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SPECTROPHOTOMETRIC AND HEMOLYTIC ANALYSIS OF AN EXTRACT OF *COSTUS SPICATUS*

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ABSTRACT: *According to the World Health Organization it is estimated that 80% of the world population, somehow, uses plants to treat diseases. Despite the wide variety of synthetic drugs (many originating related to the natural environment), interest in the knowledge of the morphology, chemical composition and pharmacological properties has increased especially when it comes to Brazilian plants. Costus spicatus plant has attracted the attention of researchers because it was found in the rhizomes just a new source of diosgenin, a precursor of steroid hormones. Also, for phytochemical studies of the aerial parts of the plant, have been described recently, two new diglycosides flavonids: a tamarixetin 3- neohesperidoside and canferol 3-O-neohesperidoside. Other quite well known compounds 3-O- neohesperidoside flavonoids quercetin and six were identified. These flavonoids diglycosides had proven anti-inflammatory activity. However, there are no studies proving the efficacy and safety of the use of this plant for therapeutic purposes. The objective of this study is to evaluate the spectrophotometric standard of a Costus spicatus extract and its effect on red cell membrane level. Wistar rat blood samples were collected and incubated with different saline concentrations and samples the buffered and unbuffered extract. The spectrophotometric reading of the extract was performed at different concentrations and the percentage hemolysis was determined. Based on the analysis of the results was possible to speculate that the extract has in its composition phenolic molecules with anti-hemolytic and saponins molecules which could be responsible for hemolytic action.*

KEYWORDS: Costus spicatus, saponins, phenolic molecules, hemolysis.

INTRODUCTION

"Since time immemorial, man looking in nature resources to improve their living conditions to thereby increase your chances of survival by improving their health. In all ages and cultures, he learned to take advantage of local natural resources "(Brazil, phytotherapy in SUS, 2006). According to the World Health Organization (WHO, 2007) it is estimated that 80% of the world population, somehow, uses plants to treat diseases. Despite the wide variety of synthetic drugs (many originating related to the natural environment), interest in the knowledge of the

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morphology, chemical composition and pharmacological properties has increased especially when it comes to Brazilian plants. Brazil is custodian of huge rich flora in raw material that has aroused the interest of researchers from various universities and institutions dedicated to herbal medicine. The Amazon region is considered inexhaustible source of potential therapies with the use of medicinal plants (Martins, 2006).

The pharmacological potential of medicinal plants may be output to a more economic treatment of various diseases including chronic diseases. In developing countries, about 80% of deaths are caused by chronic diseases (PHA, 2005). Among these Diabetes mellitus, has drawn attention to its growth in the number of occurrences. The World Health Organization estimates that annually occur 3 million deaths caused by diabetes. There are also consequences as 1 million amputations, 500,000 cases of kidney disease, blindness 300,000, 285 million people worldwide with diabetes, and in 2030 will be 435 million diabetics in most developing countries (IDF, 2009). There is an estimate that these to that disease problems generate annual spending of $ 150 billion (IDF, 2009).

In our country there are 10 to 12 million people with diabetes. Of these, 10% type I (100% of diagnosed, treated 100%) and 90% type II. Diabetes and its cardiovascular and renal consequences are now the greatest impact on public health factors leading to a high social and economic cost. According to the Ministry of Health (MOH), 1/3 of the patients buy products to treat Diabetes in pharmacy, while most government receives free products, which requires the Brazilian government high spending on the treatment of diabetes and its consequences. The MS provides an increase of 60% of deaths in cases of Diabetes in Brazil by 2025 (IDF, 2009). The choice of plants with hypoglycemic activity is mainly due to the fact that diabetes is in Brazil one of the diseases with higher mortality rates, high prevalence in adults and have high social-medical costs (IDF, 2009).

The *Costus spicatus* Swartz plant (Family: Zinziberaceae / Costaceae), popularly known in Brazil 'cane of swamp', is a native species found in humid South of Mexico, Yucatan, Costa Rica, northern Colombia and Brazil. The cane of swamp name is used to describe two species of Costus: *Costus spiralis* Rosc. and *Costus spicatus* Swartz, as both have the same use in popular therapy. Found throughout the Brazilian territory, the *Costus spicatus* is a shrub, perennial, rhizomatous, erect, unbranched, of 1-2m high, native mainly in the Atlantic Forest and Amazon region. Presents alternating leaves, membranous, Papyraceous provided with sheaths, velvety on both sides, 25-40 cm long and 6-10 cm wide. It has inflorescences in strobiliformis terminal spikes, with large showy bracts of red color, which protect the yellowish flowers. Multiply both by seeds and by rhizomes. Easy availability allows use of all parts of this plant.

One of its uses is the ornamental gardens both as to cut flower production, as can be seen in Figure 3 beauty. Its leaves, stems and rhizomes are used in traditional medicine a long time, especially in the Amazon region (GASPARRI , 2005). In this region many species are used as spices, seasonings, drugs, flavoring agents and a source of certain dyes (GASPARRI, 2005). Many species of the genus Alpinia, Amomum, Curcuma, Costus, Caempferia and Zingiber are present in traditionally prepared tonics and ingredients called 'potions', which are commercially available. It is popularly used for its purifying and diuretic, for relief of urinary infections and to expel kidney stones. Also for treating colds, sore throat, dysentery, diarrhea, and in the treatment of diabetes. Phytochemical analysis rhizomes *Costus spicatus* revealed the presence

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of flavonoids, flavoglissídios, saponins and sapogenins. Antimicrobial, anti-inflammatory and diuretic activities also proven. However, its effect was not studied hipogliceminante (ANTONIOLLI, 2007).

This plant has attracted the attention of researchers because it was found in the rhizomes just a new source of diosgenin, a precursor of steroid hormones (ANTONIOLLI, 2007). Also, for phytochemical studies of the aerial parts of the plant, have been described recently, two new diglycosides flavonids: a tamarixetin 3- neohesperidoside and canferol 3-O-neohesperidoside. Other quite well known compounds 3-O- neohesperidoside flavonoids quercetin and six were identified. These flavonoids diglycosides had proven anti-inflammatory activity. However, there are no studies proving the efficacy and safety of the use of this plant for therapeutic purposes (GASPARRI, 2005).

The objective of this study is to evaluate the spectrophotometric standard of a *Costus spicatus* extract and its effect on red cell membrane level.

MATERIAL AND METHODS

Sample Plant and Collection

We used the leaves of *Costus spicatus* collected during flowering (December-January) on the Site My Dream, the city of Itacuruça, RJ, Brazil. The choice of the time of collection was based on literature data that indicate that concentrations of flavonoids, tend to increase during this time. This is due to the action of flavonoids as attractors of pollinators and as co-pigments of anthocyanidins (DOURADO & LADEIRA, 2008). The voucher specimen of the species will be deposited in the Herbarium of the UFRJ National Museum for botanical certification by specialist.

Preparation of Extract:

The leaves of *Costus spicatus* were dried in an oven with circulating air at 40°C, manually crushed and stored in amber glass bottle. The crude aqueous extract was obtained by infusion of bark powder using distilled water as liquid extractor. The extract was lyophilized and stored in amber glass bottle under refrigeration minimum temperature of - 20°C.

Experimental animal:

The animals used in this study were male *Wistar* rats aged approximately two months, the Osmotic Fragility test. The rats were kept under the same precautions regarding the power (feed and water ad libitum) except during the experimental phase, temperature (22 ± 25°C) and 12-hour cycles light / dark in the animal house of the Foundation State University Center of the West Zone (UEZO).

Extract Effect Evaluation of Aqueous Lyophilized *Costus spicatus* in Osmotic Fragility:

Blood collection *Wistar* rats:

Wistar rats were used adult, male, approximately two months of age. The collection is performed after the animal is asleep with inhalant solution of alcohol and chloroform. The procedure was the cardiac impulse. Syringes were used 5mL (with citrate solution plus PBS) and heparinized tubes (sodium heparin vacutainer). This experiment was performed in triplicate.In the first step were removed and 3 mL of blood was added to 3 mL of each of the concentrations of the lyophilizate extract (100%, 50%, 25%, 12.5%, 6.25% and 3.12%) diluted in water distilled using 3 mL of blood diluted in 0.9% NaCl as control. The concentration regarded as 100% is 10 mg/mL and the other obtained by serial dilution. After waiting 60

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minutes of action of extract the tubes are centrifuged at 1,200 rpm for 15 minutes. We compared the mass precipitation of red blood cells in the background compared to the control.

In the second step were removed and 3 mL of blood extract added at different concentrations diluted in 0.9% NaCl solution instead of distilled water, thereby standardizing the optimal percentage for the experiment. The last step used 3 mL of blood beyond the established standard, diluted extract in 0.9% saline buffered solution pH 10 Sodium Bicarbonate for behavior comparison at higher pH. We collected 100 microliters of the RBC mass and added to 0.5 mL of the different salt concentrations of 0.1% to 0.9% after standing for 60 minutes and centrifuged for 15 minutes at 1,200 rpm the supernatant was collected and added to microwell plate (96 microwells) microplate reader and the absorbance reading made at the wavelength of 540 nm.

Scanning Spectrophotometer Lyophilized extract of *Costus spicatus* and Gross Extract:
Scanning the freeze-dried aqueous extract and crude aqueous extract was made of 200 nm to 1000 nm with 10 nm range. At wavelengths 200nm to 400 nm was used UV light and the absorbance was observed in molecular absorption spectrophotometer (LABORANA LABSP220 Digital Spectrophotometer, 200-1000nm Range From) with the extract prepared in quartz cuvette. In visible light at wavelengths from 400 nm to 1000 nm glass cuvette was used to check absorption.

Osmotic Fragility Test:
Initially, 5 mL of *Wistar* rat blood were collected and 1.5 mL blood incubated in three different solutions: 12,5 indicated as the tube containing the lyophilized extract *Costus spicatus* at a concentration of 12.5% extract in distilled water the tube indicated as 6.74, containing 12.5% diluted in water and extract with high pH 6.74 with addition of sodium bicarbonate buffer pH 10, and control tube as indicated with NaCl solution to 0 9%. The blood remained incubated for 60 minutes and then centrifuged for 15 minutes at 1,200 rpm. The initial phase of the Osmotic Fragility dilution experiment showed hemolysis with distilled water in all concentrations lyophilized aqueous extract of *Costus spicatus* even at low concentrations. By diluting the lyophilized extract of *Costus spicatus* in saline (0.9% NaCl) concentration that showed the most clear supernatant compared to the control was 12.5%. We also used the same concentration buffered with sodium bicarbonate solution ($NaHCO_3$, 2.092g/L and Na_2CO_3, 2.640g/L - Audrich Sima) pH 10, bringing the final pH of the solution to 6.74, close to the physiological for comparison results.

After exposure at different NaCl concentrations (0.1% to 0.9%) showed the absorbance of the supernatant of each extract compared to the control. The reading was performed at 540 nm and the respective absorbances were quantified as percentage of hemolysis, whereas the concentration of 0.1% NaCl solution as 100% hemolysis, and a solution of 0.9% NaCl as the saline concentration.

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Results

Table 1- Scanning spectroscopy Electronic Absorption:

Sweep Molecular Adsorption Spectroscopy (UV light / quartz cuvette)

Wave-length (nm)	Reading (absorbance)
200	**inaccurate**
210	**inaccurate**
220	**inaccurate**
230	**1.540**
240	**0.797**
250	**0.689**
260	**0.880**
270	**1.090**
280	**1.093**
290	**0.930**
300	**0.720**
310	**0.511**
320	**0.492**
330	**0.436**
340	**0.376**
350	**0.312**
360	**0.246**
370	**0.182**
380	**0.125**
390	**0.084**
400	**0.058**

Read the absorbance of the lyophilized aqueous extract of *Costus spicatus*. Wavelengths from 200 nm to 400 nm UV light.

Table 2- Average absorbance at a wavelength of 540 nm.

Osmotic Fragility			
NaCl concentration	control	buffered	test
0.10%	3.327	3.16	2.891
0.20%	3.099	3.06	2.769
0.30%	3.030	2.90	2.701
0.40%	0.423	0.716	0.358
0.50%	0.272	0.375	0.180
0.60%	0.256	0.323	0.284
0.70%	0.245	0.312	0.252
0.80%	0.224	0.241	0.232
0.90%	0.207	0.224	0.232

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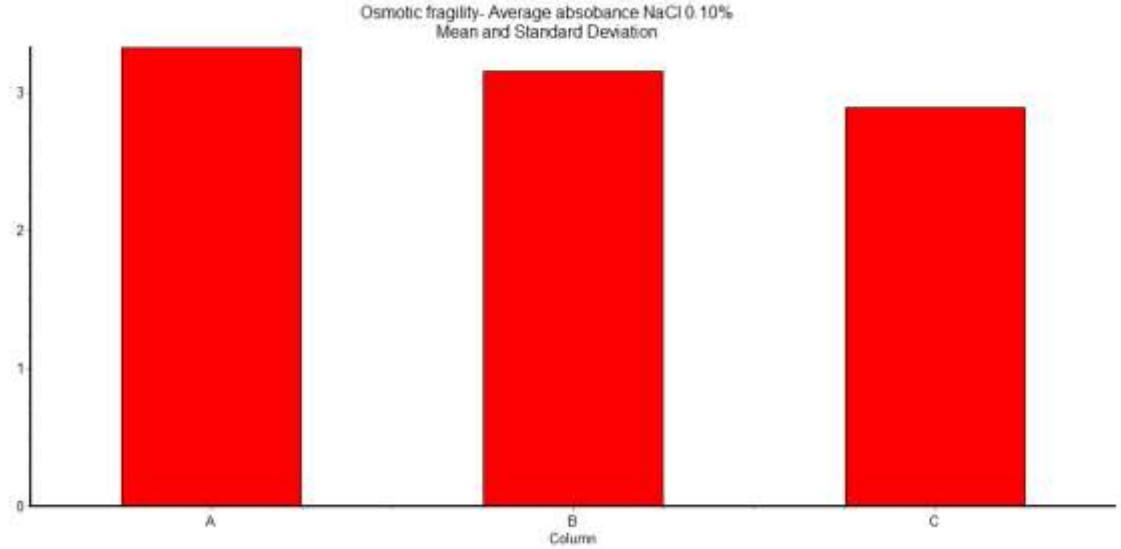
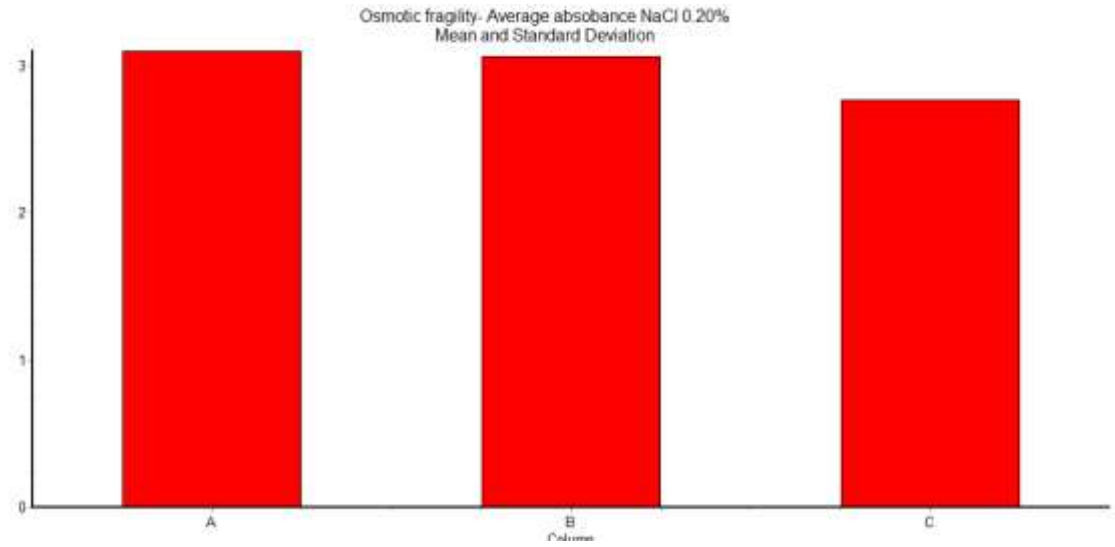
After exposure at different NaCl concentrations (0.1% to 0.9%) showed the absorbance of the supernatant of each extract compared to the control. The reading was performed at 540 nm. In the table columns indicate the absorbance values for the readings taken for the samples of the control group (different concentrations of NaCl), with buffered group (buffered extract) and the test group (extract unbuffered).

Figure 1- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.1%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and T.

Figure 2- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.2%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



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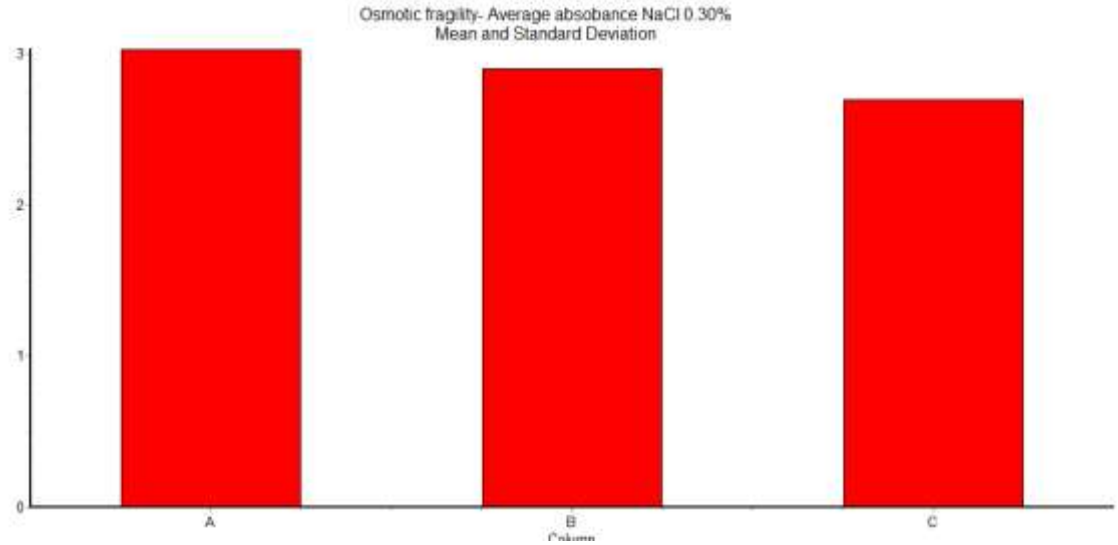
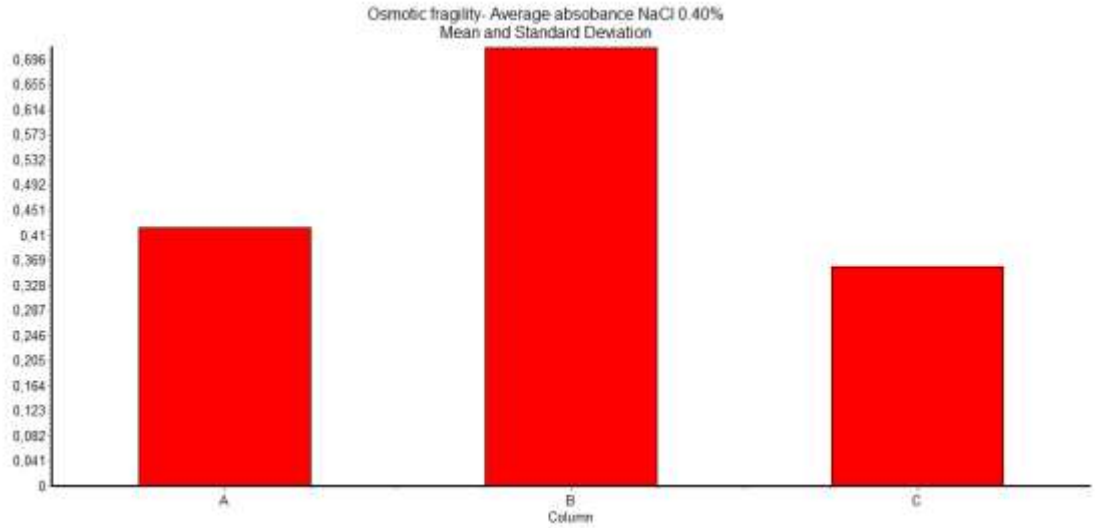
In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and T.

Figure 3- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.3%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and T. vFigure 4- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.4%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based

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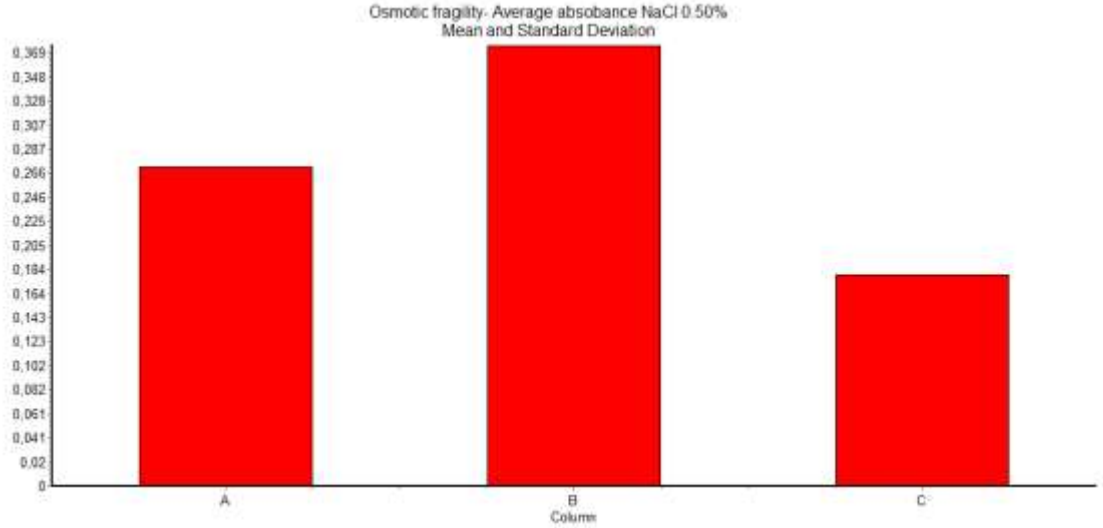
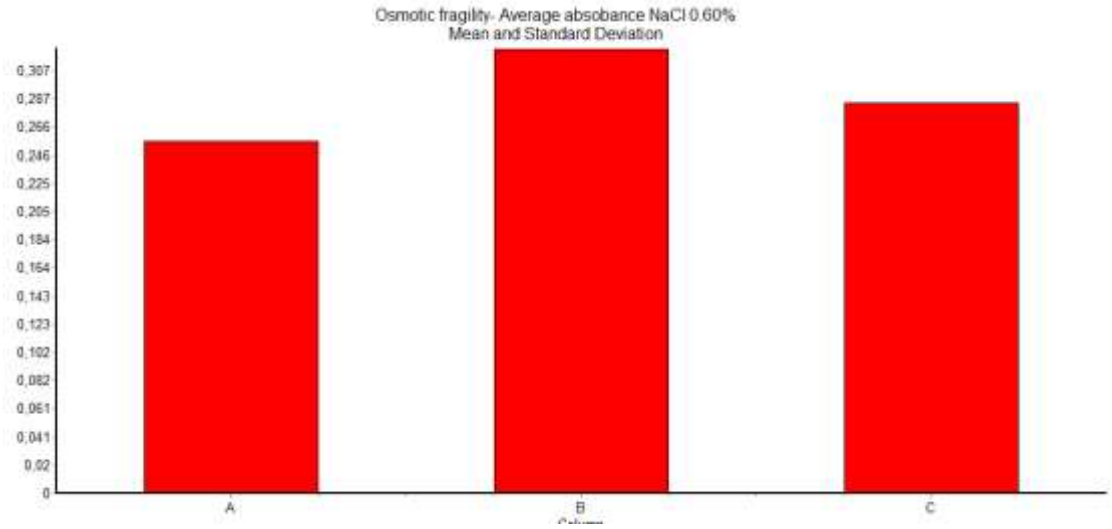
on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance (p <0.05) between measured absorbance values of group B and T. Figure 5- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.5%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance (p <0.05) between measured absorbance values of group B and T.

Figure 6- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.6%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance (p <0.05) between measured absorbance values of group C and B.

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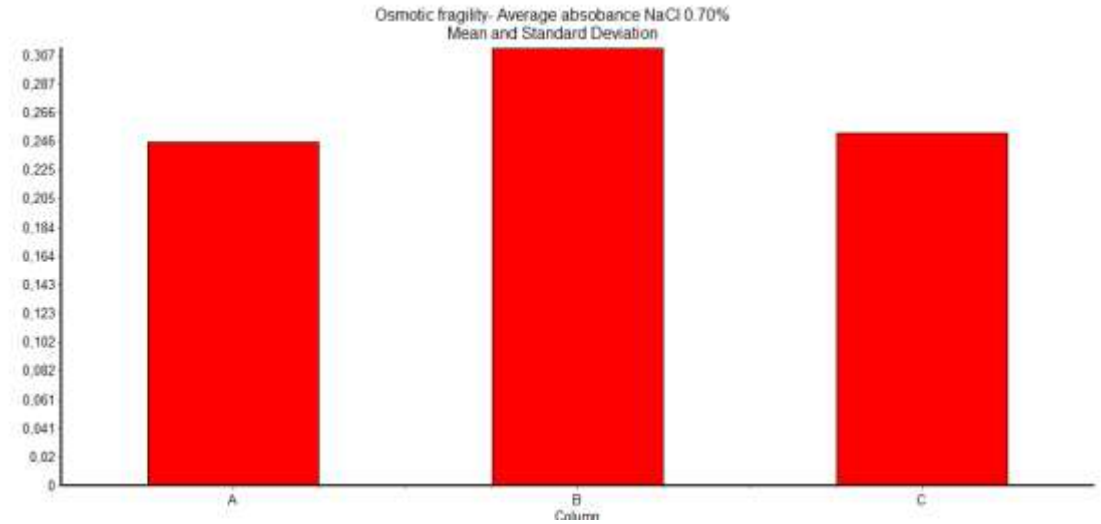
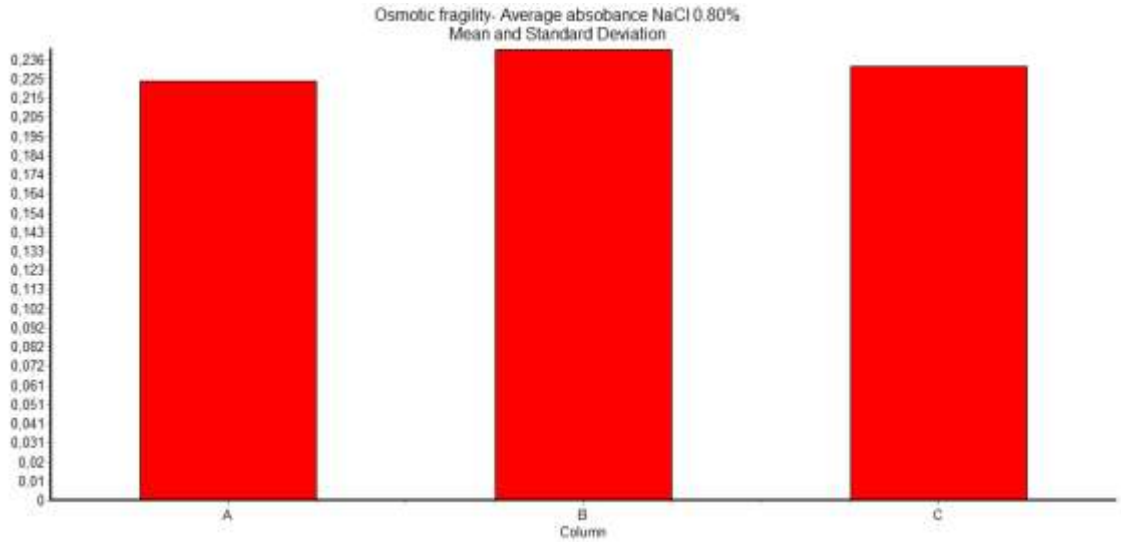
Figure 7- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.7%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and B. Figure 8- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.8%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and B.

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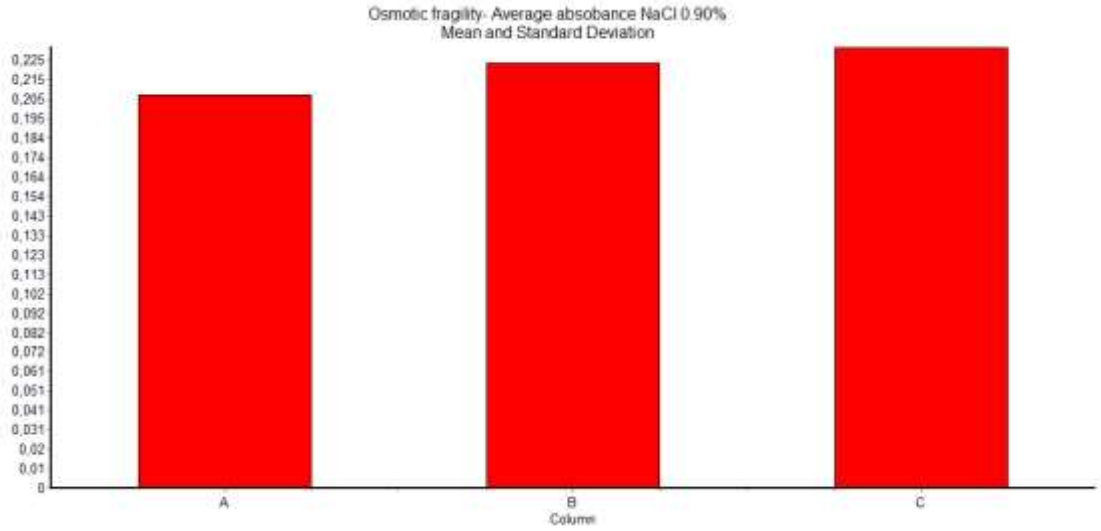
Figure 9- In the graphic below we illustrate the comparison of the mean absorbance values for the samples of the control group (C) (saline 0.9%) with buffered groups (B) (buffered extract) and test (T) (extract unbuffered).



In the graph {A} is equivalent to the control group (C), {B} is equivalent to the buffered group (buffered extract) [B] and {C} is equivalent to the test group (extract unbuffered) [T]. Based on the statistical analysis performed from the application of the multiple comparison test of Dunn was possible to observe a significance ($p < 0.05$) between measured absorbance values of group C and T.

Based on the performed statistical analysis, from the application of Multiple Comparison Test Dunn was no evidence of significance ($p < 0.05$) between the measured absorbance values of C and T group compared to saline concentrations of 0.1%, 0.2 % and 0.3%. However compared to solutions with salt concentrations of 0.4% and 0.5% a significant difference was observed between groups B and T. In Samples with saline concentrations are 0.6%, 0.7% and 0.8% a significant difference was observed in the values referring to the average of absorbance between the C and B groups. In the samples concerning the concentration of saline solution equivalent to 0.9% a significant difference was observed between the samples C and T groups.

Table 3- Average of percentage of hemolysis.

Percentage of hemolysis		
Control	Buffered	Test
100%	94.98%	86.9%
93.15%	91.97%	83.23%
91.07%	87.17%	81.18%
12.71%	21.52%	10.76%
8.18%	11.27%	5.41%
7.69%	9.71%	8.54%
7.36%	9.37%	7.57%
6.73%	7.24%	7.24%
6.22%	6.73%	6.97%

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The table shows the percentage of haemolysis levels, whereas the concentration of NaCl 0.1% as 100% hemolysis.

In relation to the mean absorbance values from the statistical analysis, which was applied a multiple regression analysis, one can observe a value of p <0.0008, demonstrating a significant difference in the absorbance values of the test group (unbuffered extract) compared to the control groups and the buffered extract. The results can be seen in the graphs of Figures 10 and 11.

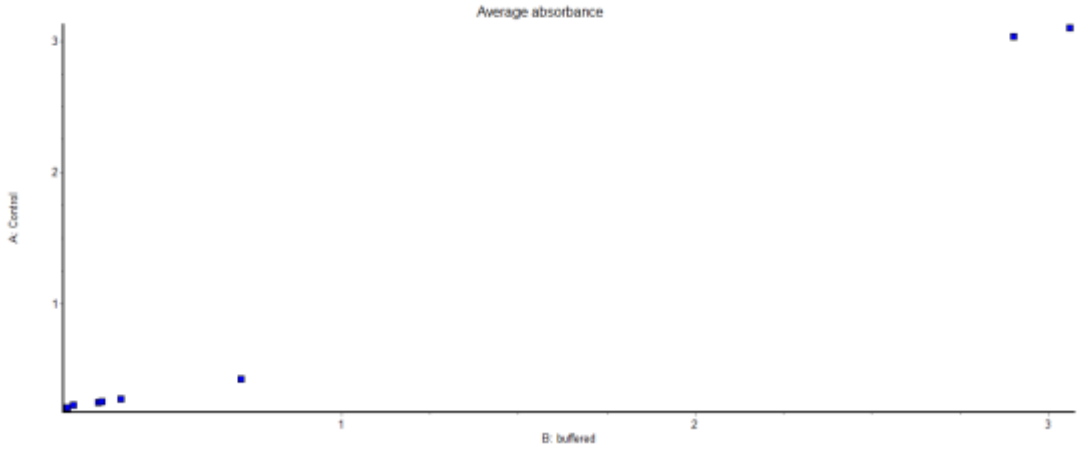

Figure 10- Average absorbance at a wavelength of 540 nm. Multiple regression analysis compared to the absorbance values of the control group (A) with the buffered extract group (B).

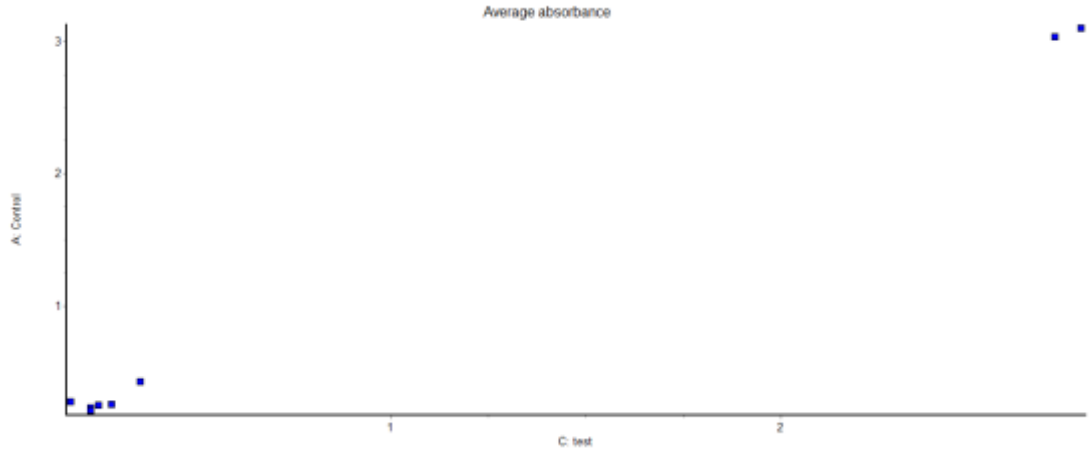

Figure 11- Average absorbance at a wavelength of 540 nm. Multiple regression analysis compared to the absorbance values of the control group (A) with the extract group unbuffered (C).

Compared with figures on the percentage of hemolysis, from the statistical analysis, where we applied a multiple regression analysis, one can observe a value of p <0.0002, showing a significant difference in hemolysis percentage values of test group (unbuffered extract) compared to the control groups and the buffered extract. The results can be seen in the graphs of Figures 12 and 13.

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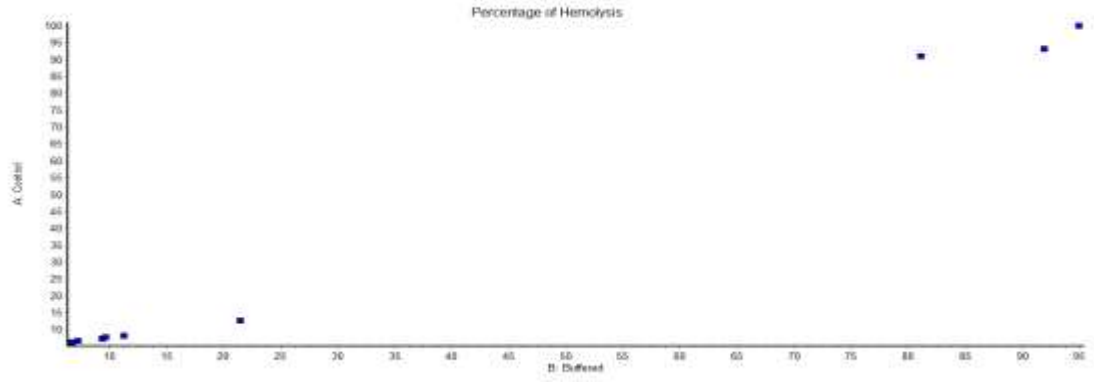

Figure 12- Percentage of hemolysis. Multiple regression analysis compared to the absorbance values of the control group (A) with the buffered extract group (B).

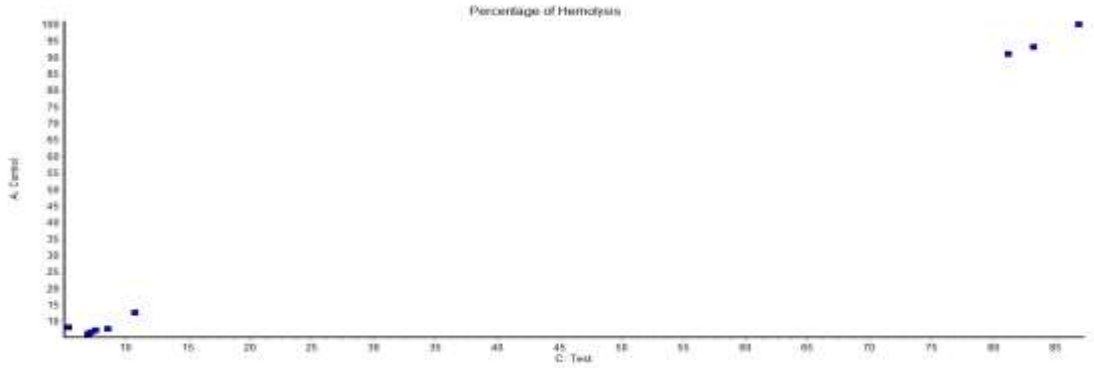

Figure 13- Percentage of hemolysis. Multiple regression analysis compared to the absorbance values of the control group (A) with the test (extract unbuffered) group (C).

When comparing the control group (A), by diluting 0.1% NaCl, buffered with the group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups A and C.

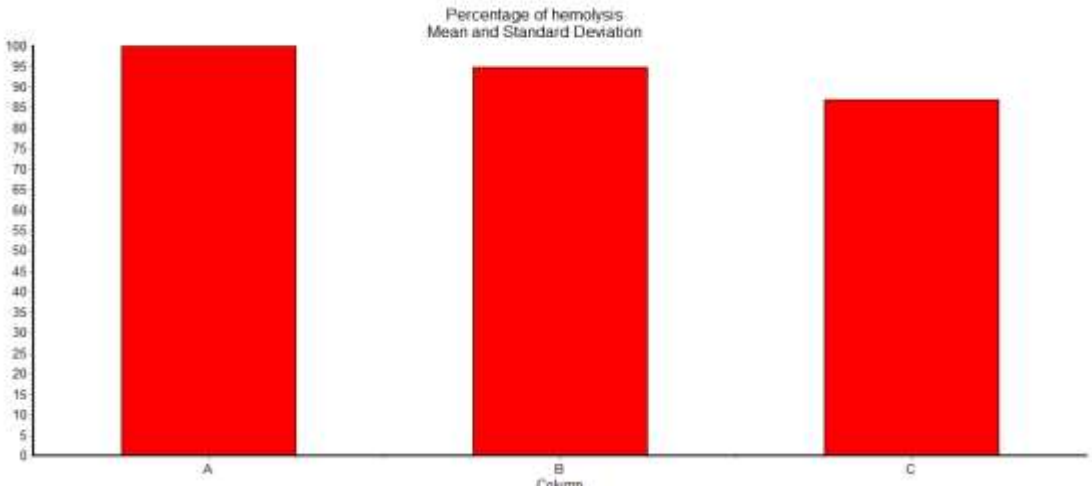

Figure 14- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

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When comparing the control group (A), by diluting 0.2% NaCl, buffered with the group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups A and C.

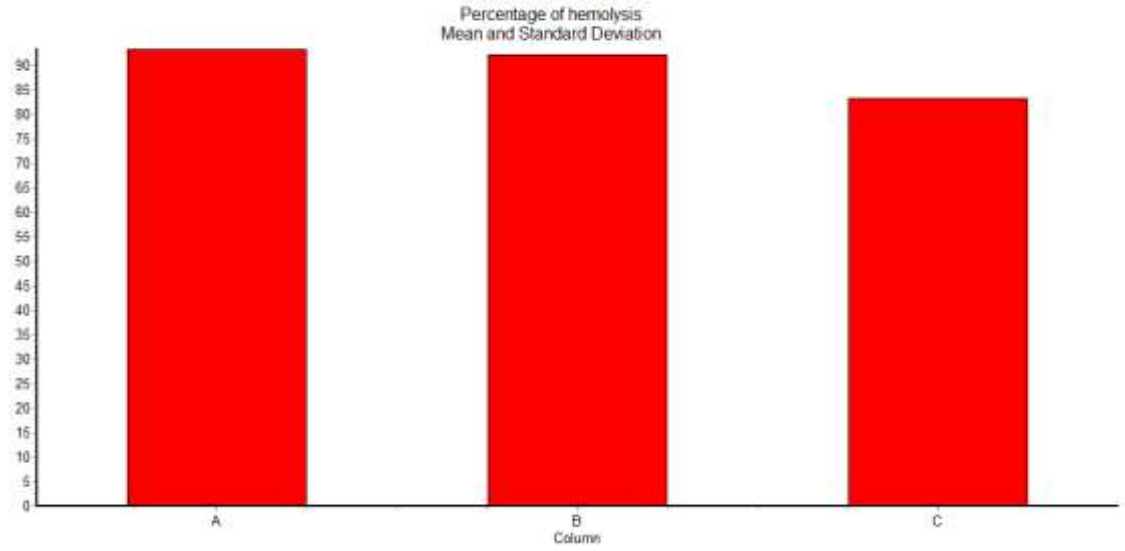

Figure 15- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

When comparing the control group (A), by diluting 0.3% NaCl, buffered with the group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups A and C.

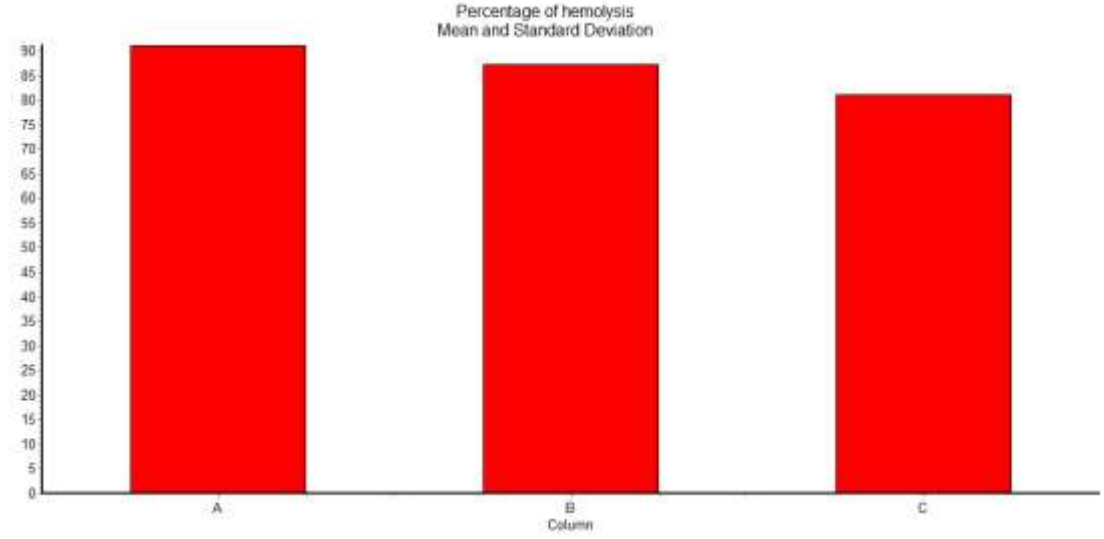

Figure 16- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

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When comparing the control group (A), by diluting 0.4% NaCl, buffered with the group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups B and C.

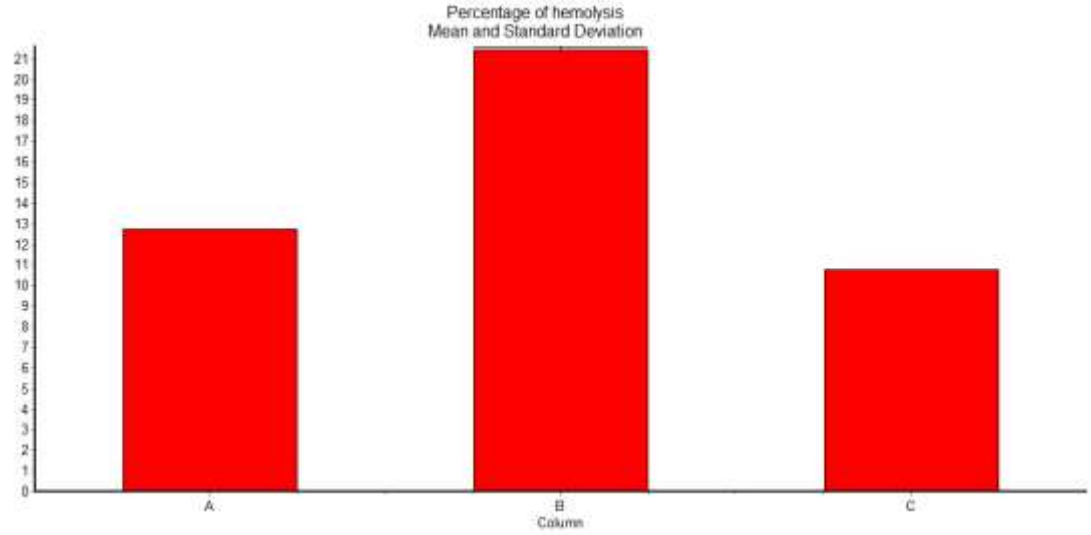

Figure 17- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

When comparing the control group (A), with 0.5% NaCl dilution with buffered group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups B and C.

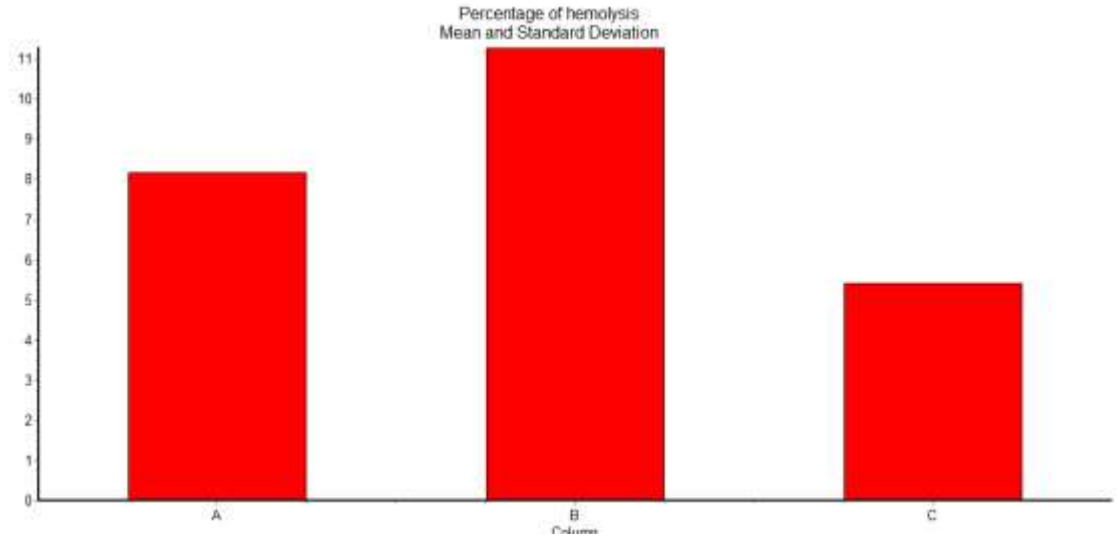

Figure 18- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

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When comparing the control group (A), by diluting 0.6% NaCl, buffered with the group (B) and the test group (C) observed a significant difference (p <0.05, Kruskal-Wallis test was applied) between groups A and B.

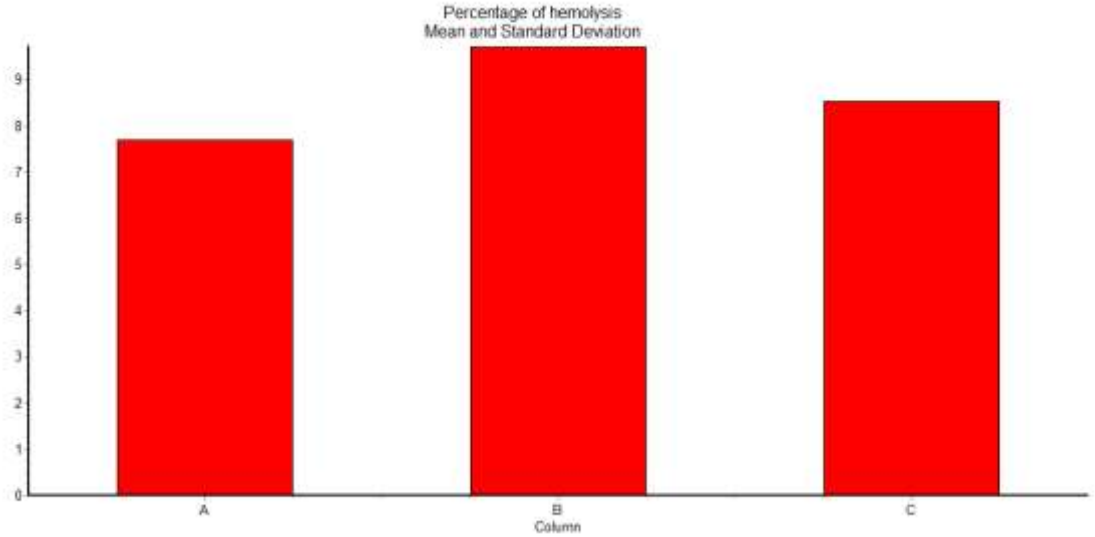

Figure 19- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

When comparing the control group (A), by diluting 0.7% NaCl, buffered with the group (B) and the test group (C) observed a significant difference (p <0.05, Kruskal-Wallis test was applied) between groups A and B.

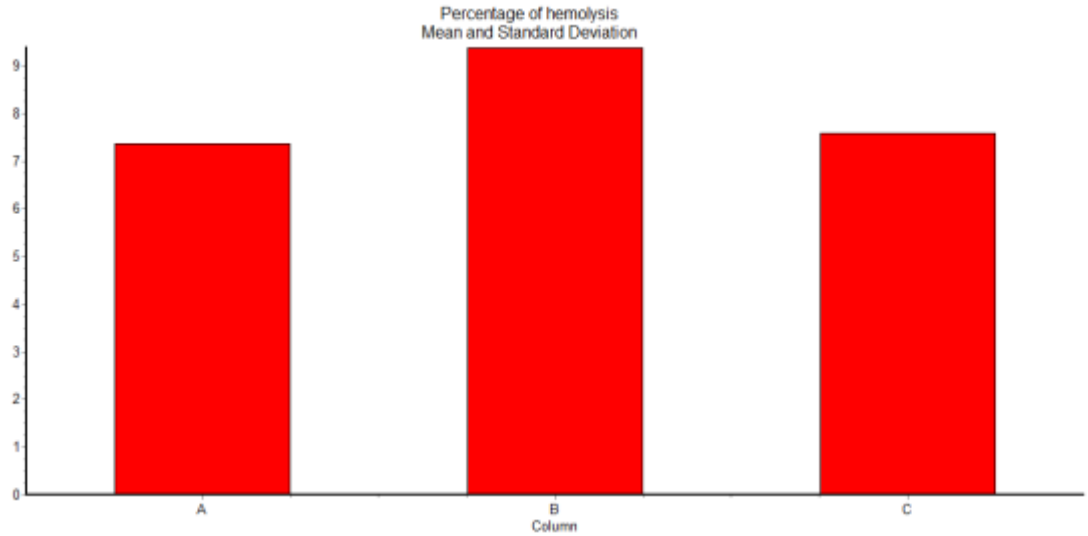

Figure 20- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

When comparing the control group (A), by diluting 0.8% NaCl, buffered with the group (B) and the test group (C) observed a significant difference (p <0.05, Kruskal-Wallis test was applied) between groups A and B; A and C.

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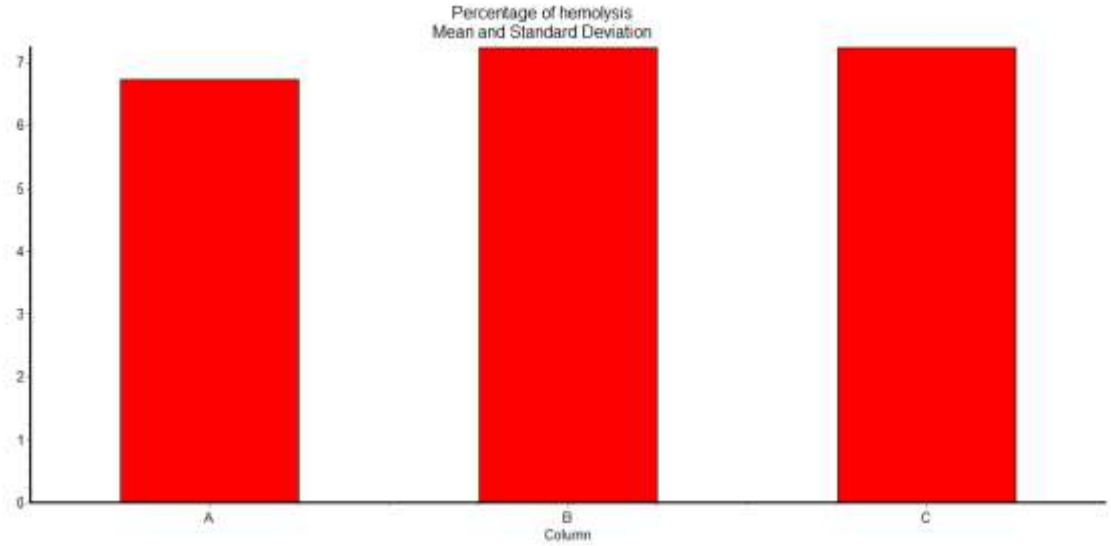

Figure 21- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

When comparing the control group (A), by diluting 0.9% NaCl, with buffered group (B) and the test group (C) observed a significant difference ($p < 0.05$, Kruskal-Wallis test was applied) between groups A and C.

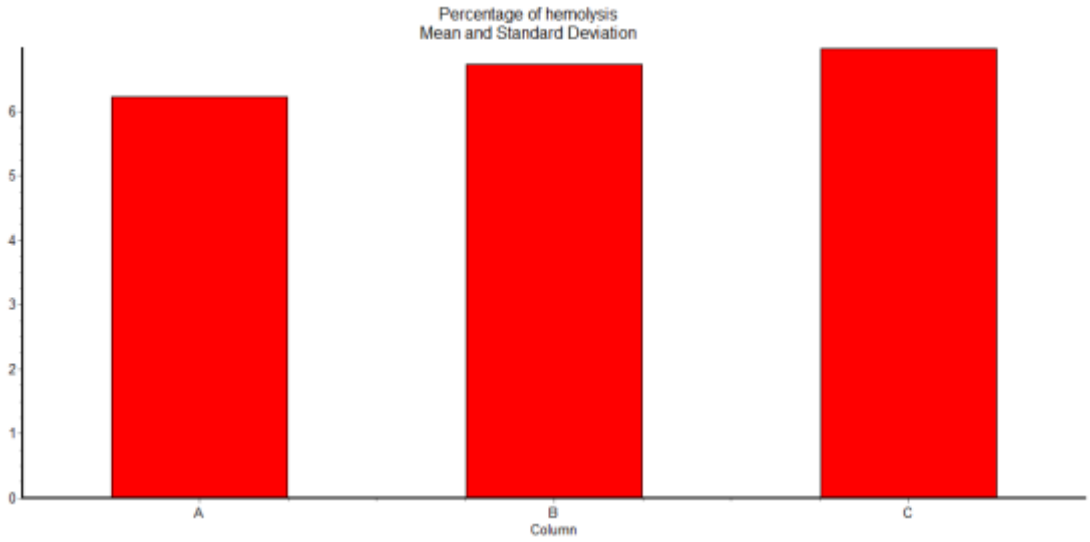

Figure 22- Statistical analysis of hemolysis percentage applying the Kruskal-Wallis test. Comparison of the mean from the reading of the control group samples (A), buffered group (buffered extract) (B) and the test group (extract unbuffered) (C).

Based on the analysis of the results can be speculated that the salt dilution related to the 0.6% and 0.7% correspond to possible concentrations where the activity of saponins hemolytic support could be triggered once the figures for the increase in absorbance in the samples matches the increase in the percentage of hemolysis observed in referring salt concentrations, when comparing the groups C and B.

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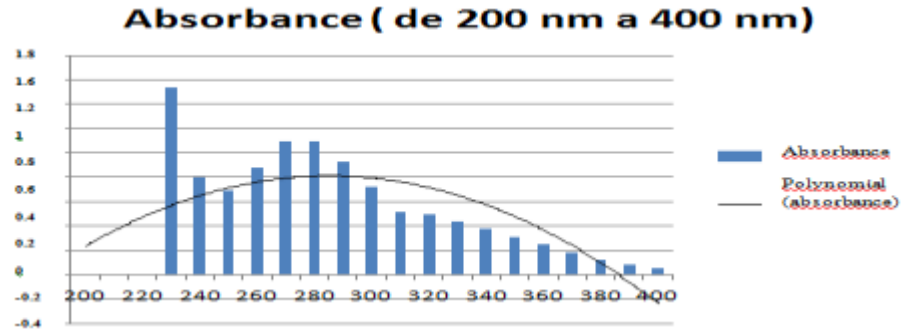


Figure 23- Absorbance curve of the lyophilized aqueous extract of *Costus spicatus* diluted to 1.06% in distilled water. Peaks in the ranges of 260 nm to 290 nm.

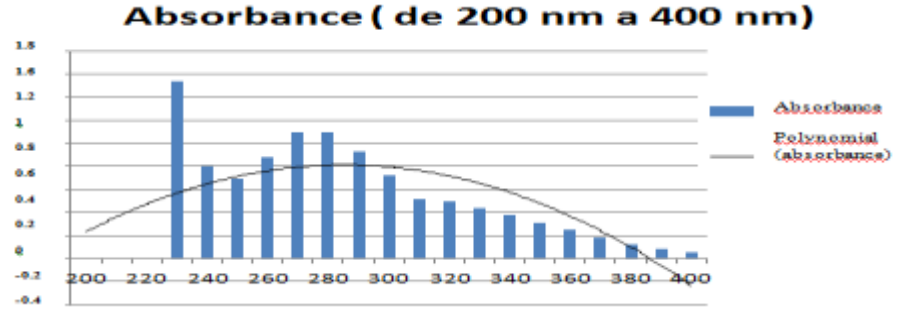
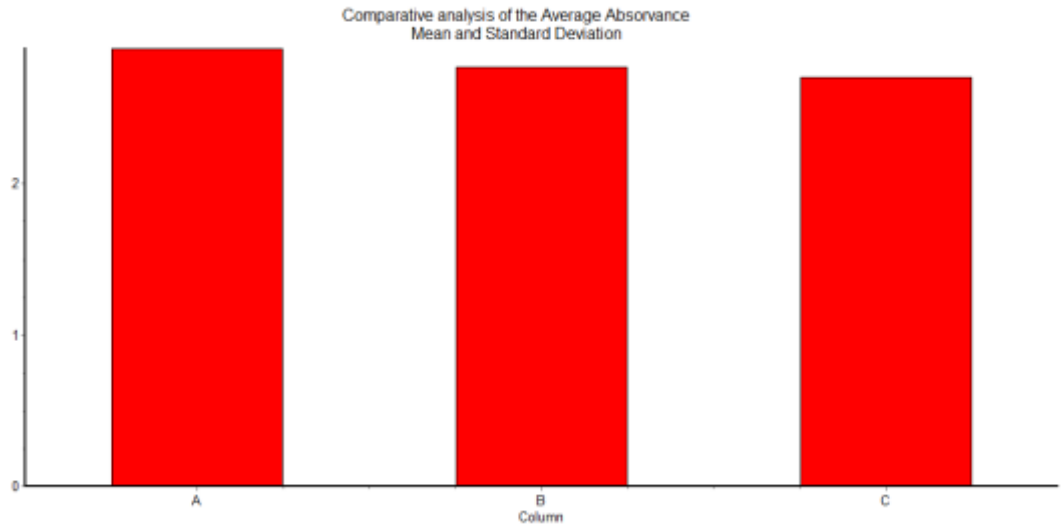


Figure 24- Absorbance curve of crude aqueous extract of *Costus spicatus* diluted to 6.25% in distilled water. Peaks in the ranges of 260 nm to 290 nm.

In our experiments we used the *Costus spicatus* extract at a concentration of 1.06% which is equivalent to 100% of the plant extract.

Figure 25- Comparative analysis of the average absorbance at a wavelength of 540 nm for the samples of the test groups.



Comparative analysis of absorbance reading performed for Test Group (extract did not buffered) saline to concentrations equivalent to 0.1% (A), 0.2% (B) and 0.3% (C) respectively. By applying the statistical test of Kruskal-Wallis was observed that there was no significant difference between the values of the different test groups ($p > 0.9999$).

These results allow us to suggest that the molecules present in the extract have a stable behavior in these saline concentrations analyzed.

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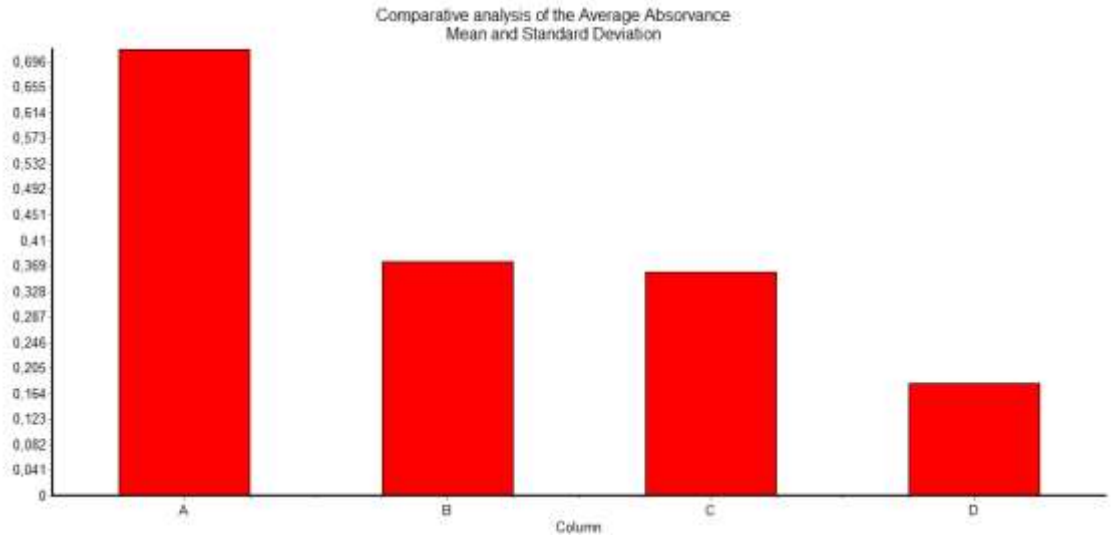
Figure 26- Comparative analysis of the average absorbance at a wavelength of 540 nm for the samples of the test and buffered groups.

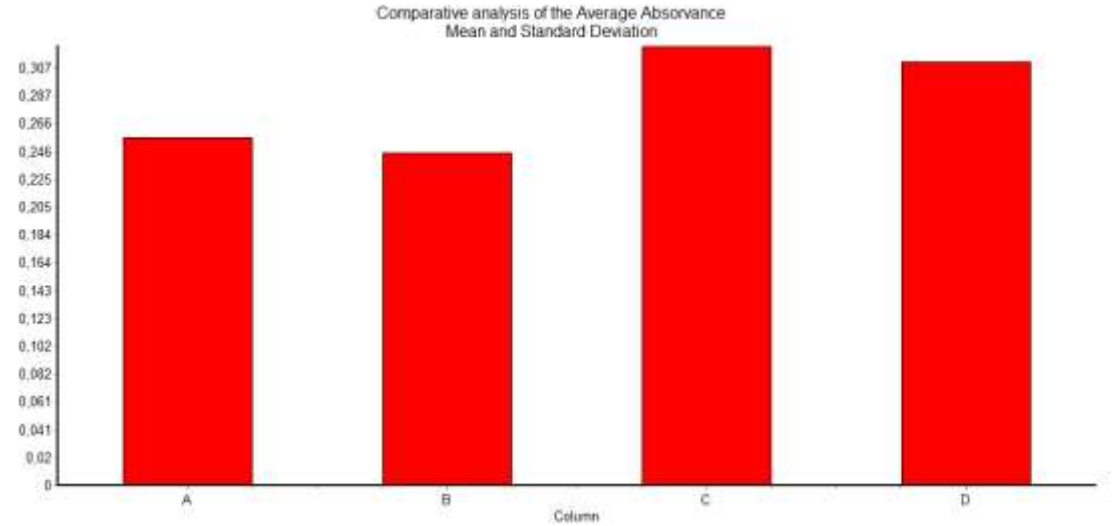


The comparative analysis of readings of sample absorbance values between buffered groups {A[0.4%] and B [0.5%]} and test {C [0.4%] and D [0.5%]} for salt concentrations of 0.4% 0.5% was held from the application of the statistical test of Kruskal-Wallis. From the results it was observed that there was no significant difference between the samples of buffered groups and between samples of the test group, however there was a significant difference when comparing the samples buffered group (0.4%) with the group of samples test (0.5%).

These results allow suggesting the stability of the molecules buffered saline and test groups in concentrations of 0.4% and 0.5%.

These results allow us to suggest a stability of the molecules of buffered and test groups in salt concentrations of 0.4% and 0.5%. It can be observed that the molecules in buffered saline at a concentration of 0.4% (A) would show a tendency ($p<0.05$) towards hemolytic action compared with molecules in unbuffered saline at a concentration of 0.5% (D).

Figure 27- Comparative analysis of the average absorbance at a wavelength of 540 nm for the samples of the control and buffered groups.



From the analysis of the results may suggest that the effect favor hemolytic referring to molecules in the buffered extract are most evident when comparing the group with the C 0.7 B

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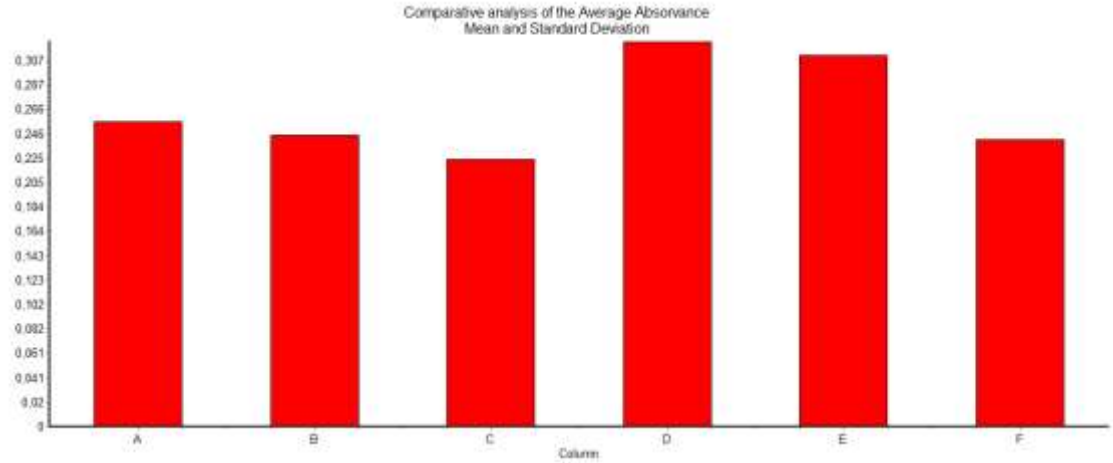
0.6 (p <0.01). The Kruskal-Wallis test was used to carry out the comparative analysis of the data.

The control (saline referring to 0.6% and 0.7%) are equivalent to (A) and (B) respectively while the buffered groups (referring to 0.6% saline, and 0.7%) are equivalent to (C) and (D), respectively.

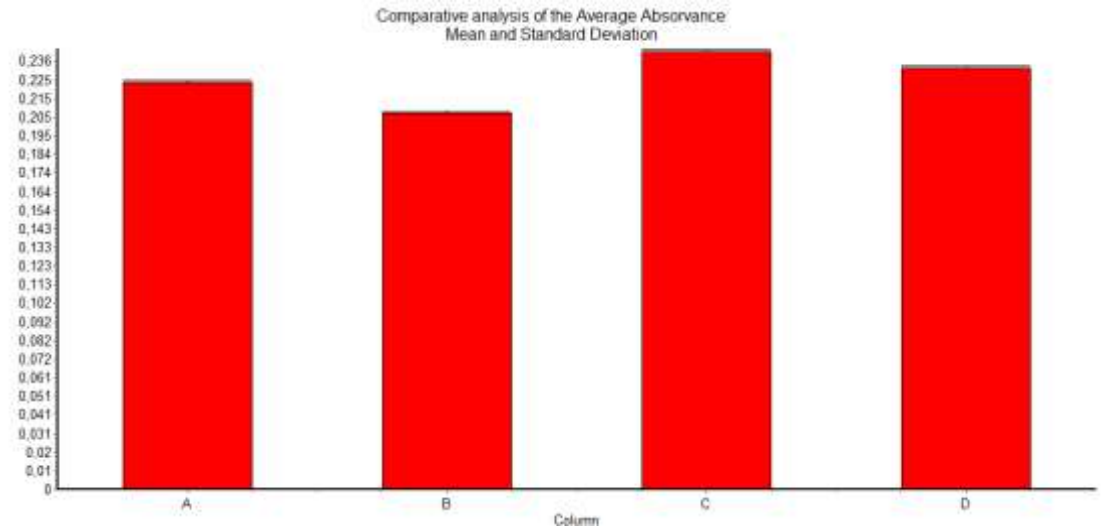
Figure 28- Comparative analysis of the average absorbance at a wavelength of 540 nm for the samples of the control and buffered groups.



From the analysis of the results may suggest that the effect favor hemolytic referring to molecules in the buffered extract are most evident when comparing the group with the C 0.8 B 0.6 (p <0.01). The Kruskal-Wallis test was used to carry out the comparative analysis of the data.

Control groups (referring to 0.6% saline, 0.7% and 0.8%) are equivalent to (A), (B) and (C) respectively while the buffered groups (referring to 0.6% saline, 0.7% and 0.8 %) equivalent to (D), (E) and (F) respectively.

Figure 29- Comparative analysis of the average absorbance at a wavelength of 540 nm for the samples of the control, buffered and test groups.



From the analysis of the results may suggest that the effect favor hemolytic referring to molecules in the buffered extract are most evident when comparing the group with the C 0.9 B

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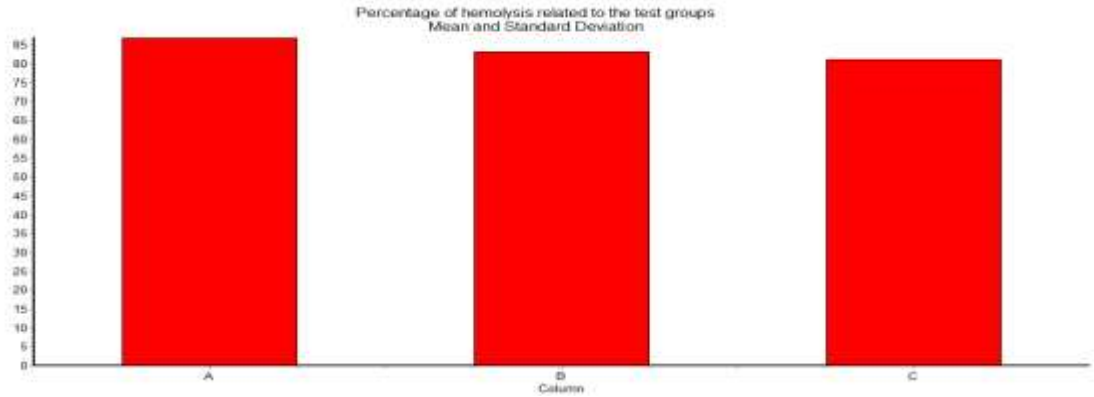
0.8 (p <0.05). The Kruskal-Wallis test and Dunn's multiple comparisons test were applied to the benchmarking analysis.

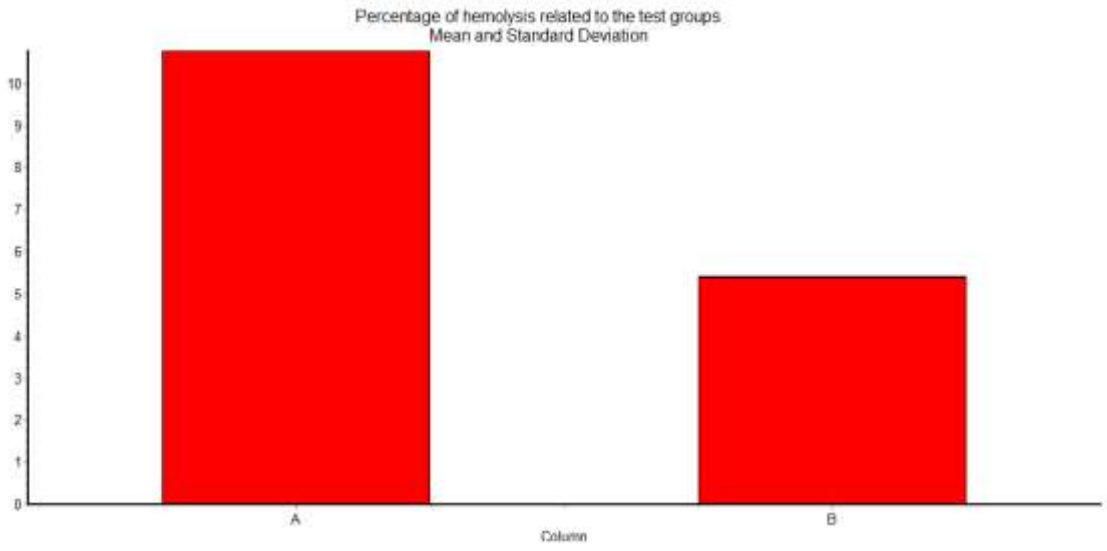
The control (related to saline 0.8% and 0.9%) are equal to (A) and (B) respectively while the buffered group (referring to saline 0.8%) is equivalent to (C) and the test group (referring the 0.9% saline solution) is equivalent to (D) respectively. From this assessment it was observed that there was no significant difference between groups B 0.8% and 0.9% T suggesting that the alleged benefit hemolytic activity related to molecular grouping present in the extract did not change but there was a decrease this effect compared to the analysis the above groups more dilute salt concentrations. We suggest that the ion concentration of the medium influence on the molecular behavior of molecules with possible hemolytic favor action.

Figure 30- Comparative analysis of the hemolysis percentage values between the test groups for saline concentrations equivalent to 0.1%, 0.2% and 0.3%.



In the chart above (A) is the test group (T 0.1%), (B) represents the test group (T 0.2%) and (C) is the test group (T 0.3%). From the analysis of the results was observed that there was a significant difference (p< 0.05) in the percentage of hemolysis between T 0.1% and T0.3%. The Multiple comparison test of Dunn was applied.

Figure 31- Comparative analysis of the hemolysis percentage values between the test groups for saline concentrations equivalent to 0.4%, and 0.5%.



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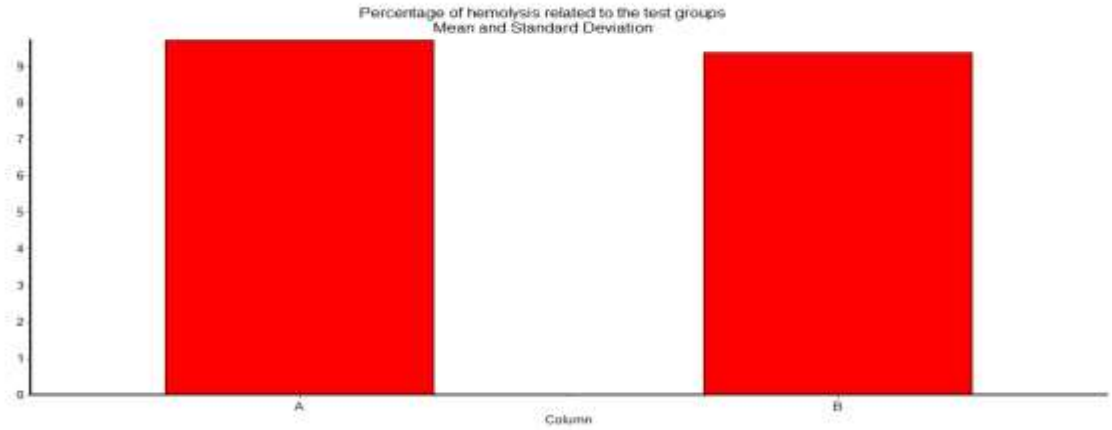
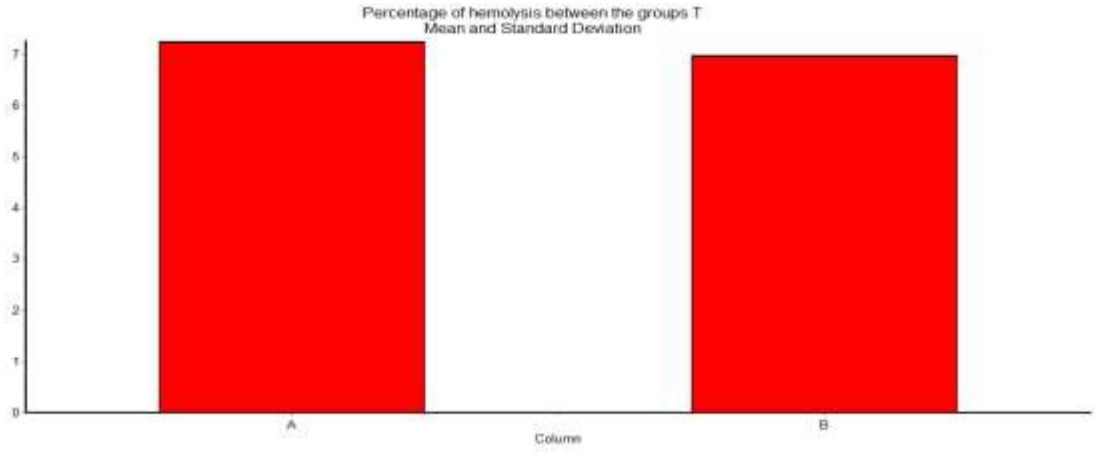
In the chart above (A) is the test group (T 0.4 %) and (B) represents the test group (T 0.5%). From the analysis of the results was observed that there was a significant difference ($p<0.0001$) in the percentage of hemolysis between T 0.4% and T 0.5%. The Paired T test was applied.
Based on the analysis of the above graphs can be seen that the hemolysis percentage decreases the extent to which there is an increase in the saline concentration to the concentration of 0.3% for the non-buffered extract (test group, T%)
For the saline concentrations equivalent to 0.4% to 0.5% was only possible to observe a significant difference between the buffered samples of the groups (% B) with the samples of the test groups (% T).
Figure 31- Comparative analysis of the hemolysis percentage values between the buffered groups for saline concentrations equivalent to 0.6%, and 0.7%.



For the saline concentrations equivalent to 0.6% to 0.7% was observed a significant difference ($p <0.0001$) between the buffered samples of the groups (% B), suggesting that the concentration of saline solution equivalent to 0.6% is the concentration ideal for the activation of possible molecules with hemolytic action referred to the buffered groups. The unpaired T test was applied to the data analysis.
Figure 32- Comparative analysis of the hemolysis percentage values between the unbuffered groups for saline concentrations equivalent to 0.8%, and 0.9%.



Based on the analysis of the graph and the results obtained can be seen that as there is increasing salt concentration and not the buffering solution there is the increased potential hemolytic activity on the molecules that exert this effect.

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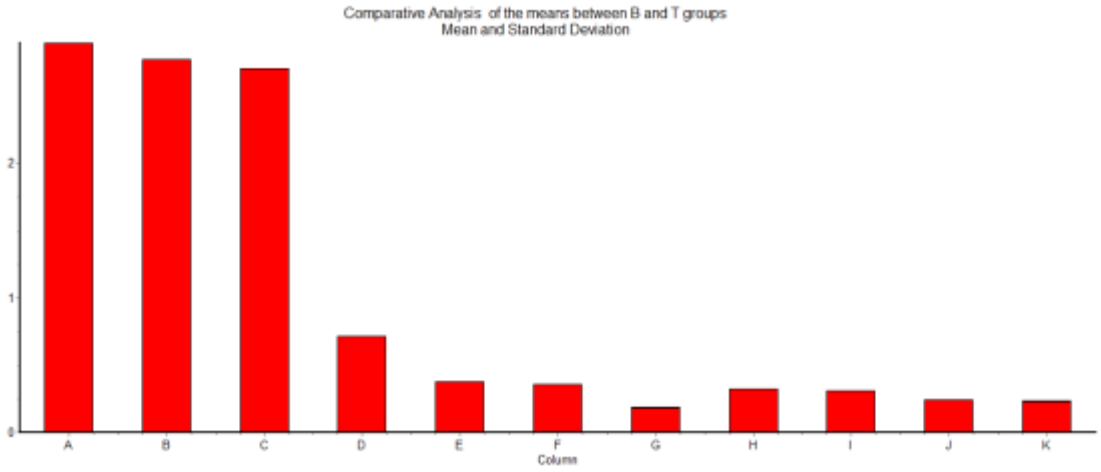
Based on the analysis of the graph and the results obtained can be seen that as there is increasing salt concentration and not the buffering solution there is the increased potential hemolytic activity on the molecules that exert this effect. In saline solution (0.9%) the buffered extract did not express significant hemolytic effect, while the unbuffered extract (T group) exhibits significant hemolytic action.

Figure 33- Comparative analysis between the mean absorbance readings of the amounts relating to the samples of B and T groups.

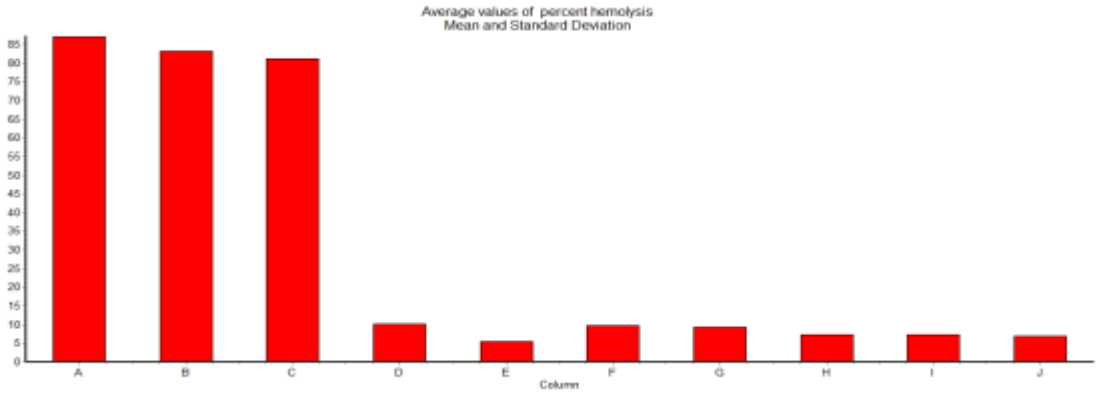


Based on statistical analysis of the results, we can say that there was a significant difference ($p < 0.001$) between the mean absorbance readings practically among all T and B groups), the exception where we can say that between the values of B6 and B7 there was less difference, although significant ($p < 0.05$) compared with the other groups, and whereas values between B8 and T9 no significant difference ($p > 0.05$). These results allow us to suggest the presence of molecules with different behaviors (hemolytic and non hemolytic) which are reportedly influenced by the salt concentration and buffering the medium. The analysis of variance of the Tukey-Kramer test was used for evaluating the data comparison.

Reading the columns. Here is the graphic consideration:

A= T 0.1%, B= T 0.2%, C= T 0.3%, D= B 0.4%, E= B 0.5%, F= T 0.4%, G= T 0.5%, H= B 0.6%, I= B 0.7%, J= B 0.8%, K= T 0.9%.

Figure 34- Comparative analysis between the mean of the percentage of hemolysis readings of the amounts relating to the samples of B and T groups.



Reading the columns. Here is the graphic considerations:

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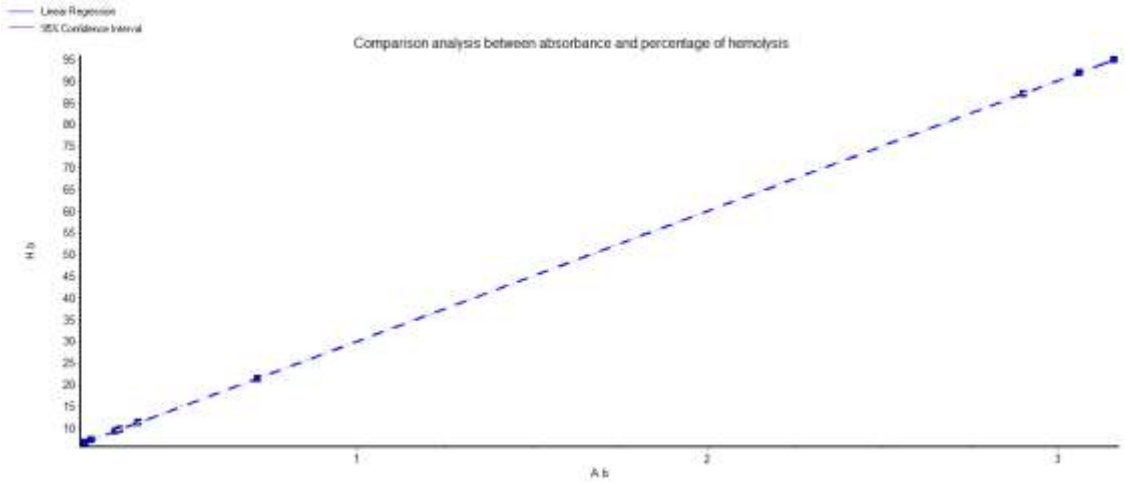
A= T 0.1%, B= T 0.2%, C= T 0.3%, D= T 0.4%, E= T 0.5%, F= B 0.6%, G= B 0.7%, H= B 0.8%, I= T 0.8%, J= T 0.9%.

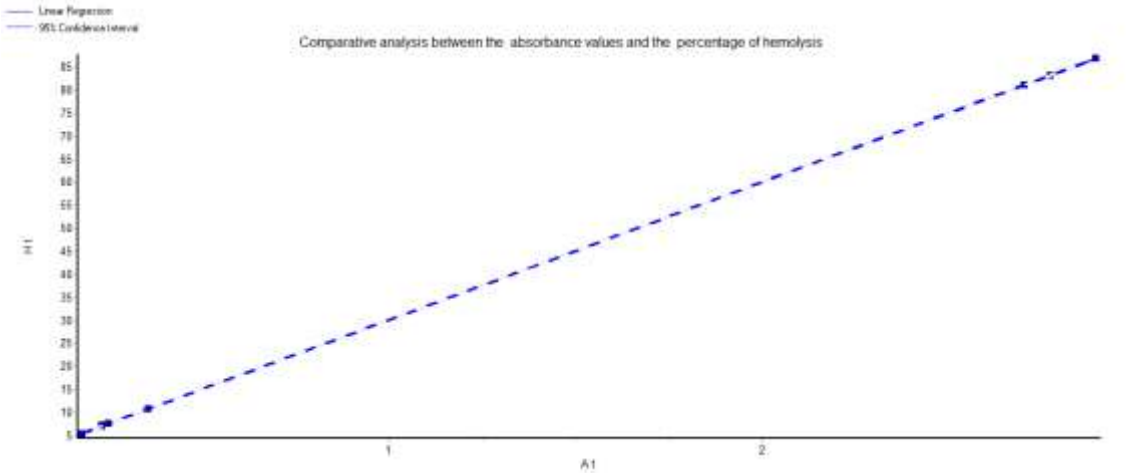
Based on the analysis of the results we observed a significant difference between T 0.1% and T 0.5% ($p<0.05$) and between T 0.2% and T 0.5% ($p<0.05$). From the results we can observe analyzed fact that T5 group expresses the lower hemolysis rate not related to the buffered extract. The Multiple comparison test of Dunn was applied.

Figure 35- Comparative analysis between the values of absorbance and the percentage of hemolysis referring to the buffered extract.



In the graph it can be noted that Hb refers to the values of the percentage of hemolysis (%) referred to the buffered extract and Ab extract refers to the absorbance values of the buffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data analysis can be seen from the linear regression analysis, there is an extremely significant correlation ($p <0.0001$) between the absorbance values and the percentage of hemolysis, increases as the percentage of hemolysis values match the increases in absorbance level.

Figure 36- Comparative analysis between the values of absorbance and the percentage of hemolysis referring to the unbuffered extract.



In the graph it can be noted that Hb refers to the values of the percentage of hemolysis (%) referred to the unbuffered extract and Ab extract refers to the absorbance values of the unbuffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data

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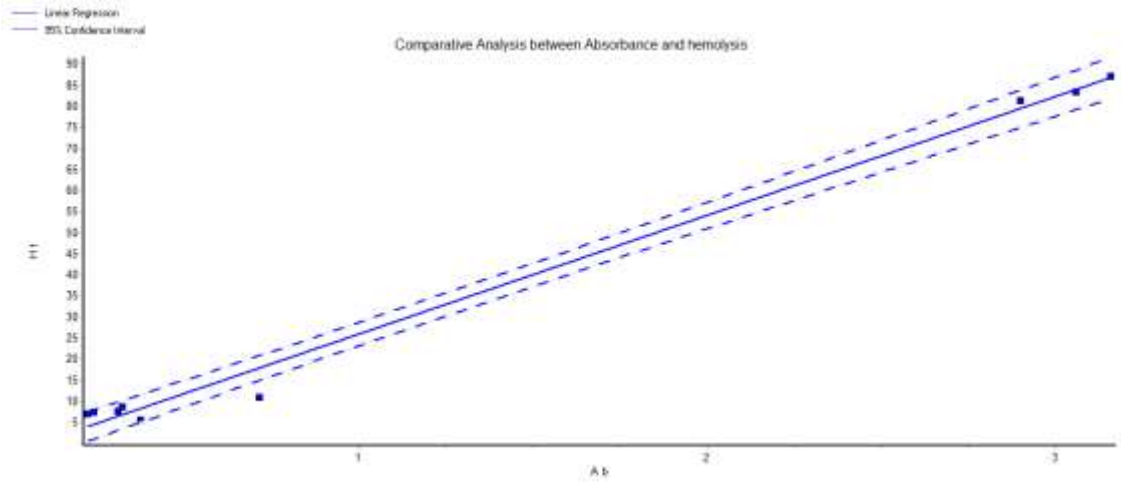
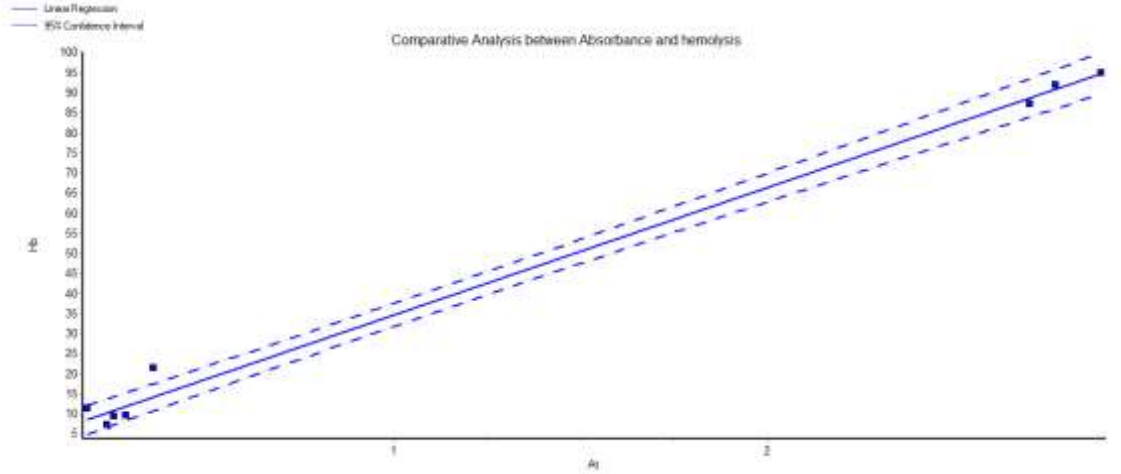
analysis can be seen from the linear regression analysis, there is an extremely significant correlation (p <0.0001) between the absorbance values and the percentage of hemolysis, increases as the percentage of hemolysis values match the increases in absorbance level.

Figure 37- Comparative analysis between the values of absorbance and the percentage of hemolysis referring to the buffered and unbuffered extracts respectively.



In the graph it can be noted that Ht refers to the values of the percentage of hemolysis (%) referred to the unbuffered extract and Ab extract refers to the absorbance values of the buffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data analysis can be seen from the linear regression analysis, there is an extremely significant correlation (p <0.0001) between the absorbance values and the percentage of hemolysis, increases as the percentage of hemolysis values match the increases in absorbance level.

Figure 38- Comparative analysis between the values of absorbance and the percentage of hemolysis referring to the unbuffered and buffered extracts respectively.



In the graph it can be noted that Hb refers to the values of the percentage of hemolysis (%) referred to the buffered extract and At extract refers to the absorbance values of the unbuffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data analysis can be seen from the linear regression analysis, there is an extremely significant correlation (p <0.0001) between the absorbance values and the percentage of hemolysis, increases as the percentage of hemolysis values match the increases in absorbance level.

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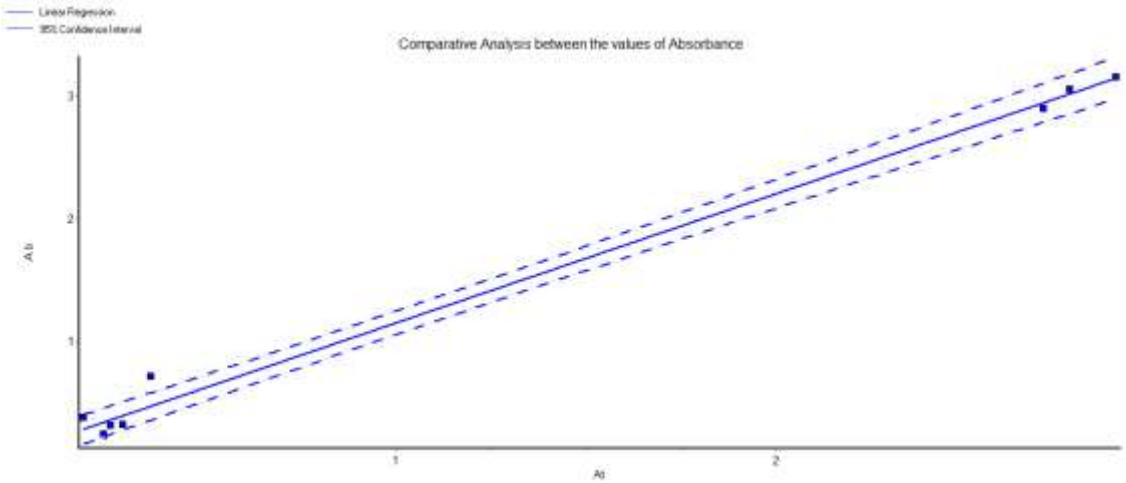
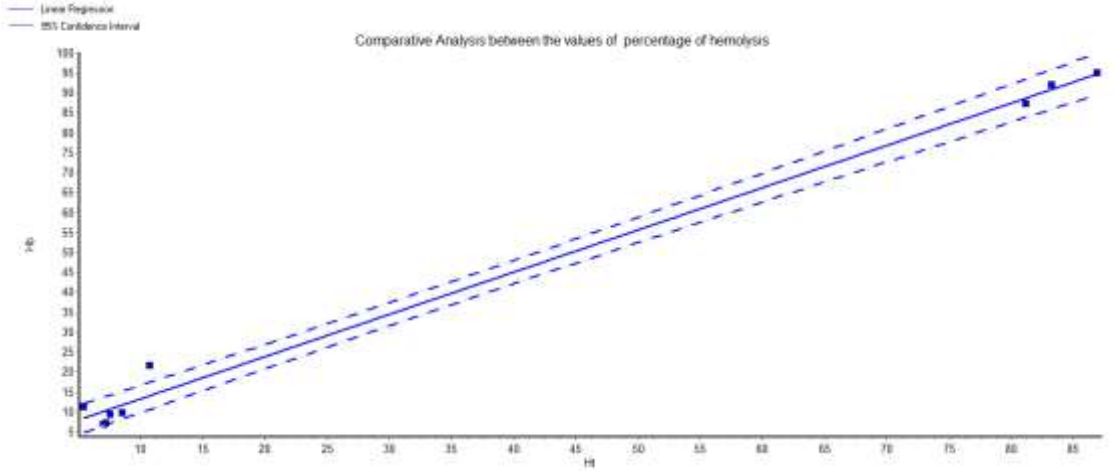
Based on the analysis of Figures 37 and 38, we can observe a statistically significant difference ($p < 0.0001$) between the absorbance values and percent hemolysis of buffered and unbuffered extracts.

Figure 39- Comparative analysis between the values of absorbances.



In the graph it can be noted that Ab refers to the values of the absorbance referred to the buffered extract and At extract refers to the absorbance values of the unbuffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data analysis can be seen from the linear regression analysis, there is an extremely significant correlation ($p < 0.0001$) between the absorbance values comparing the buffered extract with the unbuffered extract.

Figure 40- Comparative analysis between the values of percentage of hemolysis.



In the graph it can be noted that Hb refers to the values of the percentage of hemolysis referred to the buffered extract and Ht extract refers to the percentage of hemolysis values of the unbuffered extract at different concentrations of saline (from 0.1 to 0.9%). Based on data analysis can be seen from the linear regression analysis, there is an extremely significant correlation ($p < 0.0001$) between the percentage of hemolysis values (%) comparing the buffered extract with the unbuffered extract.

DISCUSSION

This study aimed to discuss the biological effects of a freeze-dried aqueous extract of *Costus spicatus* using techniques *in vitro*. The leaves of *Costus spicatus* are widely employed in folk

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medicine for the treatment of several diseases, including: malaria, hepatitis and urinary tract disease. The anti-inflammatory activity found in the leaf extract is related to the presence of flavonoids glycosides (PAES, 2013). *In vitro* techniques employed, the osmotic fragility action in blood in rats is commonly used to investigate the possible presence of compounds of pharmacological interest in extracts (PEREIRA, 2009). Red blood cells incubated with solutions of different concentrations decreasing suffer partial hemolysis from a certain concentration. This is complete hemolysis at lower concentrations (OLIVEIRA, 2010).

From the analysis of the results is possible to speculate that the aqueous extract of *Costus spicatus* demonstrated a protective effect of red blood cells in saline levels (NaCl) 0.1% to 0.3% compared to the blood without the extract. As we can see in the table 3, in NaCl solution concentrations of 0.1%, 0.2% and 0.3% of control showed a percentage of hemolysis 100%, 93.15% and 91.07% respectively. Also noting that the same concentrations the test tubes (with freeze-dried aqueous extract of *Costus spicatus*) showed a reduction in the percentage of hemolysis totaling 86.9%, 83.23% and 81.18% respectively. This protective effect is supposed therapeutic importance in clinical situations hyperglycemia, since the increase in the rate of blood sugar can result in the production of advanced glycation compounds (AGE) which oxidizing the cytoskeleton and the cell membrane, cell lysis optimizing (ANDRADES, 2010). The erythrocyte fragility feature is enhanced by the presence of oxidative stress, inflammatory processes, and increased glycation of proteins of procoagulant factors. (ANDRADES, 2010) The formation of Advanced Generation End Products (Advanced Glycation End-Products - AGE) in individuals with hyperglycemia frame has often been reported as an important factor. During Diabetes hyperglycemia is considered the main source of generation of AGE, but the participation of other reactive aldehydes, such as methylglyoxal and glycolaldehyde are also of great importance. (ANDRADES, 2010)

Through these results we suggest that the lyophilized aqueous extract of *Costus spicatus* mat have an anti-AGE action, promoting the maintenance of the cytoskeleton of spectrin of red blood cells. The antioxidant action of the species of Costus is not yet well established. However, studies show that many species of Zinziberaceae can sequester free radicals (HARAGUCHI et al, 1996).

Filho, (2010) described that in a study of the evaluation of antioxidant activity was observed that the hexane fraction flower showed a moderate activity. However, the phytochemical screening was not detected the presence of any of the classes of secondary metabolites studied, which demonstrates the presence of other compounds not searched in this fraction responsible for the antioxidant activity. It was related that can be found in the same fraction of the antioxidant activity of the samples at concentrations of 1 mg / mL, 0.1 mg / mL and 0.01 mg / mL appeared to increase with the dilutions. It is possible that the color of some samples interfere with the spectrophotometric reading, which does not associate the color intensity of the high antioxidant activity, not, therefore reflecting exactly the antioxidant activity of brightly colored samples. Another study found that a high concentration of the samples can affect the results, as it has strong intensity, interfering with the spectrophotometric reading, so extrapolating the absorbance values, dilution is necessary for the realization of these reading.
In our study we observed the possible antioxidant activity of extract related to the low percentage of hemolysis related to saline concentrations more diluted when comparing the test group (extract unbuffered) with the control (saline). With regard to the buffered group (buffered extract) we suggest that this effect was less significant, probably in relation to the

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tamponade molecules presented in the extract as alkaloids, triterpenes / steroids, flavonoids such as flavones, flavonols and flavanones and glycosides (flavonoid linked to sugars), and saponins, thus minimizing the antioxidant effect of the extract and its possible protective against hemolysis. Free radicals are produced continuously in the normal cell metabolism and in various pathological events. When in excess, may cause oxidation of the biological molecules. As a protection mechanism, the body has a complex system of antioxidant defense. The imbalance between oxidative challenge and antioxidant defense capacity is called oxidative stress. Erythrocytes play vital functions in the body which can be compromised due to oxidative stress. Red blood cells vulnerable to oxidative injury Become due to their constitution. Membrane unsaturated lipids are highly susceptible to oxidation, and the presence of oxygen free radicals and iron Enhances formation.

Furthermore, red blood cells are unable to synthesize new proteins and lipids to replace those which have been oxidized. Therefore, the maintenance of antioxidant mechanisms, such as vitamins E and C, and enzymes superoxide dismutase, catalase, glutathione system, and reductase metahemoglobin is important to prevent and repair damages.

Regarding the hemolytic activity, this is promoted markedly by the presence of hydroxyl on carbon 16, CH_2OH in Carbon 17, acetyl in Carbon 22 and still a acyl grouping in Carbon 21 among the various biological activities related to the saponins, are worth mentioning those related to increased immune response and the rupture of the membranes of erythrocytes. In general it can be seen that the immunoadjuvant activity of saponins is favored by the presence of a hydroxyl group at C-21, a methyl at C-17 and the presence of these two sugars or sugar chain attached to the aglycone, which seem to a secondary role (KAISER et al., 2010).

The flavonoids exhibit a variety of biological effects, these molecules have been the subject of attention due to its antioxidant properties as ROS (reactive oxygen species) scavengers and inhibitors of lipid peroxidation by uptake of O2 • radicals HO •, • LOO and O_2 deactivation. The erythrocyte cellular system is suitable for the study of the effects of ROS, by virtue of its structural simplicity (has no nucleus or mitochondria), accessibility and vulnerability to oxidation of its constituents. The main erythrocyte structures affected by EROS are the constituents and the membrane hemoglobin. The cell membrane is rich in poly-unsaturated fatty acids are therefore the target of lipid peroxidation. The structural membrane proteins, ankyrin and spectrin are also substrates radical reactions as well as some conveyor systems ion (anion transporter band 3 and several ATPases). Also enzymes can be inactivated membrane, particularly if they possess sulfhydryl groups. (TERAO & PISKULA, 1999).In this analysis, we observed that the extract unbuffered was able to reduce the percentage of hemolysis in dilutions of 0.1% to 0.5%.

It is important to note that as the extract was more diluted in dilution of 0.4% and 0.5%, this protective effect has become more evident, agreeing with reports in the literature where it is emphasized that the antioxidant activity observed in *Costus spicatus* flower was higher in the ethyl acetate fraction, reinforcing the need to expand the study to other metabolites of classes that can be responsible for this activity. When compared to BHT (butylated hydroxy toluene) as an external standard antioxidant activity at the same concentrations described in the literature (FILHO, 2010), the fraction of ethyl acetate flower has an antioxidant activity similar to the standard at 1.0 mg/mL and 0.1 mg/mL and higher in concentration of 0.01 mg/mL. As for the group regarding the buffered extract, noted that the dilution of 0.6% was significantly more

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hemolytic activity compared to the control group, however, this activity was expressed significantly, but less pronounced in dilution 0.7%, suggesting that the buffering provides more hydroxyl groups which behave in free form in solution can interact with these oxidation processes of generating reactive oxygen species which can oxidize proteins and lipids of biological membranes. This behavior becomes more evident in the lower dilutions of 0.8% and 0.9%, possibly where such groups hydroxyl become freer solutions, suggesting the participation of radicals in Fenton type reactions which are responsible for generating of reactive oxygen species which can peroxidize phospholipids and proteins of erythrocyte membranes. The dilution of 0.8% can be observed an equivalent behavior, hemolytic support both the buffered extract as extract unbuffered compared to the control group, suggesting this way that this would be the ideal concentration for the hydroxyl groups present themselves more free and more reactive.

Chemical studies performed with the aerial parts of *Costus spicatus* allowed the isolation of novel flavonoid glycosides di six flavonoids and other compounds such as quercetin. In its chemical composition is also recorded the presence of oxalic acid, tannins, saponins, mucilages and pectin. Saponins have high solubility in oil, but also have hydrophilic moiety, making them surfactants. In aqueous media, form a large quantity of foam. Have sour taste and can cause disruption of cell membranes (AZEVEDO et al., 2014). Based on the obtained results we can suggest that the hemolytic effects are related to the presence of saponins and anti-hemolytic are related phenolic compounds.

Costus spicatus, used in Brazilian traditional medicine to expel kidney stones, contains steroidal saponins with different chemical characteristics. In spite of its popular utilization as potent diuretic, no scientific reports correlate this activity with the chemical constituents of the extract. Therefore, two steroidal saponins (3 beta, 22 alpha, 25R)-26-(beta-D-glucopyranosyloxy)-2-methoxyfurost-5-en-3-yl O-D-apio-beta-D-furanosyl-(1-->2)-O-[6-deoxy-alpha-L-mannopyranosyl-(1-->4)]-beta-D-glucopyranoside (1) and (3 beta,22 alpha,25R)-spirostan-3-yl O-D-apio-beta-D-furanosyl-(1-->2)-O-[6-deoxy-alpha-L-mannopyranosyl-(1-->4)]-beta-D-glucopyranoside (1a), were isolated from the rhizomes of this plant and their effects on the Na+-ATPase and (Na+ + K+)-ATPase activities of the proximal tubule from pig kidney were evaluated. It was observed that 1 and 1a inhibit specifically the Na+-ATPase activity (DE SOUZA, et al., 2004). These findings could explain the effect of saponins with higher concentrations of salt solution which could additionally inhibit the ion-exchange protein-band 3 found in the erythrocyte membrane, besides promoting a possible destabilization of the cytoskeleton of spectrin which assists in maintaining erythrocyte membrane architectural, contributing to the hemolytic action is triggered.

Interestingly, the anti hemolytic action is possibly related to the presence of phenolic molecules in the extract, which would have its most obvious action spectrum in more dilute salt concentrations while saponins molecules exhibit its effect at lower concentrations saline, with its more free hydroxyl groups in this way, these hydroxyl groups may join possible towards oxidative reactions such as the Fenton reaction in the presence of heme plus interact with membrane proteins such as transport proteins band 3 and pump Na^+/K^+ATPase, besides interacting with the membrane lipid peroxidation promoting actions that could possibly contribute to achieve osmotic fragility of erythrocyte membrane and consequent hemolysis.

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New experiments and analysis are being developed in our laboratory in order that we can check the effect of this extract in animal models using diabetic rats as well as toxicological models in which bacteria strains are used for different repair mechanisms in order to study the possible oxidative stress and the potential microbicidal related natural product extract under study.

CONCLUSION

From the analysis of the results obtained and associated with reports available in the literature, one can speculate that the evaluated extract features phenolic compounds with anti-hemolytic action which can be observed in more dilute samples of NaCl and saponins molecules which would be possibly related with the hemolytic effects observed at higher concentrations of saline solutions.

REFERENCES

1. ANDRADES, Michael Everton; 2010 glycolaldehyde Effect of coagulation protein damage parameters, Federal University of Rio Grande do Sul, Department of Biochemistry, 2010.
2. AZEVEDO, L.F.P.; FARIA, T.S.A.; PESSANHA, F.F.; ARAUJO, M.F.; LEMOS, G.C.S. **Phytochemical screening and antioxidant activity of *Costus spicatus* (Jacq.) Sw.** Brazilian Magazine medicinal plants, vol. 16 nº 2 Botucatu, Apr./June 2014. < http://dx.doi.org/10.1590/S1516-05722014000200007>
3. Brazil. Ministry of Health. Secretariat of Science, Technology and Strategic Inputs. Department of Pharmaceutical Services. The herbal medicine in the NHS and the Research Program on Medicinal Plants of the Central Drug / Ministry of Health, Department of Science, Technology and Strategic Inputs, Department of Pharmaceutical Services. - Brasilia: Ministry of Health, 2006.
4. DE SOUZA, A. M.; LARA, L da S.; PREVIATO, J. O.; LOPES, A. G.; CARUSO-NEVES, C.; DA SILVA, B. P.; PARENTE, J. P. Modulation of sodium pumps by steroidal saponin. Z Naturforsch C. May/Jun, 59 (5-6):432-6. 2004
5. DOURADO, R. S .; LADEIRA, A.M. Identification of flavonoids in Hypericum cordatum (Vell.) N. Robson (Clusiaceae). Rev. bras. Bot. 31 (4) 611-620 Oct. / Dec.2008.
6. FILHO, R. B. Phytochemical contribution to the development of an emerging country. New Chemistry, vol. 33, no. 1, 2010.
7. GASPARRI, Susana. Study of antioxidant and mutagenic / antimutagenic activities induced plant extract of Costus spicatus, 2005.
8. HARAGUCHI, H., KUWATA, Y., INADA, K., SHINGU, K., MIYAHARA, K., NAGAO, M., YAGI, A. Antifungal activity from *Alpinia galanga* and the composition for incorporation of unsaturated fatty acid in cell growth. **Planta Medica**, v. 62, p. 308-313, 1996.
9. IDF, Internacional Diabetes Federation – IDF Diabetes Atlas - 4th Edition, 2009
10KAISER, Samuel; PAVEI ,Cabral; ORTEGA, George G. Study of the structure-activity relationship of adjuvant and/or hemolytic saponins by use of multivariate. Brazilian Magazine medicinal plants, vol. 20 nº 3 Curitiba, June/July 2010. <http://dx.doi.org/10.1590/S0102-695X2010000300003>
11. MARTINS, Nelson Lúcio Parada. Comparative analysis of skin healing with the intraperitoneal use of aqueous extract of Orbignya phalerata (rubs) controlled study in rats, 10, ORIGINAL ARTICLE, 66, Brazilian Surgical Acta, Vol 21 (Supplement 3) 2006

ISSN 2053-406X(Print), ISSN 2053-4078(Online)

12. OLIVEIRA, Antonio M. Altair, osmotic resistance of erythrocytes, 2010.
13. PAES, L.S.; MENDONÇA, M. S.; CASAS, L.L. Structural and Phytochemical aspect from vegetative part of *Costus spicatus* (Jacq.) Sw (Costaceae). Brazilian Magazine medicinal plants. vol.15 no.3 Botucatu 2013 http://dx.doi.org/10.1590/S1516-05722013000300011
14. PAH, the Pan American Health. Overview, Canada, 2005.
15. PEREIRA, Dewson Rocha, 2009 osmotic fragility of the aqueous extract of leaves indigofera suffruticosa on erythrocytes of rats 2009.
16. TERAO, J.; PISKULA, M. Flavonoids and Membrane Lipid Peroxidation Inhibition, Nutrition, 15, 790-791. Terao, J., Piskula, M. Flavonoids and Membrane Lipid Peroxidation Inhibition, Nutrition, 15, 790-791. 1999
17. WHO, World Health Organization, World Health Report, 1st edition, 2007.

ISSN 2053-406X(Print), ISSN 2053-4078(Online)