al. 1996). Studies show that pomegranate, because of its high amount of phenolic compounds can act as a preventive measure to oxidative processes, in other words, its composition is a great antioxidant (LANSKI AND NEWMAN, 2007; JURENKA 2008).

Studies have reported that an extract of *Punica granatum* contains portions of Ellagic Acid and other ellagitannins. Such substances are able to induce vasodilatation, scavenging free radicals, besides presenting potential lipid-lowering, anti-inflammatory and anticancer (USTA *et al*., 2013)

According NODA and colleagues (2002), the fruit contains anthocyanins (delphinidin, cyanidin and pelargonidin), quercetin, phenolic acids (caffeic, catequínico, chlorogenic, and ortho paracumárico, ellagic, gallic and quinic acids) and tannins (punicalagin). In addition to these substances, experiments argue that *Punica granatum* has flavonoids able to prevent the activity of cyclooxygenase and lipoxygenase enzymes oxidizing (F. A. JARDINI e J. MANCINI FILHO, 2007)

2. Objectives.

1.1. Overall goal.

Study the chemical, biological and toxicological properties of the hydroalcoholic extract of Punica granatum.

1.2. Specific goals.

1. Get hydroalcoholic extract from the processing of dried peel of the fruit of *Punica granatum*.
2. Perform biochemical and biophysical characterization of the hydroalcoholic extract of *Punica granatum.*
3. Evaluate the genotoxic, cytotoxic and mutagenic action of the hydroalcoholic extract processed from the dried peel of the fruit of *Punica granatum*.

3.Materials and Methods

3.1. **Obtain the extract.**

The hydroalcoholic extract of *Punica granatum* was supplied by EMBRAPA Food. The same was obtained

from the processing of peels of pomegranate fruit. First the fruit was pulped, their shells passed by dehydration at a cabin on a laboratory scale dryer temperature of 40 ºC and air velocity of 1 ms-1. Subsequently, the shells (1 kg) were crushed. The obtained powder was extracted with a Soxhlet submitted (novatécnica) with the solvent ethanol: water (80:20, v / v). The alcoholic extract was placed in a rotary evaporation (rv10 digital, Ika) the temperature of 70 and 250 rpm rotation.

3.2. Organoleptic characterization.

The extract was evaluated for its organoleptic characteristics. Visual, taste and smell the same aspects were observed.

3.3. Characterization of Biochemistry and Biophysics Extract.

The hydroalcoholic extract was characterized biophysically and biochemistry. The procedure was performed by spectrophotometric reading ultraviolet (UV-2450, Shimadzu), and determination of pH and conductivity in two stages.

The extract was first subjected to a serial dilution, so that it conforms to the law of Lambert and Berr. This law directs that the value of the wavelength related to the absorbance scan is kept at values lower and / or equal to 1 absorbance.

Dilution 1: 1 mL of concentrated extract (1.67g / mL) in 10 mL of distilled water (0.167g /mL).
Dilution 2: 100 uL of thr 1st dilution (0.167g / mL) in 10 mL of distilled water (0.0167g / mL).
Dilution 3: 10mL of then 2nd dilution (0.0167g / mL) in 10 mL of distilled water (0.00167g / mL).
Dilution 4: 20 mL of the 3rd dilution (0.00167g / mL) in 100 mL of distilled water (0.0000167g /mL).
Dilution 5: 120mL of the 4th dilution (0.0000167g/mL) in 60 mL of distilled water (0.0000002783g/mL).
The first step consisted in the dilution and reading of the sample in the ultraviolet spectrophotometer (UV-2450, SHIMADZU), wavelength 200-800 nm. Measurements were made using acrylic cuvettes. The pH and conductivity were checked in bench top pH meter (NT-PHN, NOVATÉCNICA).

Table 1: Conditions for phase 2 Characterization of Biochemistry and Biophysics

1	Open tube.	4	Vacuum tube
2	Closed tube.	5	Vacuum and protected from light.
3	Closed and protected from light tube.	6	Closed tube after heating.

The step of determining pH, conductivity and spectrophotometric reading was performed on the day following the phase 1 as it is the same procedure of that phase, and dilution of the extract is performed in different physical condition, and measurements are recorded after 24 hour period.

Two groups of four 15mL conical tubes and four vacuum tubes under the conditions described in Table 1 were used, with one exposed to room temperature and kept under refrigeration another group, totaling 12 tubes. Tests were performed in duplicate.

3.4. Cytotoxicity and Genotoxicity tests.

Tests for cytotoxicity and genotoxicity of the extract were performed using the disc diffusion method. The bacterial strains used are of *Staphylococcus aureus* (S. aureus ATCC 8096) strain. The bacterial concentration was standardized using the range of 0.5 Mc Farland (Laborclin).

The cytotoxicity and genotoxicity tests were conducted in four steps:

Step 1: Removal of colonies and transfer to selective medium white medium (nutrient agar, Merck). Incubation was placed in a bacteriological incubator (Ps -101, scientific Solab) at 35 °C for 24h (maintenance of colonies).

Step 2: Withdrawal of colony maintenance, in addition saline (NaCl 0.9%) and turbidity compared to the equivalence scale with 0.5 MC Farland.

Step 3: Sowing the material solubilized in saline solution in plates (Petri dishes for up to 50mL) for the disk diffusion test.

Step 4: Performing the disk diffusion test.

The disc diffusion test was performed by depositing paper discs in sterile plates with different volumes in the extract at a concentration of 0,167g /mL, plus the use of antibiotics such as amoxicillin (500 mg / mL), chloramphenicol discs (30 mg / disc) discs ampicilin (10 mg / disc) discs gentamicin (10 mg / disk) and other substances such as H_2O_2 (hydrogen peroxide, 3 = volume%) and stannous chloride ($SnCl_2$ Vetec). For insertion of the material in the plates, automatic pipettes (Gopet 0, 5-10µL II, and 20-200µL 100-1000µL) were used. All antibiotics employees are also encouraged to use the SUS through RENAME (National List of Essential Medicines).

Below is an outline of the test:

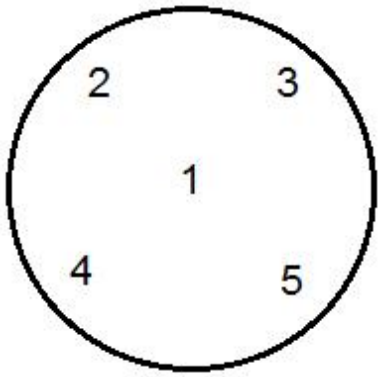


Plate 1 (duplicates A and B):
1- 24 µL NaCl (0.9%)
2 -24µL of diluted extract (0,167g /mL).
3- 12µL of diluted extract (0,167g /mL).
4- 24µL amoxicillin (50 mg / mL).
5-12µL of the diluted extract (0,167g /mL) + 12µL of amoxicillin (50 mg /mL)

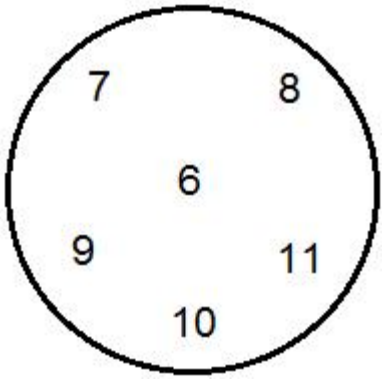


Plate 2 (duplicates A and B):
6 -Disc Chloramphenicol.
7- Disc Chloramphenicol + 12µl of diluted extract (0,167g / mL).
8-Disc Ampicilin.
9-Disk Ampicilin + 12µl of diluted extract (0,167g / mL).
10-Disc Gentamicin
11-Disc Gentamicin + 12µl of diluted (0,167g /mL) extract.5-12µL of the diluted extract (0,167g /mL) + 12µl of amoxicillin (50 mg / ml)

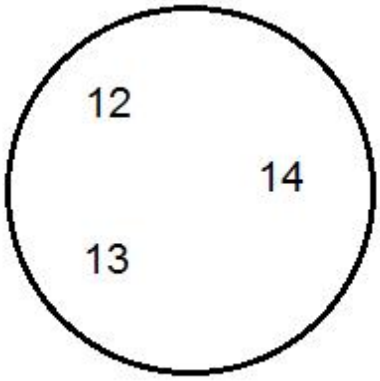


Plate 3 (duplicates A and B):
12-24µL of hydrogen peroxide (H_2O_2 volumes = 3%)
13-12µL of hydrogen peroxide (H_2O_2 volumes = 3%)
14-12µL of hydrogen peroxide (H_2O_2 volumes = 3%) + 12µL of diluted extract (0,167g /mL). .

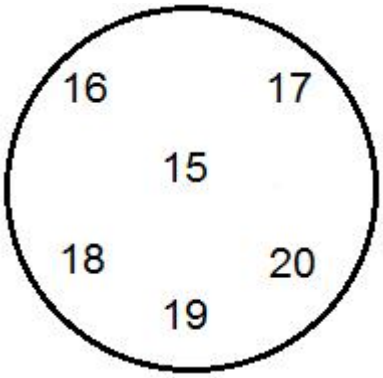


Plate 4 (duplicates A and B):
15-24 µL stannous chloride ($SnCl_2$) (5 mg /mL)
16-12µL of stannous chloride ($SnCl_2$) (5 mg /mL)
17-8µL diluted extract (0,167g mL).
18- 8µL hydrogen peroxide (H_2O_2 volumes = 3%)
19-8µL stannous chloride ($SnCl_2$) (5 mg /mL)
20-8µL stannous chloride ($SnCl_2$) (5 mg /mL) + 8µL of dilute extract (0,167g /mL) + 8µL of hydrogen peroxide (H_2O_2 volume = 3%).

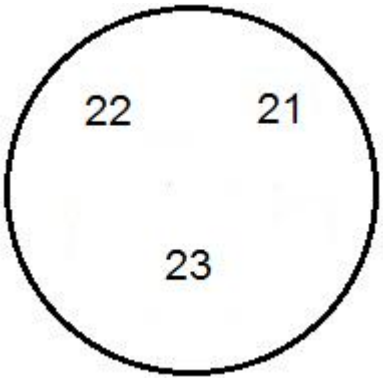


Plate 5 (duplicates A and B):
21-12µL of diluted extract (0,167g /mL) + 12µL $SnCl_2$ (5 mg /mL)
22-12µL of diluted extract (0,167g /mL) + 12µL of H_2O_2 (5 mg /mL)
23-12µL $SnCl_2$ (5 mg /mL) + 12µL of H_2O_2 (volume = 3%)

3.5. Statistical Tests

The results were statistically analyzed with the help of Graph Pad software InStat3. The analyzes were performed according to the Tukey-Kramer test in combination with ANOVA for changes for absorbance wavelength between phases 1 and 2 and the measures of halos; and according to the test of Kruskal Wallis ANOVA with respect to changes in pH and conductivity.

4. Results

4.1. Concentration of the extract.

The aqueous residue obtained *Punica granatum* concentration of the hydroalcoholic extract showed the final concentration of 1,67g /mL.

4.2. Organoleptic characterization.

Table 2: Characterization Organoleptic hydroalcoholic extract of *Punica granatum*.

CHARACTERISTICS EVALUATED	RESULT
Visual aspects	Liquid with high viscosity and dark brown coloring
Smell	Characteristic
Flavor	Astringent

4.3. a. Characterization Biochemistry and Biophysics.

Tests of biophysical and biochemical characterization of the aqueous extract of *Punica granatum* showed the following guidelines for pH, conductivity and spectrophotometry:

Table 3: Characterization Test Biochemistry and Biophysics of Extract (phase 1)

pH	Conductivity (mV)
6.12	58.25

Table 4: Characterization Test extract of Biochemistry and Biophysics - room temperature (Phase 2)

Tube	Condition	pH	Condutivity (mV)
1	Open tube	5.7	42.4
2	Closed tube	5.91	52
3	closed/ Protect from light tube	6.07	47.2
4	Closed/ Vacuum tube	6.35	37.4
5	Closed/ vacuum/ protect from light tube	6.47	37
6	Closed after heating tube	6.4	44

Table 5: of the Statement Characterization Test Biochemistry and Biophysics - refrigerated (phase 2)

Tube	Condition	pH	Condutivity (mV)
1	Open tube	6.61	34.25
2	Closed tube	5.76	49.2
3	closed/protect from light tube	5.97	50.5
4	Vacuum tube	6.2	41.5
5	Vacuum/protect from light tube	6.51	36.55
6	Closed after heating tube	5.98	57.35

Graphic representation of the characterization of the extract (phase 1)

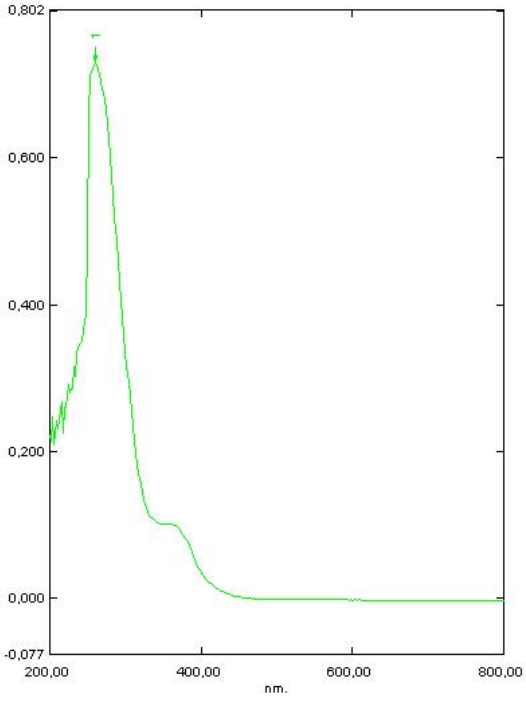


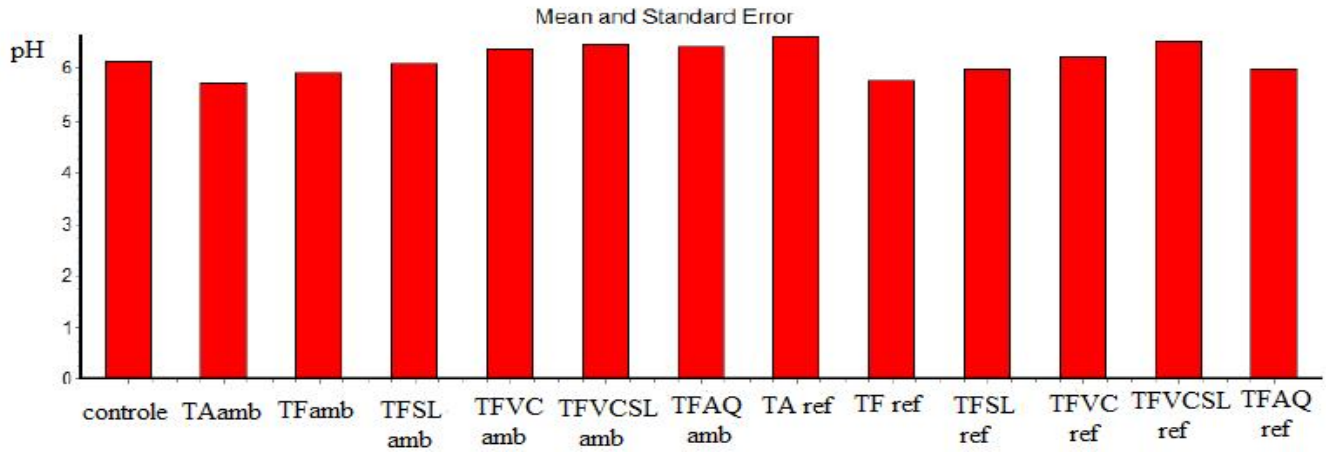
Table 1: Scan Phase 1

Wavelengths (nm)	absorbance
250	0.79

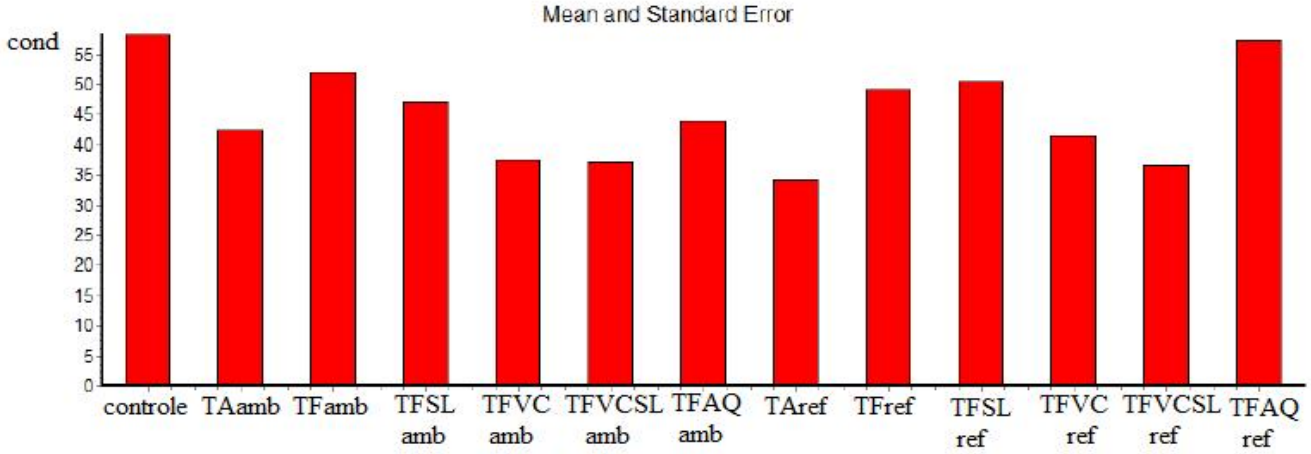
Note: peaks at about 352 and 258nm / 354nm, although not detected by the unit are present.

4.4. Statistical analysis.

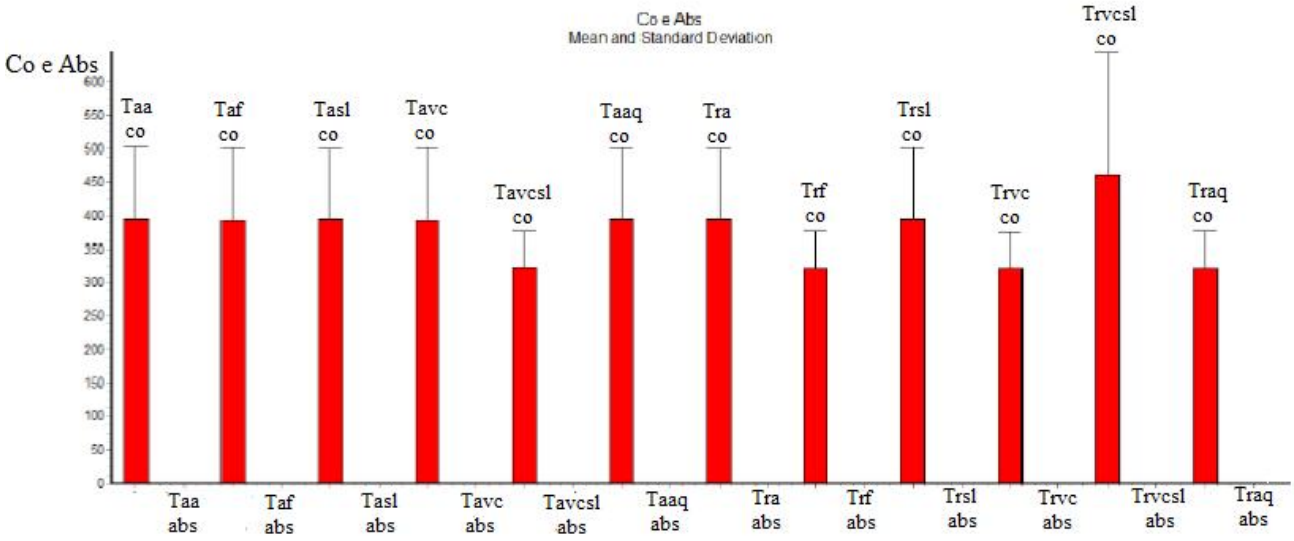
4.4.1. a. Graphic representation of the characterization of the extract pH (Statistical Analysis)



4.4.2. b. Graphic representation of the characterization of the Extract Conductivity (Statistical Analysis)



4.4.3. c. Graphical Representation of characterization Extract as Absorbance and Wavelength (Statistical Analysis)



4.5. Teste Disk Diffusion.

The disk diffusion tests with *S. aureus* (ATCC 8096) showed halos with the following dimensions:

Table 7: Results of Disk Diffusion (Duplicates Plate 1)

	Plate 1 A Approximate values	Plate 1 B Approximate values
1	No Halo	No Halo
2	Between 28 and 30 mm.	Between 28 and 30 mm.
3	Between 20 and 25 mm.	Between 20 and 25 mm.
4	>50 mm	>50 mm
5	>50 mm	>50 mm

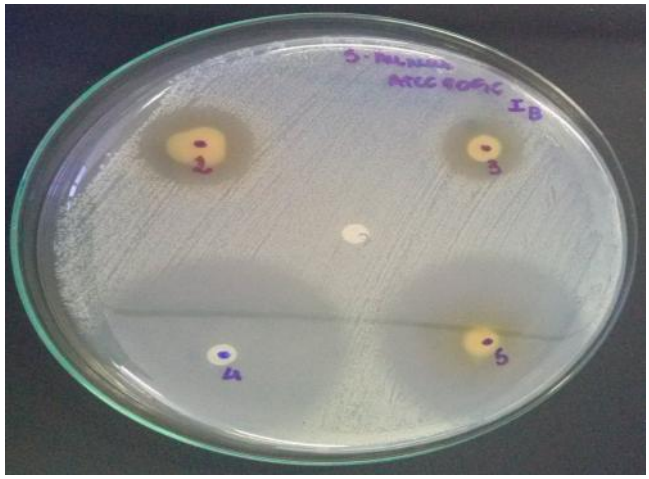


Figure 1: plate IB, 1- 24 uL NaCl (0.9%) ; 2- 24 uL diluted extract (0.167g /mL), 3- 12µL of diluted extract (0.167g /mL); 4 - 24µL of amoxicillin (50 mg /mL); 5- 12 uL amoxicillin (50 mg / mL + 12µL of diluid extract (0.167g /mL). Presence of "stain" on the discs concerning halos 2 and 3 Source: author

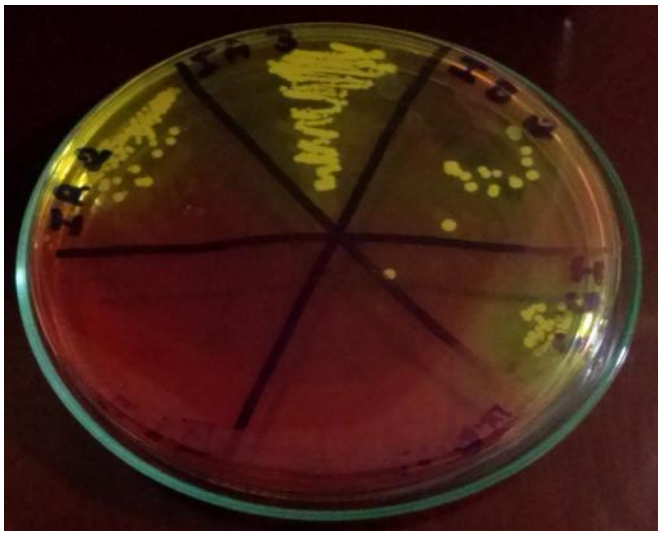


Figure 2: sowing the material bump the "stain" present in plates 1 and 2 in the middle salty mannitol (selective medium for *S. aureus*) strains resistant to investigation of the application of the extract. Source: author.

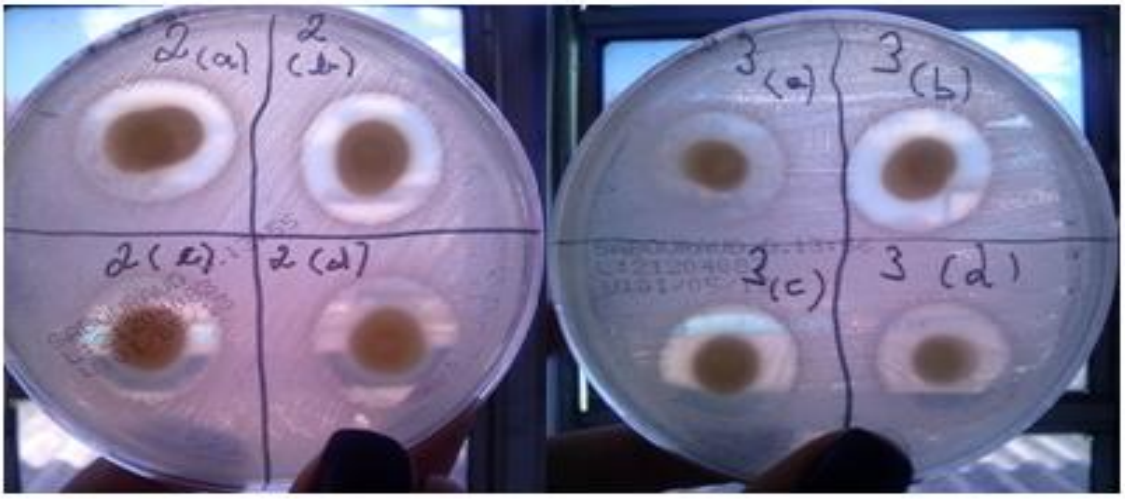


Figure 3: New disk diffusion only to the boards regarding guidelines of halos that grew in selective medium for *S. aureus,* material collide salty mannitol medium and seeded in white, showing that there was the same behavior. Source: author

The presence of spots in halos 1, 2 and 3 of the plate 1 led to speculation that it might exist resistant bacteria therein. To investigate this possibility, was performed one sowing of harvested material from spots in mannitol salt medium and growth was observed on selective medium.

Was made new disk diffusion with the strains that grew in the middle mannitol in order to know if the bacteria would have the same behavior as the first broadcast. As a result, it was found that the bacteria followed the same orientation as the first test.

Table 8: Results of Disk Diffusion (Duplicates Plate 2)

	Plate 2 A Approximate values	**Plate 2 B Approximate values**
6	Between 30 and32 mm	Between 30 and 32 mm
7	Between 30 and 32 mm	Between 30 and 32 mm
8	Between 35 and 40 mm	Between 35 and 40 mm
9	Between 40 and 45mm	Between 40 and 42mm
10	20 mm	20 mm
11	15 mm	15 mm

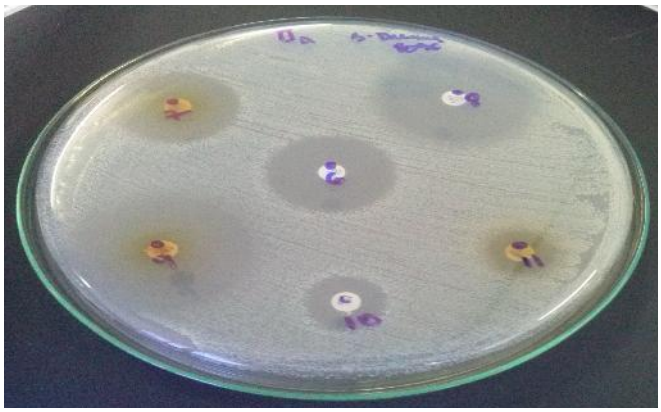


Figure 4: Plate IIA. 6 disk Chloramphenicol, Chloramphenicol disc 7 + 12µL of diluted extract (0,167 g / mL), 8-disk ampicillin, ampicillin 9-disk + 12µL of diluted extract (0.16 g / ml), 10-disk gentamicin, 11-disc gentamicin+ 12µL of diluted extract (0,167g / mL) Source: author.

Table 9: Results of Disk Diffusion (Duplicates Plate 3)

	Plate 3 A Approximate values	Plate 3 B Approximate values
12	>50 mm	>50 mm
13	50 mm	Between 45 and 50 mm
14	>50 mm	>50 mm

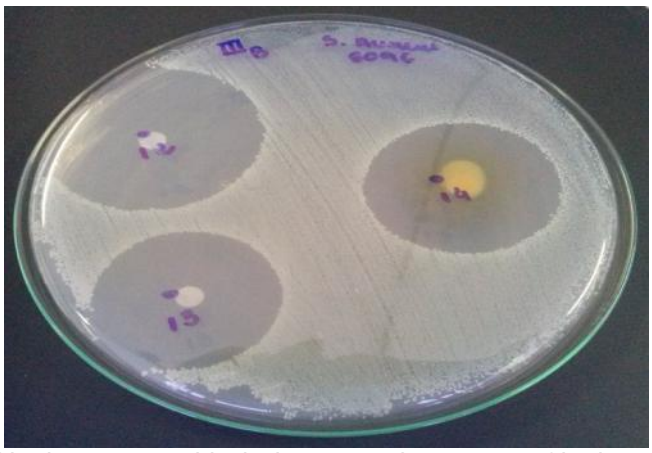


Figure 5: Plate IIIB. 11-24µL of hydrogen peroxide (volume = 3%), 12-12µL of hydrogen peroxide (volume = 3%), 13-12µL of hydrogen peroxide (volume = 3%). Source: author.

Table 10: Results of Disk diffusion (Duplicates Plate4)

	Plate 4 A Approximate values	Plate 4 B Approximate values
15	Between 15 and20 mm	Between *15 and 20 mm*
16	Between 10 and 12 mm	Between *10 and 12 mm*
17	Between 20 and 22 mm	Between *20 and 22 mm*
18	Between 45 and 50 mm	Between *45 and50 mm*
19	Between 10 and 12 mm	Between *10 and 12 mm*
20	Between 48 and 50 mm	Between *48 and 50 mm*

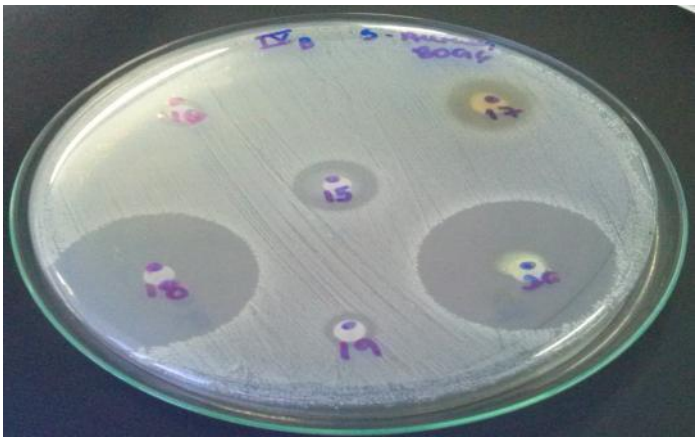


Figure1 6: Plate IV B.15- 24µL of stannous chloride (5 mg / mL), 16-12µL stannous chloride (5 mg / mL), 17-8µL diluted extract (0.167g / mL), 18 8µL hydrogen peroxide (volume = extract 3%), 19-8µL stannous chloride (5 mg / ml), 20-8µL of stannous chloride solution (5 mg / ml) 8µL + hydrogen peroxide + 8µL of the diluted extract (0,167g / mL) Source: author.

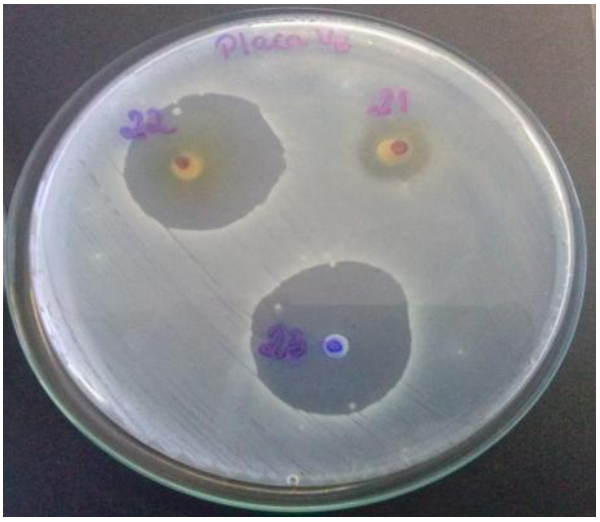


Figure 7: Plate VB.21- 12µL of diluted extract (0.167g /mL) + 12µL of stannous chloride (5 mg /mL), 22-12µL of diluted extract (0.167g /mL) + 12µL of hydrogen peroxide (volume = 3%) , 23-12µL stannous chloride (5 mg /mL) + 12µL of hydrogen peroxide (volume = 3%). Source: author

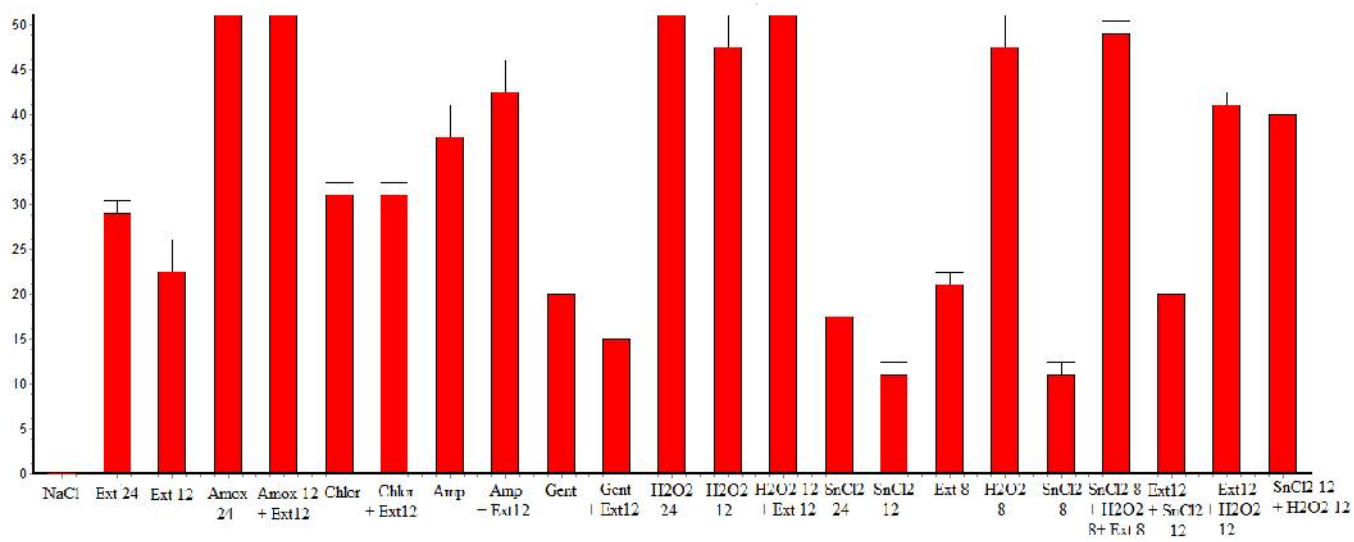


Figure 8: Statistics of disk diffusion. A: 24 µL of NaCl (0.9%), B: 24µL of diluted extract C: 12µL of the diluted extract, D: 24 µL of amoxicillin (50 mg / ml) E: 12µl of diluted extract + 12µL of amoxicillin F: Disk Chloramphenicol, G: Disc Chloramphenicol + 12µL of diluted extract, H: Disc Ampicillin, I: Disc Ampicillin + 12µL of diluted extract, J: Disc Gentamicin, K: Disc Gentamicin + 12µL of diluted extract L: 24µL of Hydrogen Peroxide (H_2O_2 = 3 volume%), F: 12µL of Hydrogen Peroxide (H_2O_2 volume = 3%), N: 12µL of Hydrogen Peroxide (H_2O_2 = 3 volume%) + 12µL of the diluted extract, O: 24 µL of stannous chloride ($SnCl_2$) at 5 mg /mL, P: 12µL of stannous chloride ($SnCl_2$) at 5 mg /mL, Q: 8µL extract diluted, R. 8µL of hydrogen peroxide (H_2O_2 volume = 3%), S: 8µL of stannous chloride ($SnCl_2$) at 5 mg /mL, T: 8µL stannous chloride ($SnCl_2$) at 5 mg /mL + 8µL extract diluted+ 8µL of Hydrogen Peroxide (H_2O_2 volume = 3%), U: extract 12µL 12µL of diluted + $SnCl_2$, V: 12µL + 12µL of extract diluted H_2O_2, W: 12µL of $SnCl_2$ + 12µL of H_2O_2

5.Discussion

Statistical analysis was able to identify the absence of changes considered significant in pH, conductivity, wavelengths and the absorbance, indicating that the different conditions to which the extract was submitted in phase 2 provide no degradation of bioactive compounds even giving this characteristic of stability.

Through literature and this work, which results in the sensitivity of S. aureus to alcoholic extract *Punica granatum* independent of dose, it can be stated that this, as well as dyes derived from fruit, has antimicrobial activity (TRINITY *et al*., 2009; WERKMAN *et al*., 2008; RMR CATO *et al*., 2006; PEIXOTO, 2014; PEIXOTO, 2015), giving this cytotoxic potential.

Gentamicin and chloramphenicol act on bacterial protein synthesis (Rang, Dale, Ritter, 2001), it is suggested that the hydroalcoholic extract of pomegranate has a similar action to those due to similarity in the extent of halos assigned by statistical analysis beyond its best interacting with them when compared to antibiotics ampicillin and amoxicillin. Previous studies such as those reported by Canton et al (2010), and Adams et al (2013) show that there is a synergistic effect of the extract of *Baccharis dracunculifolia* DC and *Allium sativum*, respectively, gentamicin against S. aureus; Another study describes the possibility of using the extract of *Punica granatum* as an alternative to combat diseases in which the causative agent is resistant to the action cloranfenicol, as with Salmonella tiphy (WERKMAN, C., *et al*., 2008 CITED PEREZ, C., AND ANESINI C., 1994). One may suggest that the same mechanism can be used against *S. aureus*, as in the present study, the extract showed similar action to chloramphenicol.

The amoxicillin and ampicillin are antibiotics which inhibit cell wall synthesis (RANG; DALE AND RITTER, 2001). In this paper, we realize there is a possibility that the extract be able to preserve the action of these antibiotics or small proportions of extract may have lower effects ampicillin, but not the same potentiates the action of these antibiotics nor interact so well when comparing with the chloramphenicol or gentamycin. Penicillin may have limited effect on the bacteria in question, given that Staphylococcus aureus is resistant to oxaciclina and meticiclina (CATO *et al*., 2006). Thus, it can be suggested that the relative limitation observed between the penicillin's and the extract is not attributable to the effects of Punica granatum, but is related to the interaction of the antibiotic the microorganism.

Studies describe that *Punica granatum* contains a variety of phenolic compounds such as ellagic acid moieties and ellagitannins (USTA *et al*., 2013), anthocyanin, quercetin, phenolic acids, tannins (NODA *et al.,* 2002; PEIXOTO, 2015) or that has flavonoids in its composition (WERKMAN, C. et al., 2008; PEIXOTO, 2015), which can giving it a potential antioxidant. In the present study, it was observed that pomegranate extract, depending on the proportions used, similar action has or is able to potentiate the effects of $SnCl_2$, which is a powerful reducing agent capable of inactivating E. coli strains, and through the generation of free radicals in vitro, can break bonds in the DNA plasmids (GIUSEPPE *et al*., 2007).

As with $SnCl_2$, the extract showed similar action on hydrogen peroxide (H_2O_2), which, like all detergents oxidants, has germicidal capacity arising from the production of nascent oxygen resulting from contact with catalase (enzymes present in blood and tissues), which makes it a great help in the sterilization of wounds from the mechanical action of this oxygen released (MORIYA T, Modena JLP, 2008). Furthermore, it is known that H_2O_2 is one of the components of the Fenton reaction, which induces oxidative stress processes, through the production of free radicals (BARREIROS *et al*., 2006).

Peixoto (2014) showed that the prokaryotic cell model, the extract induces oxidation, which could help in the development of reactive oxygen species, which explains its bactericidal action previously tested by Trinity and colleagues (2009), which proves the antimicrobial activity of the dye - S. aureus front pomegranate rind depicting the presence of halos with values between 8 and 13 mm from the well by the method.

In spectrophotometric scan the wavelengths of 260, 342, 362 and 510nm in most representatives in the extract were detected. The literature states that the wavelengths between 240 to 280nm and 300 are characteristic of the presence of flavonoids (BOBIN RAYMOND M, MARTINI MC, 1994), and the wavelength of 510 nm is characteristic of condensed tannins (ROCHA, 2011) or that this characterizes the wavelength of maximum absorbance value for cyanidin-3-glucoside (ABE, 2007).

It can be seen then that the extract in question, the analytical methods used were able to demonstrate only the presence of flavonoids and tannins or a type of anthocyanin in minor proportions, taking into account that in length from 510nm absorbance values were observed very small.

Condensed tannins also known as proanthocyanidins, are secondary metabolites which have characteristics as input for astringent taste of food and protein precipitation (QUEIROZ, *et al.,* CRAA., 2002). Already flavonoids have the characteristic inhibit the action of cyclooxygenase and lipoxygenase, which are two closely related enzymes of the inflammatory process. Anthocyanins are one type of flavonoid widely present in nature and are assigned to these blue, violet and red colors of fruits (ABE, 2007), moreover, the anthocyanins are considered one of the most significant natural antioxidant (SANTIAGO *et al*., 2011)

The absence of other bioactive compounds that, according to literature, are present in the composition of *Punica granatum* may be related to the fact that seasonal factors such as soil composition, humidity, temperature can interfere with the identification of these, which influence the level of phenolic compounds plant. (MONTEIRO *et al.*, 2006; SUZANA, C SANTOS *et al*., 2006) or even can relate the fact of the extract was obtained from the processing of peels to that phenolic compounds are present throughout the factor plant but in different proportions (JARDINI *et al.,* 2010).

The *Punica granatum* has a cytotoxic effect, which is related to the sensitivity of S.aureus effect of the extract. It is speculated that this extract has genotoxic potential, which can be attributed to the good interaction and increase this leads to the action of H_2O_2 and $SnCl_2$ suggesting that the extract has the ability to induce the generation of reactive oxygen species and provide changes the bacterial genome. Such modifications in the DNA of the microorganism can also be linked to the possible action of the extract on ribosomal subunits in order to extract features, probably similar to the antibiotics gentamicin and chloramphenicol mechanism of action, which are able to inhibit protein synthesis bacteria by inhibition of ribosomal subunits coupling or through the production of aberrant proteins (RANG, DALE AND RITTER, 2001). One may suggest that the extract has mutagenic action considering that possibly this is able to interfere with bacterial genes, however, so that this potential is better understood, it is necessary to incorporate more specific tests related to this feature.

6.Conclusion

From the results presented, it can be suggested that, against *Staphylococcus aureus*, alcoholic extract of *Punica granatum* possibly have cytotoxic, genotoxic, oxidative, inhibitory action of protein synthesis, enabling the incorporation of pomegranate extract as a potential alternative to treatment of staphylococcal diseases.

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